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ORIGINAL ARTICLE

Production of humic acids by solid-state fermentation of *Trichoderma reesei* in raw oil palm empty fruit bunch fibers

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Abstract
Humic acids (HA) are organic macromolecules of high structural complexity and are primarily obtained from non-renewable carbon sources such as peat and coal. HA is widely used in agriculture but is known to have therapeutic properties, which are still poorly explored. Previous studies have shown the potential of biotechnological processes in the production of HA in submerged fermentation (SF) and solid-state fermentation (SSF) using pre-treated fibers of oil palm of empty fruit bunch (EFB) for the cultivation of *Trichoderma* strains. EFB is an agro-industrial residue that is readily available at a low cost. The present study aimed to study the production of HA by *Trichoderma reesei* in the SSF of raw fibers of EFBs from two different palm oil producers. Profiles of HA production, cellular protein, pH, glucose, moisture, and oxygen transfer were obtained during SSF by EFB with and without lipids, as well as, images by electron microscopy of fibers. Results showed efficient HA production in the raw fibers of the EFBs. HA production followed the cellular protein (6 g HA per 100 g of fibers) evolution of the fermentations in the absence of lipids, while the composition of lipids greatly affected its production. The best HA production (350 mg HA per 100 g of fibers) was obtained from EFB that was richer in lignocellulosics and the residual lipids were similar to the fractions of palm and kernel oils, while EFB with lower lignocellulosic presented a production 110 mg HA per 100 g of fibers.

Keywords Humic acids · Empty fruit bunch · Agroindustrial residue · *Trichoderma reesei* · Solid-state fermentation

Introduction

Humic substances (HS) compose 85–90% of the total organic carbon reserve present in the soil and are characterized by stable complexes of dark staining, high molecular mass and different solubilities (Senesi and Loffredo 2001). Humic acids (HA) are a fraction of HS that are soluble in alkaline medium and precipitate in an acid medium (pH < 2), forming dark crystalline and amorphous structures. HA presents a complex molecular structure with various chemical groups, which define their properties. Agriculture is the one that stands out most as using HA, mainly because they increase the absorption of nutrients, stimulate plant growth, improve soil structure and generate benefits for productivity (Borsari 2013; Busato et al. 2010).

Currently, commercial HA is extracted from non-renewable carbon sources such as peat and coal. Solid residues generated from agricultural activities have available nutrients that can be converted into commercial products by fermentation. Mimicking their natural environment, filamentous fungi have the capacity to grow in low water levels, and thus, solid-state fermentation is a promising process for these residues (Couto and Sanromán 2006).

The processing for the production of palm oil generates two types of fibers, including those from the empty fruit bunch (EFB) after separation of the fresh palm fruits, and the mesocarp fibers, which are waste materials left after the oil extraction. The fibers from EFB are commonly used for composing materials and various other applications, because they are readily available at a low cost (Hassan et al. 2010; Rozman et al. 2005). The raw EFB fibers contain residual

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oil whose composition depends on the processing for the removal of palm fresh fruits.

EFB is an agroindustrial residue composed of 45–50% cellulose, approximately 25–35% hemicellulose and 10–15% lignin in addition to minor components like xylose, mannose, galactose, silica, copper, calcium and manganese (Hassan et al. 2010; Deraman et al. 2010; Abdul Khalil et al. 2006). *Trichoderma* is a complex genus of fungus characterized by the rapid growth of colonies, reaching a diameter of 2–9 cm after 4 days of growth at 20 °C. *Trichoderma* ssp. is ubiquitous colonizers of cellulosic material (Schuster and Schmoll 2010).

The oil palm EFB fibers are a promising renewable source to produce HA by *Trichoderma* strains. Motta and Santana (2014a) studied the production of HA in submerged fermentation (SF) by *Trichoderma reesei* and *Trichoderma viride* strains cultured in pre-treated oil palm EFB fibers. The HA production was higher than the microbial growth in both cases, and *T. reesei* produced three times more HA than *T. viride*. A relationship between *Trichoderma* sporulation and HA production was observed (Motta and Santana 2012, 2013). In subsequent studies, Motta and Santana (2014b) also used the same EFB fibers for HA production in solid-state fermentation (SSF). In this case, fast sporulation occurred, and no vegetative form was observed throughout the fermentation time.

The present study extended the previous findings of the potential HA production from oil palm EFB in SSF by evaluating the use of the raw EFB fibers for the production of HA by *T. reesei* (Motta and Santana 2013, 2014b). The fermentations were set up with EFB fibers from two different palm oil producers. The palm trees were cultivated in different soils and weather conditions of north and northeast Brazil, and the EFBs resulted from different processing conditions. The differential of this research is the effect of the residual lipids of the EFBs in the production of HA. With the previous characterization of these lipids, it was possible to observe the difference in the production of HA of one type of EFB and with or without of lipid. The raw EFB fibers were previously classified as higher lignocellulosic content and residual oil similar to the palm oil and kernel oil fractions (Agropalma EFB) and lower lignocellulosic content and residual oil similar to palm oil (Bahia EFB).

