Penicillium commune spore production in solid-state fermentation of coffee pulp at laboratory scale and in a helical ribbons rotating reactor

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Abstract: ***Penicillium commune*** **was grown on coffee pulp (CP) by solid-state fermentation (SSF). The effects of the duration of CP thermal treatment and the effects of incubation temperature on spore production yield were studied at laboratory scale. The effect of mixing during fermentation was assayed at pilot plant scale in a 70 L stainless steel non-aseptic reactor equipped with helical ribbons for mixing solids. For thermal treatments of CP at 121 °C for 10, 20, 30 and 40 min, no significant difference in spore production yield was observed. Maximum sporulation yield was found at 25 °C; when the incubation temperature was higher than 30 °C, the sporulation yield decreased significantly. A spore production yield of 3.7 x 10^9 spores g^{-1} dry CP was obtained when continuous mixing (0.25 rpm) was used at pilot plant scale; however, a decrease in spore yield (1.4 x 10^9 spores g^{-1} dry CP) was observed under static conditions. Spore production was not affected when a scale factor between 79 and 105 was assayed from laboratory to pilot plant; at this level, the productivity obtained was 3.1 x 10^7 spores g^{-1} dry CP h^{-1}. This value is similar to that found in other reports using natural substrates but working at a smaller scale.**

Keywords: *Penicillium commune*; spores; rotating reactor; solid-state fermentation (SSF); coffee pulp

INTRODUCTION

Solid-state fermentation (SSF) processes simulate the natural conditions of filamentous fungi developed on wet substrates. For this reason, SSF is the cultivation technique used in cases where morphological differentiation is required, as in the production of conidiospores. Besides simulating the natural growth conditions, the biological and environmental advantages of SSF can overcome the engineering problems. Theoretically, SSF processes can be operated economically on a large scale despite the engineering difficulties involved when the technical demands (e.g. in non-sterile processes) and the production costs are low.[1] For such processes, the demand for spores to be used as inoculum (10^6–10^7 spores g^{-1}) is high at a large scale.

Coffee pulp (CP) is rich in soluble sugars, but it spoils easily and is difficult to handle; its utilization is limited by its content of anti-physiological (caffeine) and anti-nutritional (tannins and polyphenolic) compounds. Thus, there exists a need for a new technology that can handle and detoxify large amounts of CP.[2] SSF has been used for specific biological detoxification of coffee pulp using filamentous fungi at laboratory scale.[3] For laboratory scale processes, spores are usually produced in agar slopes and Petri dishes. However, this kind of culture cannot satisfy the demand at higher scales. On the other hand, the scale-up of SSF processes introduces heat transfer limitations.[4] Substrate mixing during fermentation has been suggested as a way to overcome such limitations and to improve the performance of reactors.[5–7] Adverse effects, however, have also been reported due to mycelium damage.[8] Reports have described spore production on natural supports such as buckwheat seeds,[9] oats[7] and rice bran,[10] yet it is difficult to predict the behavior of a particular microorganism in a mixed system, particularly when hyphae have no septa.

In this study, the effect of duration of substrate thermal treatment and the effect of incubation temperature on spore production yield (SPY) was evaluated at laboratory scale. The effect of mixing the solids was also evaluated, using a helical ribbons reactor (70 L capacity). The degrading caffeine and polyphenolic compound fungus *Penicillum commune* was used in this study.

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MATERIALS AND METHODS

Fungal strain and its maintenance

Penicillium commune V33A25, IRD-UAM collection, was used for CP solid-state fermentation. For short time periods, the microorganism was grown on a coffee infusion medium prepared as follows: sucrose, 2.0 g L^{-1}; KH_2PO_4, 1.3 g L^{-1}; Na_2HPO_4, 0.12 g L^{-1}; $MgSO_4$ $7H_2O$, 0.3 g L^{-1}; $CaCl_2$ $2H_2O$, 0.3 g L^{-1}; all components were dissolved in an infusion (40 g L^{-1}) of commercial ground coffee (Grand Mère ''familial'', café Grand Mère S.A., Vélizy-Villacoublay, France) in distilled water. After adding 20 g L^{-1} of agar, the medium was sterilized at 121 °C for 15 min. The cooled sterilized medium was inoculated with spores (1×10^6 mL^{-1}) and incubated at 30 °C for 6 days. The spores produced were suspended in 0.1% Tween-80 by magnetic agitation, and the resulting suspension was used to inoculate the SSF at laboratory and pilot plant scales. Spore concentration was quantified using a Malassez counting chamber.[11]