Materials and methods

Inoculum

The *Trichoderma reesei* strain (CCT 2768) from the Tropical Culture Collection-CCT (Campinas, São Paulo, Brazil) was used in SSF. The cultures cultivation were carried out based on the previous work of the research group, in which initially, the culture were sporulated in test tubes containing potato-dextrose agar tilted for 2 weeks at 24 °C. After that, a glycerol solution was added to each tube, which was then scraped of the middle surface for the release of the spores. The solution of glycerol was stored in cryotubes of 1.2 mL in an ultrafreezer (Motta and Santana 2014a; Volpi et al. 2018).

Solid support

The raw EFB fibers used in SSF were the same that was used in others works of our research group (Volpi et al. 2018, 2019), i.e., from Agropalma (Limeira, São Paulo, Brazil) and the cooperative of small palm oil producing industries (Muniz Ferreira, Bahia, Brazil). Table 1 shows the lignocellulose content and the initial moisture of the fibers, and the main residual lipids are presented in Tables 2 and 3, according to the previously performed lipid characterization (Volpi et al. 2019). The range of sizes of the EFB fibers used in the fermentations (355–710 µm) was obtained for those fibers retained in the 24 and 42 Tyler sieves.

The residual lipids were extracted from EFB fibers for characterization and use in the control fermentations. The lipids were extracted according to the methodology AM 2-93 (AOCS 2009), using petroleum solvent in the Soxhlet Extractor.

Solid-state fermentations

The SSFs were performed in packed-bed column bioreactors (Raimbault Columns), as previously established in Motta and Santana's studies (2014b). The system was composed of five cylindrical glass columns 15 cm high and 3 cm in diameter, coupled to an air humidifier at the entrance of the column. The air supply was through a compressor. The airflow at the column input was adjusted to 0.4 L min^{-1} and monitored by air flow meter. The measurement of the inlet and outlet oxygen was performed by YSI® Model 5300 oximeter. The columns were arranged in a microbiological oven to keep at 30 °C. The preparation of the culture medium was performed according to the data from Motta and Santana (2014b). The columns were withdrawn as samples at 24-h

Table 1 Lignocellulosic and moisture compound contents of Agropalma and Bahia EFBs

EFB	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Moisture (%)
Agropalma	31.9	19.8	24.1	11.45
Bahia	17.9	15.2	13.7	18.94

The lignocelullosic method was performed using the neutral detergent fiber (NDF) and acid detergent fiber (ADF) from the Association of Official Analytical Chemists AOAC (2016), and the moisture content according to AACCI methods (2010)

Table 2 Composition of the oil residual lipids in the EFBs

Composition of lipid	Agropalma EFB[a] (%)	Bahia EFB[a] (%)
Fatty acids	Oleic acid—37.60 ± 2.39	Palmitic acid—65.90 ± 4.01
Triacylglycerols	POP—28.12 ± 0.22	POP—36.7 ± 0.28
Diacylglycerols	18.23 ± 0.69	7.17 ± 0.31
Monoacylglycerols + free fatty acids	6.84 ± 1.14	47.28 ± 1.47
Free fatty acids	9.31 ± 0.16	51.63 ± 1.12
Peroxid value	12.41 ± 1.27	67.49 ± 2.9
Iodine value	110.2	82.1

All analyses were determined by AOCS method (2009)

P palmitic acid, *O* oleic acid

[a]Mean of three repetitions ± standard deviation

Table 3 Content of carotenoids, phytosterols and tocopherols in the residual lipids in the EFBs

	Agropalma EFB[a]	Bahia EFB[a]
Carotenoids (mg kg^{-1})	890.24 ± 0	81.53 ± 5.43
Phytosterols (mg kg^{-1})	5270.65 ± 150.67	1102.35 ± 13.03
Tocopherols (mg kg^{-1})	1188.87 ± 5.46	–

All analyses were determined by AOCS Methods (2009)

–: not detected

[a]Mean of three repetitions ± standard deviation

intervals, and the soluble fermented materials were extracted with distilled/deionized water, where 2.0 g of sample was weighed and 4.0 mL of water was added.

Fungal growth and HA production

Cell protein concentration

Fungal growth was accounted for by an indirect method through the determination of the protein concentration, adapting what was described by Motta and Santana (2014b). Samples of the fungal extract (3.0 mL) were collected and centrifuged for 10 min at 16,770×*g* to obtain the pellets. The supernatant was collected, and the pellets were resuspended and washed twice with distilled water. The pellets were suspended in 3.0 mL of 1.0 N NaOH and heated at 100 °C for 10 min for the release of intracellular proteins. After cooling, the solution was centrifuged at 16,770×*g* at 5 °C, the supernatant was recovered, and the protein was determined with the bicinchoninic acid assay take a commercial Pierce BCA kit (Thermo Scientific, USA). Two mL of standard reagent was added to 0.1 mL of sample, and the mixture was incubated at 37 °C for 30 min. After cooling to room temperature, the absorbance was read at 562 nm. The protein concentration was determined from a calibration curve previously constructed with bovine serum albumin (BSA).

Quantification of humic acids

The quantification of humic acids was performed as described by Badis et al. (2010), where the samples were centrifuged for 15 min at 4193×*g* and the supernatant was filtered using the Microfilter syringe filter (Thomapor®-Membranfilter, 5FP 025/1). The supernatant was diluted five times in 0.5 M NaOH solution and then diluted ten times in water (pH 4.5 ± 0.01), and the absorbance was measured at 350 nm. The concentration of HA was determined by a standard curve that was previously constructed.

Oxygen transfer

Oxygen transfer was evaluated by the rate *N* through the gaseous balance at the inlet and outlet of the columns, according to Eq. 1 (Thibault et al. 2000):

$$N = \left(F_{\text{in}} \times C_{\text{O}_2\text{in}} - F_{\text{out}} \times C_{\text{O}_2\text{out}}\right)/V, \quad (1)$$

where F_{in} is the air flow at the inlet of the column, F_{out} is the air flow at the outlet, $C_{\text{O}_2\text{in}}$ is the oxygen concentration at the column inlet, $C_{\text{O}_2\text{out}}$ is the oxygen concentration at the outlet, and V is the volume of liquid in the fermenter, calculated take into account the moisture content (found in item 2.4.6) present in the volume of medium.

pH

The pH of the fungal extract was measured after fermentation by a pH meter after vigorous shaking of 5.0 g of fermented medium and 5.0 mL of distilled water.

Glucose

The glucose concentration of the fungal extract was determined using the enzymatic glucose-oxidase kit, LABORLAB. Aliquots of 10 µL of the extract were added to 1 mL of the standard reagent and incubated at 37 °C for 5 min.

After cooling to ambient temperature, the absorbance was read at 505 nm. The assays were performed in triplicate. HA productivity was calculated by difference of production in the time.

Moisture content

The moisture content on a wet basis was performed by drying approximately 3 g of the fermented sample at 105 °C, until constant weight.

Scanning electron microscopy (SEM)

The crude and fermented fibers after 72 h were submitted to scanning electron microscopy (Leo 440i, LEO Electron Microscopy, England). The methodology followed what was described by Motta and Santana (2014b).

Recovery of HA

Recovery of HA was performed according to the protocol of the International Humic Substances Society (IHSS), which consists of three steps: alkaline extraction, separation of HA precipitates and removal of HA silicates and salts and oven-drying for removal of inorganic compounds. The oven step was not performed due to the absence of inorganic compounds from the fermented extract. Thus, HA was calculated by Eq. 2:

$$\mathrm{HA} = \frac{\mathrm{HAmass}}{\text{total sample mass}} \times 100 \quad (2)$$

Results and discussion

Solid-state fermentation

Figure 1 shows the kinetic profiles of SSF in terms of HA production, cellular protein, pH, glucose, moisture of the solid medium and oxygen transfer rate for the fermentations with Agropalma EFB fibers.

According to Fig. 1a, HA was produced in the raw EFB fibers containing residual lipids and in degreased EFB fibers used as a control. In both cases, HA production was higher than cellular protein, evidencing the efficient use of the EFB fibers. The cellular protein evolution (Fig. 1b) was similar for both fermentations, but the maximum concentration (6.0 g protein 0.100 g^{-1} of fibers) was delayed (96 h) in the presence of lipids compared with control (5.5 g protein 100 g^{-1} of fibers in 48 h). HA production followed the cellular protein evolution, but the highest concentration (350 mg HA 100 g^{-1} of fibers) was obtained in the presence of lipids, at approximately 72 h. Although there were mass transfer limitations in the presence of lipids, the utilization of these lipids as an additional carbon source may justify the higher HA production obtained.

Figure 1c shows that the pH of the microbial HA production ranged from 4.5 to 6.6, a range that is optimal for fungal growth, i.e., 2–6 approximately (Kredics et al. 2003).

Figure 1d presents the glucose excess in the medium along the fermentation time. Due to imprecision in the measurements, similar behavior was considered for both EFBs. However, in both cases, the greatest drop in glucose occurred around 72 h, suggesting that the glucose from the breaking of the lignocellulosic compounds was consumed preferentially in the presence of lipids.

The moisture in the fibers remained around 65%, even for the highest HA production (Fig. 1e). This is in agreement with fungal SSFs as reported by Bastos et al. (2016). A low moisture content may lead to poor nutrient accessibility and poor oxygen availability, resulting in poor microbial growth (Pandey et al. 2003; Rajagopalan and Modak 1995).