Substrate treatment

At laboratory and pilot plant scales the fermentation material used was prepared by uniformly mixing sun dried CP and distilled water until 50% moisture content was attained. The medium was heated in an autoclave, maintaining the steam temperature at 121 °C for different time periods (10, 20, 30 and 40 min); then it was cooled to room temperature. The initial moisture content of the medium was adjusted to 64.2 ± 0.26% by adding sterilized distilled water.

Inoculation

The treated CP was inoculated by adding the spore suspension described above. At laboratory scale this was done manually, whereas at pilot plant scale the suspension was sprayed over the CP, with continuous mixing of the solids in a helical ribbons reactor (70 L capacity). The average inoculum size was $7.0 \times 10^6 \pm 1.1 \times 10^6$ spores g^{-1} wet CP. The average pH of the CP was 4.3 ± 0.02 after inoculation. Experiments were performed at least in duplicate.

SSF at laboratory scale

Fermentation was carried out in small glass column fermenters (4.8 cm diameter × 15 cm height) which were filled with 100 g inoculated wet CP. The columns were incubated in a water bath at constant temperature. Saturated air was supplied at 1.0 mL air g^{-1} wet CP min^{-1} (1 vgm).

SSF at pilot plant scale

The whole SSF system consists of a reactor, air conditioning system and instrumentation for online CO_2 and temperature analysis. Figure 1 shows a schematic diagram of the system. The air conditioning system consists of a humidification column packed with Raschig rings, which uses water at constant temperature and provides a fixed aeration rate (10 L min^{-1}).

The 70 L capacity helical ribbons reactor (length 75.6 cm, width 32.6 cm and depth 38 cm) consists of a horizontal stainless steel tank (Fig. 1). The reactor capacity is nearly 30 kg wet CP. A rotating shaft was mounted in the center of the vessel with an agitation device that has two pairs of helical ribbons (internal and external). Two external ribbons move the solids from the edge of the vessel to the center, while the two internal ribbons move solids in the opposite direction (center to edge). The shaft was rotated by means of a motor and gearbox with a speed capacity between 0.25 and 1 rpm. A cover on the vessel enables charging of the reactor. A perforated pipe allows distribution of the spore suspension over the solids. Perforations on the bottom of the tank allow for the supply of conditioned air.

The fermenter was charged with 10.5 and 7.9 kg of wet CP, and the temperature of the water jacket was maintained constant at 25 °C. A glass column fermenter packed with the substrate taken from the reactor was also run in parallel as a control.

Experimental strategy

Laboratory scale. The effects of duration of thermal treatment (10, 20, 30 and 40 min at 121 °C) and the effects of constant incubation temperature (25, 28, 30, 32 and 35 °C) on SPY were evaluated. Additionally, in order to evaluate the effect of temperature changes on this yield, cultures were first incubated at a constant temperature (30 °C) for 20 h, then at 25, 28, 30, 32 or 35 °C until fermentation ended.

Pilot plant scale. Spore production was evaluated for both static and continuously mixed cultures (0.25 rpm after 20 h of incubation). At the end of fermentation, samples were taken from the central zone of the reactor. Samples were maintained at 4 °C until analysis.

Experimental data were analyzed using the ANOVA procedure; different means were compared using the Duncan test at $\alpha < 0.05$.

Spore production measurements

After sampling, fermented solids were suspended in Tween 80 (0.1%) in the proportion 1:10, and then mixed for 20 min to separate the spores from the CP. The suspension was diluted in sterile water and the concentration of spores was determined using a Malassez chamber.[11] Concentration was expressed in terms of the number of spores g^{-1} dry CP.