Figure 1f shows the highest oxygen consumption for the control condition (without lipid), at 48 h, in agreement with fungal growth. In the presence of lipids, the overall rate of oxygen transfer remained around 3 mg L^{-1} h^{-1} along fermentation time, suggesting the storage of oxygen by the residual lipids in EFB.

Figure 2 shows the kinetic characterization of SSF in terms of HA production, cellular protein, pH, glucose, moisture and oxygen transfer for Bahia EFB.

Figure 2a shows that the highest HA production was obtained for the control (EFB without lipid) at 48 h, with approximately 110 mg 100 g^{-1} of fibers. Conversely, maximum fungal growth (7.5 g of protein 100 g^{-1} of fibers) was observed for EFB with lipid (Fig. 2b). One possible explanation could be that the fungi are using the lipid layer as a solid support for growth, as filamentous fungi such as *Trichoderma* have the characteristic of growing in wide range of different environmental conditions and substrates. Therefore, it may be that the fungus took better advantage of the lipid layer for its growth and not for the metabolism and consequent production of HA. The pH curves were similar (Fig. 2c), dropping in 48 h and remaining in the range of 5–6 up to 96 h. This concurred with the fungal growth (Fig. 2b) and glucose consumption (Fig. 2d). The moisture remained constant at around 50% during fermentation (Fig. 2e). Moreover, higher oxygen consumption at approximately 72 h was in agreement with fungal growth (Fig. 2f).

Specific growth rates and HA productivity

Table 4 shows specific patterns of growth for *T. reesei* from Agropalma and Bahia EFBs. For both, fibers with lipids