Gas analysis

Carbon dioxide production was used as an indirect measurement of *Penicillium commune* growth. The CO_2 concentration in the dry air stream output from the fermenters was monitored using a gas chromatograph (Gow-Mac 580, Gow-Mac Instrumentation Co., Bethlehem, PA, USA) equipped with a thermal conductivity detector and an automatic injector. The respiratory activity rate (μ_{CO2}) was calculated from the natural logarithm of CO_2 total production during

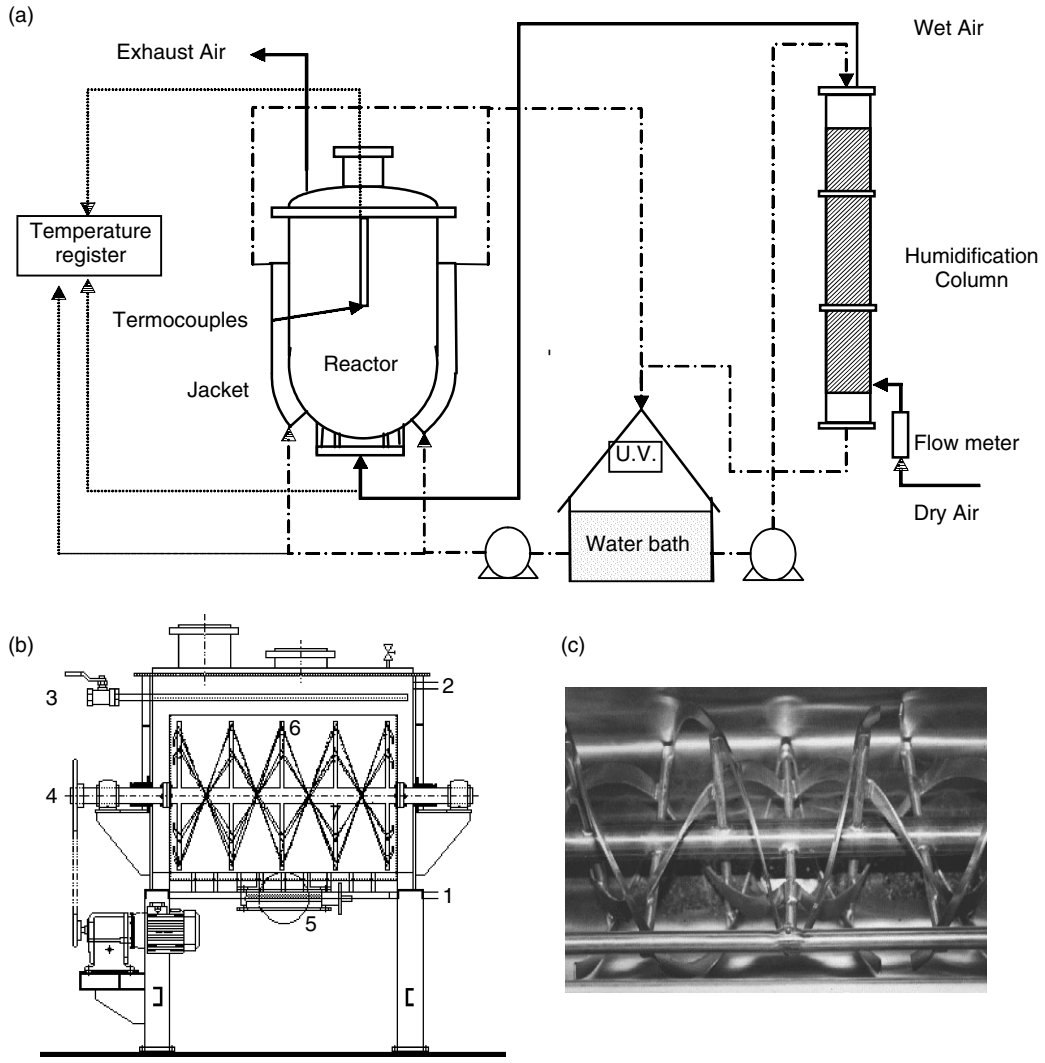

Figure 1. (a) Schematic diagram of the set-up of the pilot plant system. (b) Schematic overview of the 70 L capacity helical ribbons reactor used for SSF of coffee pulp at pilot scale; 1 – air inlet, 2 – air outlet, 3 – inoculum inlet, 4 – drive shaft, 5 – discharge port, 6 – external ribbons, 7 – internal ribbons. (c) Photograph of the helical mixing ribbons.

the exponential growth phase; it was estimated as indicated elsewhere.[12]

Estimation of external temperature of coffee pulp

The external CP temperature during thermal treatment was estimated considering the heating of a solid body under nonsteady-state conditions without superficial resistance and using steam as the heating fluid. We used Heissler plots for sphere heating.[13] The overall heating time was divided into 7 min periods.

Table 1 shows the CP and steam physical properties used. The convective transfer coefficient (h), the Nusselt number (Nu) and the Grashoff number (Gr) were calculated using Eqns (1), (2) and (3), respectively.[15]

$$h = \frac{Nu \cdot k_{steam}}{D} \tag{1}$$

$$Nu = 2 + 0.6(Gr^{1/4} \cdot \mathrm{Pr}^{1/3}) \tag{2}$$

$$Gr = \frac{D^3 \cdot \rho_{steam}^2 \cdot g \cdot \beta \cdot \Delta T}{\mu_{steam}^2} \tag{3}$$

Table 1. Coffee pulp and steam physical properties used to estimate external temperature of coffee pulp during heat treatment

Property		Value	Reference
α_{pulp}	Coffee pulp thermal diffusivity	4.5×10^{-3} m^2 s^{-1}	Heldman and Singh[14]
k_{pulp}	Thermal conductivity of coffee pulp	0.37 W m^{-1} K^{-1}	Heldman and Singh[14]
k_{steam}	Thermal conductivity of steam	0.2 W m^{-1} K^{-1}	Welty *et al.*[13]
ρ_{steam}	Steam density	0.59 kg m^{-3}	Welty *et al.*[13]
μ_{steam}	Steam viscosity	12.7 Pa s	Welty *et al.*[13]
D	Coffee pulp sphere diameter	0.18 m	This study
Pr	Steam Prandtl number	1.06	Welty *et al.*[13]
G	Gravitational constant	9.81 m s^{-2}	

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The coefficient of expansion, β, is the reciprocal of the average of external CP temperature (T_o) and heating fluid temperature (T_∞), while ΔT is the difference between T_∞ and T_o. Other variables are defined in Table 1.

RESULTS AND DISCUSSION

Effect of thermal treatment on spore production at laboratory scale

In order to reduce native microflora coffee pulp requires thermal treatment before it can be used as substrate for SSF. Figure 2 shows the steam and estimated external CP temperatures that were registered during the thermal treatments used. The profile of CP surface temperature was not affected by the different test conditions. The CP temperature was not higher than 100 °C in any of the cases. Under these conditions the contaminants were expected to be preserved, yet the CP was only pasteurized. However, no evident contamination of the medium was observed during the entire course of fermentation in all cases. The use of high inoculum levels (10^7 spores g^{-1} initial dry CP) could have avoided the growth of contaminants. Therefore, the use of similar pre-treatments at pilot plant scale under non-aseptic conditions was expected to result in non-contaminated fermentation.

The CO_2 production for the four CP thermal treatments is shown in Fig. 3. A significant decrease in total CO_2 production was observed when the thermal treatment was performed for 20 min and 40 min (Table 2). A previous study demonstrated that the specific growth rate (μ) could be estimated accurately from the respiratory activity rate (μ_{CO2}), calculated from the natural logarithm of total CO_2 production, given a practical method to determine these parameters for SSF cultures.[12] In this sense, the μ_{CO2} values were similar for 20 and 30 min thermal treatments; the maximum value was obtained with 10 min thermal treatment, showing a significant difference from the other cases. Table 2 shows that no significant difference regarding spore production was noticeable in these treatments.