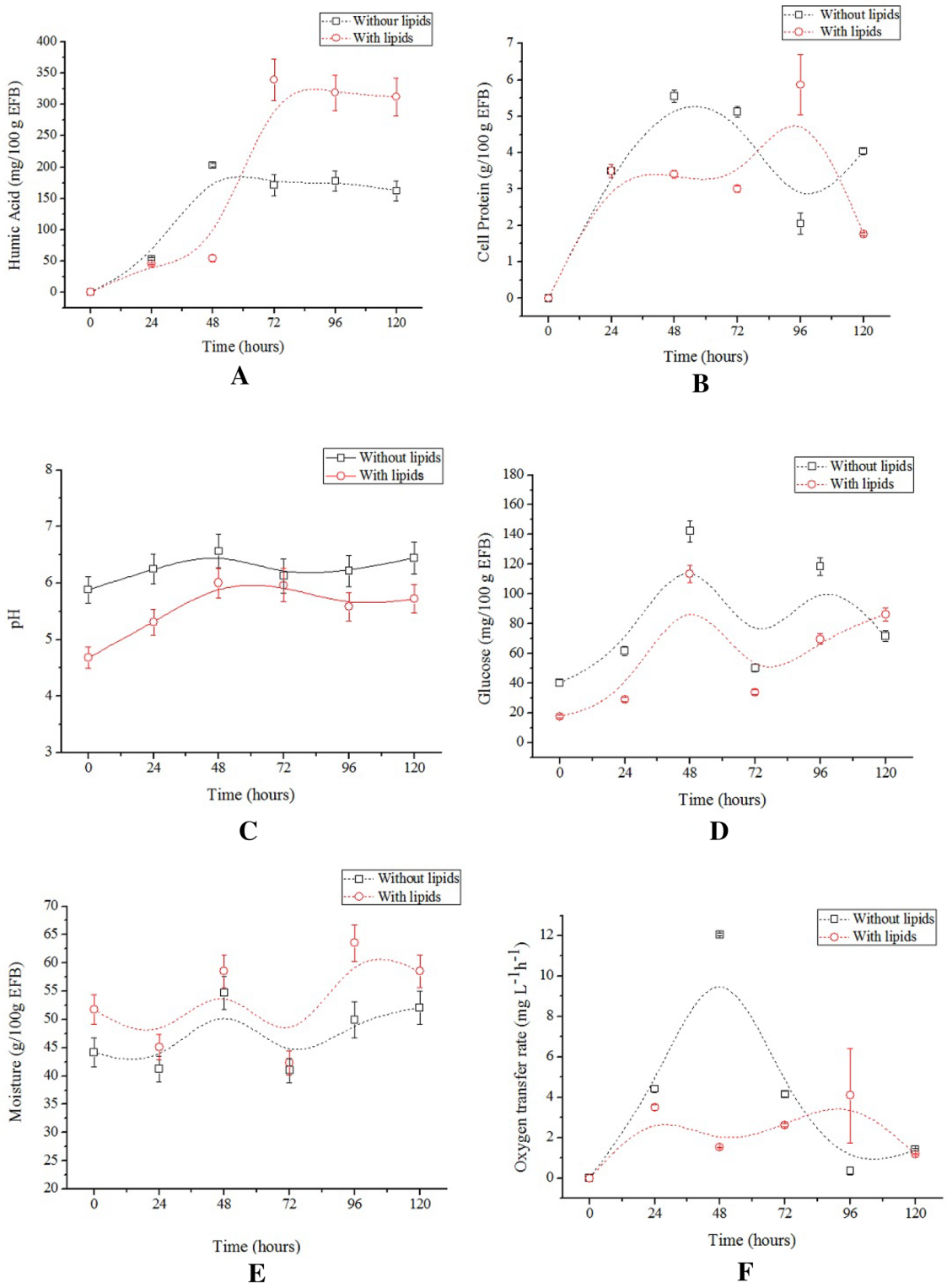

Fig. 1 Profiles of the AH content (**a**), cell protein (**b**), pH (**c**), glucose concentration (**d**), solid moisture (**e**) and oxygen transfer (**f**) for SSF by *T. reesei* from Agropalma EFB with and without lipids

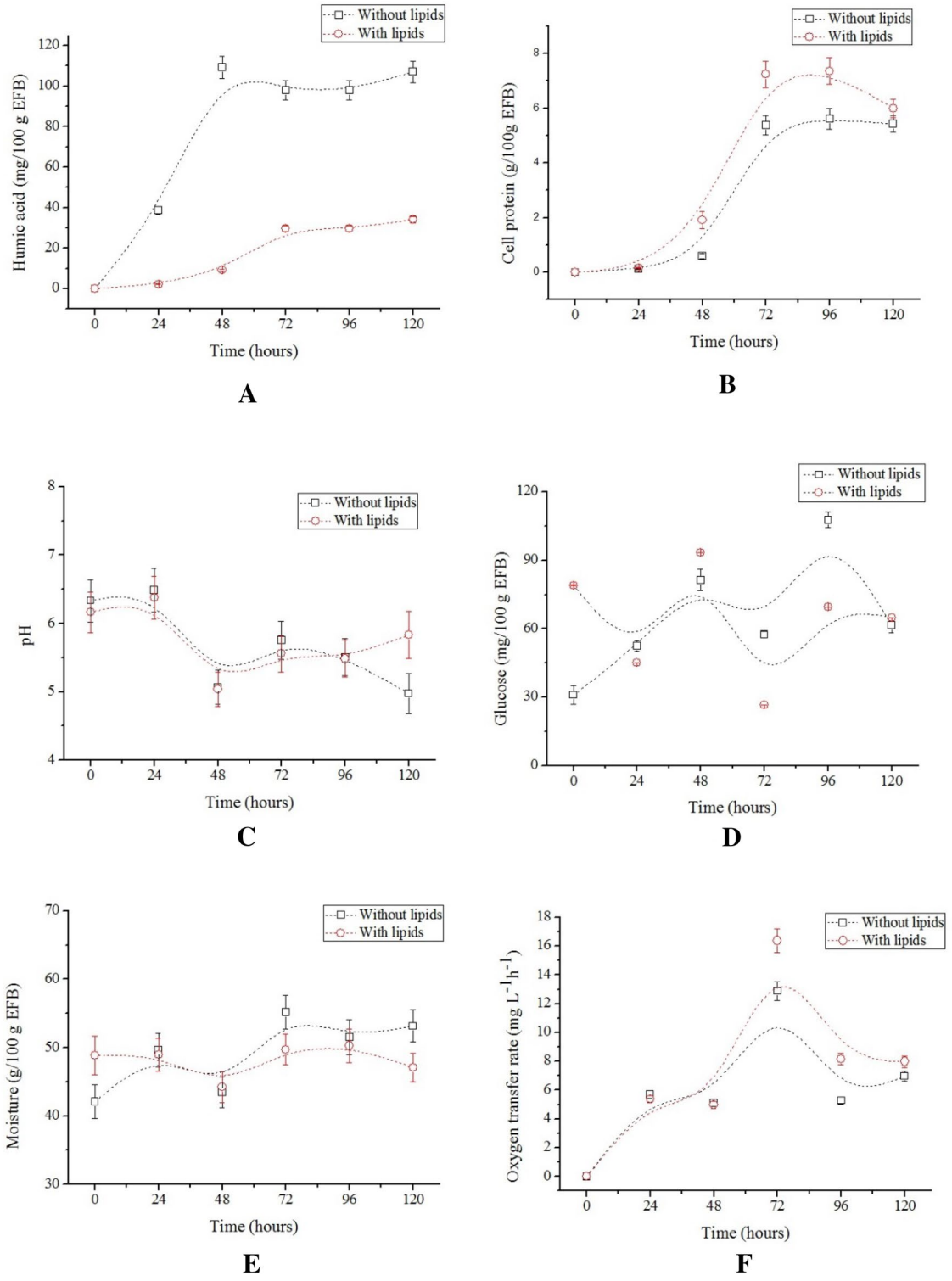

Fig. 2 Profiles of the AH content (**a**), cell protein (**b**), pH (**c**), glucose concentration (**d**), solid moisture (**e**) and oxygen transfer (**f**) from Bahia EFB with and without lipids

showed approximately the same specific growth rates of 0.0080 h^{-1}. The highest specific growth rate was obtained for the Agropalma EFB without lipid (0.019 h^{-1}) and can be seen in Fig. 1b, with an intense fungal growth in 48 h. In fiber with lipids, the behavior of the fungus is almost constant, showing peak growth at 96 h. For Bahia EFB,