In previous studies on the sporulation of fungi in SSF, the substrate was steam-treated prior to cultivation.[7,10,16–18] However, only a few cases are fully described and thus reproducible. In addition, these treatments were often carried out at laboratory scale and are not suitable at higher scales.

Effect of temperature on spore production

The application of SSF processes has been limited due to engineering problems; scale-up is one such problem because of the different gradients observed, especially temperature.[1] In this sense, it is important to assess the effect of incubation temperature on SPY.

Table 3 shows a significant ($\alpha < 0.05$) decrease in SPY at incubation temperatures above 30 °C. Even when maximum values for CO_2 production and

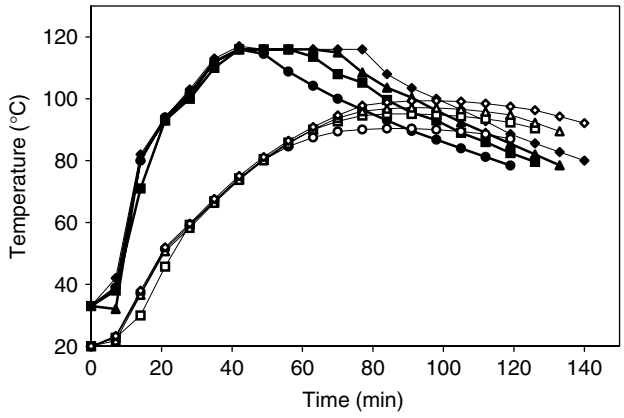

Figure 2. Estimated coffee pulp temperature (open symbols) and actual steam temperature (filled symbols) during different thermal treatments. Maintenance period: (○, ●) 10 min; (□, ■) 20 min; (△, ▲) 30 min and (◇, ◆) 40 min.

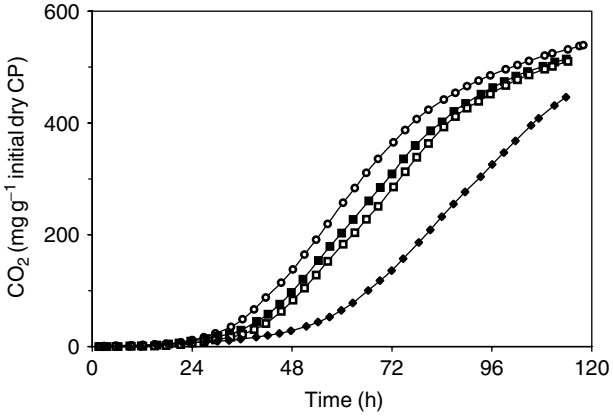

Figure 3. Carbon dioxide evolution during solid substrate cultivation of *Penicillium commune* on coffee pulp at laboratory scale during different thermal treatments. Maintenance period: (○) 10 min, (■) 20 min, (□) 30 min and (◆) 40 min. Initial culture conditions: moisture content, 64.7%; inoculation, 7×10^6 spores g^{-1} wet CP; pH 4.35; aeration 1 vgm.

Table 2. CO_2 and spore production data for *Penicillium commune* on coffee pulp at laboratory scale using different thermal treatments

Duration of thermal treatment (min)	CO_2 production (mg CO_2 g^{-1} initial dry CP)	μ_{CO2} (h^{-1})	Spore production (spores g^{-1} dry CP)
10	458.2 ± 17.7^a	0.11 ± 0.004^a	$2.1 \pm 4.6 \times 10^{9a}$
20	392.6 ± 31.2^b	0.10 ± 0.001^b	$1.8 \pm 6.1 \times 10^{9a}$
30	$408.5 \pm 12.8^{a,b}$	0.10 ± 0.003^b	$2.3 \pm 3.0 \times 10^{9a}$
40	255.6 ± 11.5^c	0.07 ± 0.006^c	$2.5 \pm 1.8 \times 10^{9a}$

[a,b,c] Same letter indicates no significant difference ($\alpha < 0.05$).

respiratory activity rate (μ_{CO2}) were obtained in the range 28–30 °C, no significant differences were found between 25 and 30 °C.

In order to simulate the effect of temperature changes on culture variables, a second approach was implemented. Cultures were first incubated at 30 °C for 20 h. This temperature allowed rapid growth, as shown by the high values of CO_2 production and the μ_{CO2} obtained (Table 3), and could have promoted an accelerated germination of spores. Then, the incubation temperature was changed to 25, 28, 30, 32 or 35 °C and kept constant until fermentation ended. For this kind of assay, the maximum SPY was attained when the second temperature was 25 °C (2.8×10^9 spores g^{-1} dry CP). However, there was no significant difference over the range 28–30 °C. The SPY decreased significantly at temperatures above 30 °C. These data show that there was no significant difference in SPY when the germination temperature was changed (Table 3). These results support the idea that accelerated germination at 30 °C has little or no effect on the overall process of spore production. The respiratory activity rate (μ_{CO2}) was not estimated in this set of assays because it is a temperature-dependent parameter (see below). All fermentation parameters (Table 3) were considerably affected at incubation temperatures above 30 °C, both when the incubation temperature was kept constant and when the germination of spores was first carried out at 30 °C.

Most reports indicate that the incubation temperature for cultures of the genus *Penicillium* is in the range 23–30 °C. For example, Gutiérrez-Sánchez *et al.*[19] reported 30 °C to be the incubation temperature for *P. commune* in submerged and solid-state cultures for caffeine degradation. Maheva *et al.*[20] found 23.5 °C to be the optimum temperature for sporulation of *Penicillium roqueforti* on buckwheat seeds. Larroche *et al.* reported the use of incubation temperatures of 27 and 25 °C for spore production on buckwheat seeds[9] and pozzolano,[11] respectively. Pascual *et al.*[21] used 25 °C as the incubation temperature for *Penicillium oxalicum* spore production in aerial and submerged fermentations. Our results agree with these studies.

On the other hand, the respiratory activity rate (μ_{CO2}) was fitted to the expression proposed by Esener *et al.*[22] in order to simulate the increase and decrease of μ_{CO2} as a function of temperature (Eqn (4)), a prediction that cannot be carried out with the conventional Arrhenius model. Figure 4 shows the experimental values and values calculated using the Esener *et al.*[22] model.

$$\mu_{CO_2} = \frac{Ae^{\frac{-E_{a_1}}{RT}}}{1 + Be^{\frac{-E_{a_2}}{RT}}}$$

The estimated values of the parameters were:

$$A = 5.27 \times 10^{13}\ h^{-1} \qquad B = 1.25 \times 10^{62}$$

$$Ea_1 = 82.6\ kJ\ mol^{-1} \qquad Ea_2 = 359.0\ kJ\ mol^{-1}$$

The calculated energy of activation (Ea_1) was in the range reported by Wang *et al.*[23] for microbial growth – that is, between 72 and 83.6 kJ mol^{-1}. These data suggest that there is no problem of mass limitation during fermentation at laboratory scale.

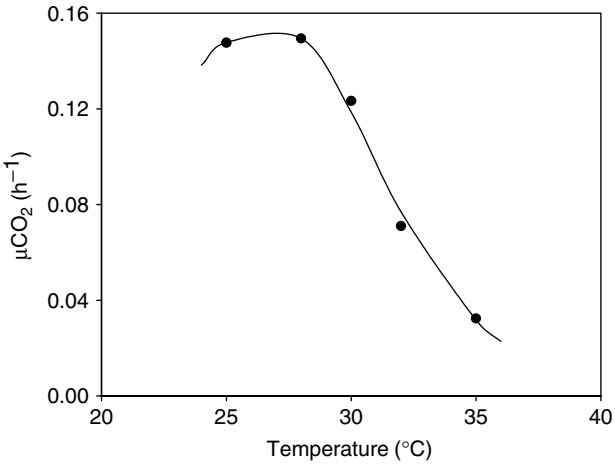

Figure 4. Effect of incubation temperature on respiratory activity rate (μ_{CO2}) of *Penicillium commune* on coffee pulp. Symbols show the experimental data, and line shows the Esener model fit. Calculated model parameters: $E_{a1} = 82.6$ kJ mol^{-1}, $E_{a2} = 359.0$ kJ mol^{-1}, $A = 5.27 \times 10^{13}\ h^{-1}$, $B = 1.25 \times 10^{62}$ (dimensionless).