Table 4 Specific growth rates and log-phase period for *T. reesei* from Agropalma and Bahia EFBs, with and without lipids

EFB	With lipids	Without lipids
Agropalma EFB	0.0082 h^{-1} (0–96 h)	0.019 h^{-1} (0–48 h)
Bahia EFB	0.0080 h^{-1} (0–96 h)	0.0057 h^{-1} (0–96 h)

the highest specific growth rate was in the fiber with lipid, which had the same behavior as the cellular protein production (Fig. 2b). Kupski (2012) reported that *T. reesei* in SSF with bark and rice bran produced cellulases at a specific growth rate of 0.002 h^{-1}, that was lesser than that obtained in this study.

Comparing the behavior of Agropalma and Bahia EFBs (Figs. 1, 2), the best HA production was with lipid for Agropalma EFB and without lipids for Bahia EFB. Therefore, the composition of the residual lipids plays an important role in HA production. Apparently, *T. reesei* utilizes the lipids from Bahia EFB preferentially for growth instead of HA production. Regarding Motta and Santana's work (2014b), *T. reesei* produced 75 mg HA per 100 g of fibers in 72 h, while the highest HA production in Agropalma EFB fibers was 350 mg HA per 100 g of fibers in 72 h in the presence of lipids. For Bahia EFB, the highest production (110 mg per 100 g of fibers in 48 h) was similar to that reported by Motta and Santana because of the absence of lipids. The cellular protein did not vary significantly for the best conditions (with lipids), as it was 7.5 g 100 g^{-1} of fibers for Bahia EFB and 6 g 100 g^{-1} Agropalma EFB. These values were higher than results obtained by Motta and Santana (2014b), which were 4.92 g 100 g^{-1} of fibers. Therefore, the lignocellulosic content and lipid composition contribute to both fungus growth and HA production.

The productivity of HA for Agropalma EFB in the best growing condition with lipid was 2.6 mg 100 g^{-1} of fibers