Table 3. CO_2 and spore production data for *Penicillium commune* on coffee pulp at laboratory scale using different incubation temperatures

			Spore production yield (spores g^{-1} dry CP)	
Incubation temperature (°C)	CO_2 production* (mg CO_2 g^{-1} initial dry CP)	μ_{CO2}* (h^{-1})	Constant temperature incubation*	Germination at 30 °C**
25	375.4 ± 25.1^a	0.15 ± 0.03^a	$2.8 \pm 0.3 \times 10^{9a}$	$2.8 \pm 0.6 \times 10^{9a}$
28	389.5 ± 108.6^a	0.15 ± 0.01^a	$2.3 \pm 0.4 \times 10^{9b}$	$2.5 \pm 0.6 \times 10^{9a}$
30	417.4 ± 52.4^a	0.13 ± 0.01^a	$2.1 \pm 0.5 \times 10^{9b}$	$2.1 \pm 0.5 \times 10^{9a}$
32	228.16 ± 26.4^b	0.07 ± 0.01^b	$1.5 \pm 0.9 \times 10^{8c}$	$2.3 \pm 1.8 \times 10^{8b}$
35	44.6 ± 7.6^c	0.03 ± 0.01^c	$8.4 \pm 1.1 \times 10^{7c}$	$2.1 \pm 3.0 \times 10^{8b}$

[a,b,c] Same letter indicates no significant difference ($\alpha < 0.05$).

* Incubation temperature kept constant throughout the culture.

** Cultures incubated at 30 °C for 20 h, and then temperature changed to the indicated incubation temperature.

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Spore production at pilot plant scale

Mixing has been suggested as a factor eliminating heat accumulation SSF,[5–7] especially at large scales. Mixing in SSF reactors has been carried out following different approaches – that is, utilization of rotating drums with inner baffles, or use of reactors equipped with a paddle mixer. A mixed bed reactor has been reported for koji and enzyme production.[24] Also, a conical vessel mixed with a ribbon at the wall was used at pilot plant scale.[24] However, mixing can damage the mycelium and thus reduce the activity of the fungi,[9] a problem that the use of unicellular microorganisms does not present.

In this study, the reactor was designed to allow adequate aeration and the gentle mixing of solids, while limiting damage to the mycelium. Continuous mixing of CP at 0.25 rpm was applied after 20 h of cultivation. Based on previous results, the SSFs at the pilot plant were performed applying a 10 min thermal treatment, in which the water jacket temperature was maintained at 25 °C during the process. No contamination was observed during assays under these conditions. In addition, control fermentation processes at laboratory scale were run in parallel.

Table 4 shows the CO_2 and SPY (spores g^{-1} dry CP) data for the different assays carried out at laboratory and pilot plant scales. The production of CO_2 was similar at pilot plant and laboratory scales under static conditions, whereas the respiratory activity rate (μ_{CO2}) was higher at pilot plant scale under the same conditions. In this case, SPY decreased considerably (to less than 50%) from laboratory to pilot plant scale without mixing.

The SPY at pilot plant scale was higher than that at laboratory scale when the CP was mixed. In this case, SPY at 120 h was $2.9 \pm 0.5 \times 10^9$ and $3.7 \pm 1.6 \times 10^9$ spores g^{-1} dry CP at laboratory and pilot plant scales, respectively. The SPY was not affected when a factor of 79–105 was assayed from laboratory to plant scale; furthermore, an incremental tendency of 26% was observed (Table 4). Also, the average final moisture content was $70.5 \pm 1.1\%$, suggesting an important production of metabolic water during the process.

The low value of SPY obtained at pilot plant scale without mixing was probably due to the typical problems that arise when scale-up is performed – i.e. mass and heat transfer limitations. This was evident at the end of culture when the center of the reactor bed presented poor growth compared with its surface. When mixing was applied, however, no formation of agglomerates and growth was present inside the CP bed; perhaps shear stress was low because of the gentle mixing. Nevertheless, some mycelium damage occurred as indicated by the low values of CO_2 production and μ_{CO2} observed (Table 4).

There are no recent reports concerning *Penicillium* spore production at pilot plant scale. Table 5 presents a set of data related to spore production of the genus *Penicillium* on SSF using different substrates at laboratory scale. Data from the literature and our data were compared on the basis of the productivity of spores (spores g^{-1} dry CP h^{-1}). In the present study, we obtained a productivity of 2.4×10^7 spores g^{-1} dry CP h^{-1} at laboratory scale. This value is close to that obtained with *Penicillium roquefortii* on buckwheat seeds, but is lower than that of *P. frequentans* on a peat:vermiculite mixture. At pilot plant scale, we obtained a productivity of 3.1×10^7 spores g^{-1} dry CP h^{-1}, which is similar to those obtained at laboratory scale using a natural substrate (buckwheat seed). Even though spore productivity in CP is lower than in peat:vermiculite, CP is a byproduct of the wet coffee process and is a simpler natural substrate for spore production.[19] Under the conditions studied, 1.9 kg dry fermented CP (or 6.5 kg wet basis) was required to inoculate 1 tonne of wet CP at a level of 7×10^6 spore g^{-1} CP on a wet basis.

Oostra *et al.*[7] showed that mixing did not have a detrimental effect on the spore production of

Table 4. CO_2 production and spore production of *Penicillium commune* at pilot plant and laboratory scale with two mixing strategies

Type of culture	CO_2 production (mg CO_2 g^{-1} initial dry CP)	μ_{CO2} (h^{-1})	Spore production yield (spores g^{-1} dry CP)
Continuous mixing at pilot plant scale	208.3	0.07 ± 0.001	$3.7 \pm 0.7 \times 10^{9a}$
Static culture at pilot plant scale	272.3	0.16 ± 0.004	$1.4 \pm 0.1 \times 10^{9b}$
Laboratory	276.3 ± 91.4	0.10 ± 0.022	$2.9 \pm 0.2 \times 10^{9a}$

[a,b] Same letter indicates no significant difference ($\alpha < 0.05$).

Table 5. Spore production on SSF of genera *Penicillium* at laboratory scale

Microorganism	Substrate	Spore productivity (spores g^{-1} dry matter h^{-1})	Reference
P. frequentans	Peat:vermiculite	8.3×10^7	De cal *et al.*[25]
P. roquefortii	Buckwheat seed	3.1×10^7	Larroche and Gros[9]
P. roquefortii	Pozzolano	7.5×10^6	Maheva *et al.*[20]
P. commune	Coffee pulp	2.4×10^7	This study at laboratory scale
P. commune	Coffee pulp	3.1×10^7	This study at pilot plant scale

Coniothyrium minitans using oats as a substrate, and suggested that mixed systems are superior to non-mixed systems at large scale. This could have been the result of better aeration and temperature control in the bed. They reported a productivity of 1.1×10^7 spores g^{-1} dry matter h^{-1} using a 1.5 L rotating reactor; comparison is not possible because of the different reactor size and microorganism used. Nevertheless, these data are useful for comparing rotating SSF reactors. In contrast, Larroche *et al*.[9] suggested that spore productivity is not affected by low rotation frequency and that this is probably due to better aeration of the mycelium.

CONCLUSIONS

Results obtained in the present study show that the temperature of CP attained during heat treatment allowed only pasteurization of the substrate, and that the high inoculation used in SSF prevented the development of contaminants during culture. The Esener equation was used to describe the variation in respiratory activity rate (μ_{CO2}) as a function of incubation temperature. Under the selected culture conditions, spore production was not affected when a scale factor of 79–105 was assayed from laboratory to pilot plant. A spore production yield of 3.7×10^9 spores g^{-1} dry CP was obtained when continuous mixing (0.25 rpm) was used at pilot plant scale after 120 h cultivation. This information could play a role in overcoming limitations in the application of the SSF process at large scales.

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