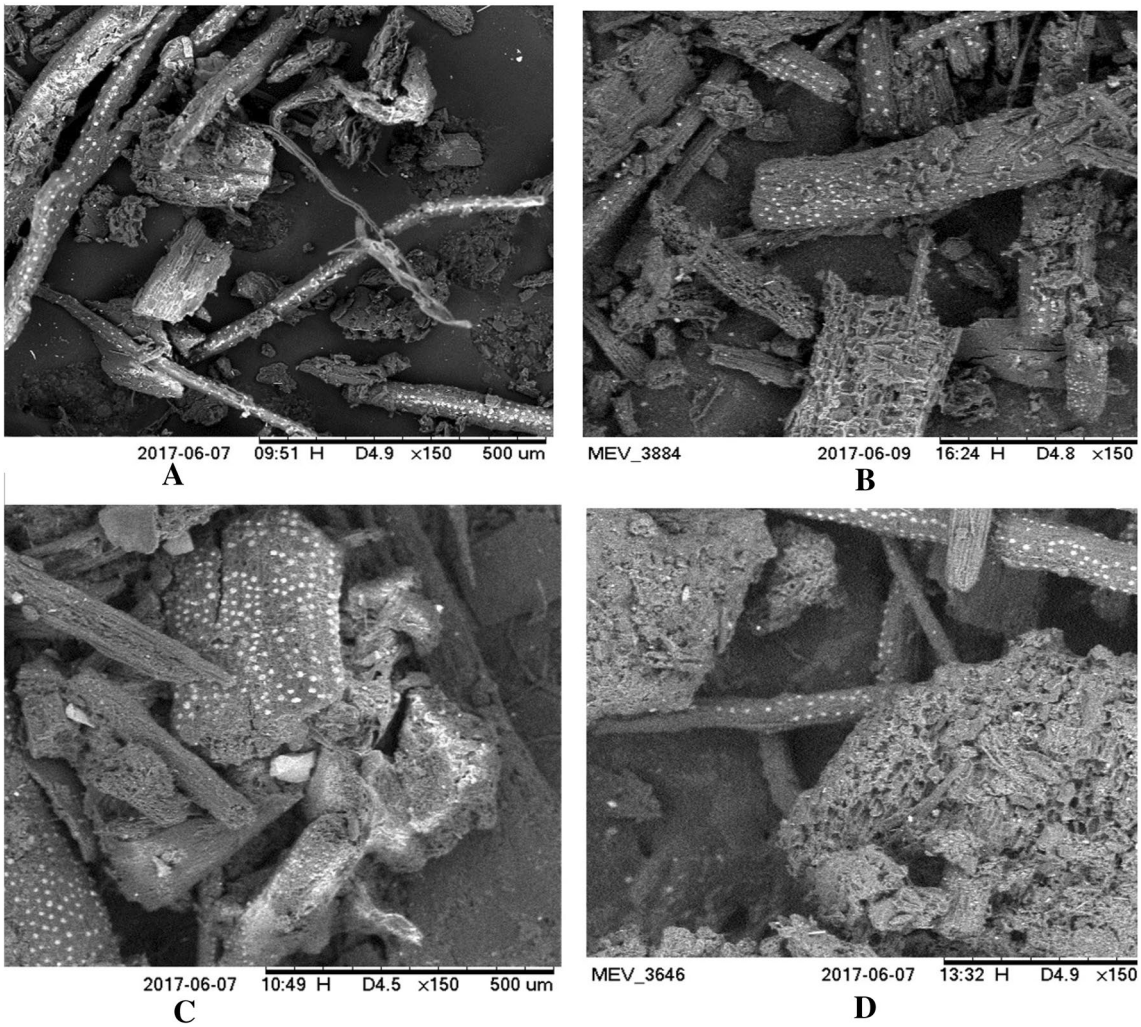

Fig. 3 SEM images of Agropalma EFB fibers before SSF: control (without lipids) (**a**) and with lipids (**b**). Fibers after 72 h of SSF: control (without lipids) (**c**) and with lipids (**d**)

per hour, while for Bahia EFB in the best growing condition without lipid, it was 2.27 mg 100 g^{-1} of fibers per hour. Motta and Santana's (2014b) presented (0.73 mg 100 g^{-1} h^{-1}) were three times lower than these results. In relation to the biomass productivity for Agropalma EFB, it was 0.06 g 100 g^{-1} h^{-1} for fiber with lipids and 0.1 g 100 g^{-1} h^{-1} for the Bahia EFB with lipids, which were higher than reported by Motta and Santana (2014b) (0.07 mg 100 g^{-1} h^{-1}). Thus, Bahia EFB functions better as a solid support for fungal growth, as reported in previous studies.

Images of EFB fibers

Figure 3 shows the scanning electron microscopy (SEM) from Agroplasma EFB before (A and B) and after (C and D) 72 h of microbial action. Similarly, Fig. 4 shows the images of SEM from Bahia EFB before (A and B) and after (C and D) 72 h of SSF. Comparing both figures, it is possible to visually understand how much each fiber was degraded during the microorganism growth.

The images show no difference between the fibers with and without lipids before cultivation. The white dots present in the EFB fibers are silica bodies, which are generally found in EFBs. They are usually circular, uniform, and are present on the surface of the fibers. After SSF, pores appeared on the fibers indicating consumption of the lignocellulosic compounds (Figs. 3d, 4c). These images are similar to those obtained by Motta and Santana (2014b).

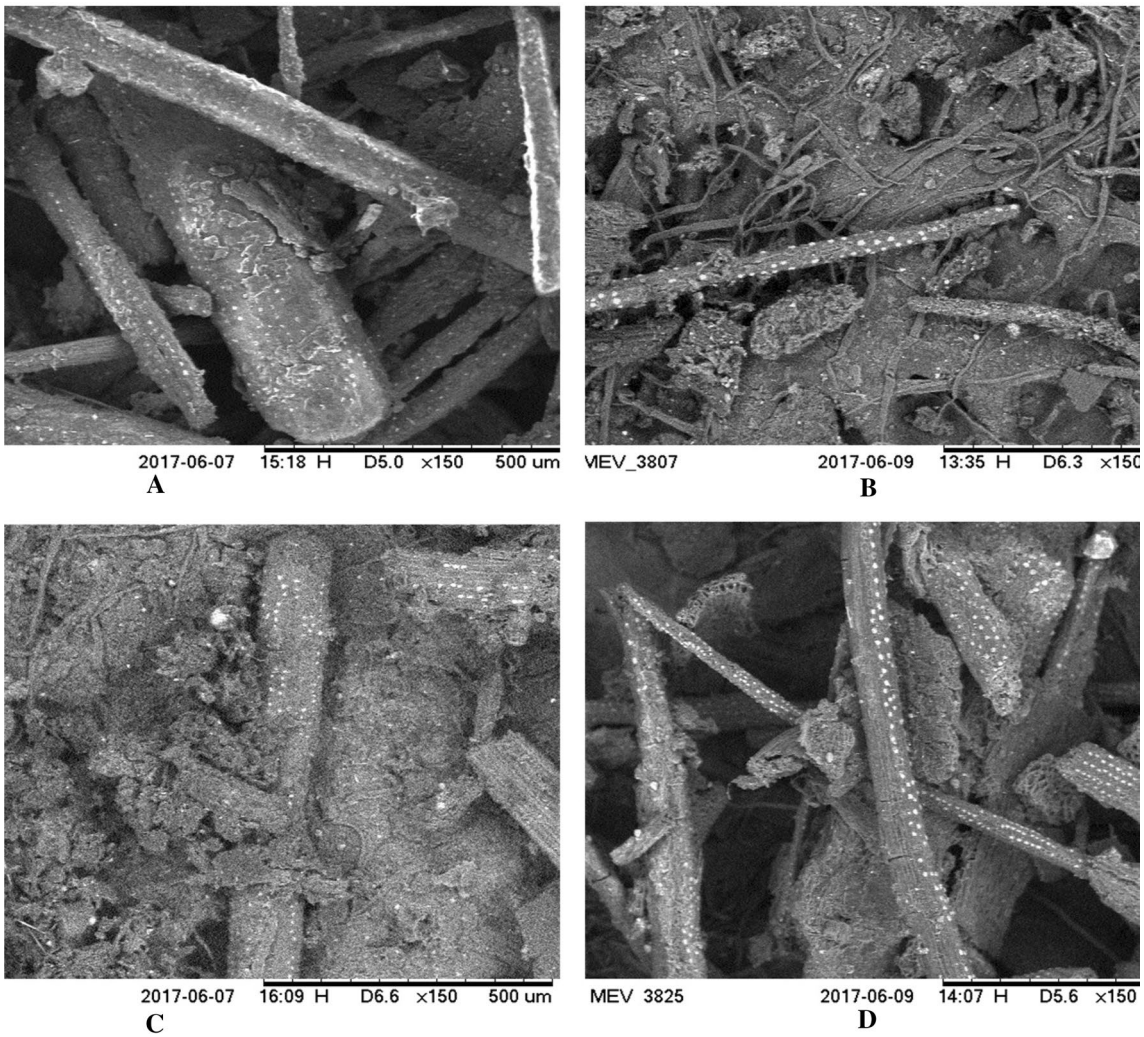

Fig. 4 SEM images of Bahia EFB fibers before SSF: control (without lipids) (**a**) and with lipids (**b**). Fibers after 72 h of SSF: control (without lipids) (**c**) and with lipids (**d**)

Table 5 HA recovery produced in SSF

EFB (better SSF condition)	Recovery HA (%)
Agropalma EFB with lipid 72 h	63.11
Agropalma EFB without lipid 48 h	60.82
Bahia EFB with lipid 72 h	49.35
Bahia EFB without lipid 48 h	58.75

HA recovery

The recovery of HA obtained from the SSF for the two EFBs varied around 50–60% as shown in Table 5. For Agropalma EFB, no great difference in HA recovery was obtained for the fibers with or without lipids. For Bahia EFB, the highest HA recovery was for the fibers without lipids. The difference in HA recovery was mainly due to the lower HA production in Bahia EFB fibers. Thus, raw fibers from palm oil EFBs are efficient substrates for the production of HA by *T. reesei* cultivation in SSF. The main importance of this finding is in making the production of HA even cheaper, by not requiring pre-treatment of the fibers for greater adhesion of microorganisms or absorption of water, as in other processes. Moreover, the use of fibers in SSF adds value to the high volume of EFB wasted from palm oil processing. The removal process of palm oil fresh fruits from bunches plays an important role in the composition of the residual lipids in the raw fibers. Thus, the presence of palmitic acid, carotenoids, lower free fatty acids, and lower oxidation in Agropalma EFB benefited HA production compared with lipids in Bahia EFB.

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Compliance with ethical standards

Conflict of interest The authors have no financial conflicts of interest to declare.

References

AACCI American Association of Cereal Chemists International (2010) Approved methods, 11th edn. AACC International, St Paul

Abdul Khalil HPS, Siti Alwani M, Mohd Omar AK (2006) Chemical composition, anatomy, lignin distribution, and cell wall structure of malaysian plant waste fibers. BioRes 1(2):220–232

AOAC (2016) Official methods of analysis of AOAC International, 20th edn. AOAC International, Rockville

AOCS (2009) Official methods and recommended practices of the American Oil Chemist's Society, 6th edn. AOCS Press, Champaign

Badis A, Ferradj FZ, Boucherit A, Fodil D, Boutoumi H (2010) Removal of natural humic acids by decolorizing actinomycetes isolated from different soils (Algeria) for application in water purification. Desalination 259:216–222. https://doi.org/10.1016/j.desal.2010.04.001

Bastos RG, Motta FL, Santana MHA (2016) Oxygen transfer in the solid-state cultivation of *D. monoceras* on polyurethane foam as an inert support. Braz J Chem Eng 33:793–799. https://doi.org/10.1590/0104-6632.20160334s20150262

Borsari F (2013) Experiências de campo demonstram os benefícios para a produtividade do uso de ácidos húmicos na agricultura intensiva. Agro DBO 46:44–45. https://issuu.com/eriklm/docs/agro_ed_46/44

Busato JG, Zandonadi DB, Dobbss LB, Façanha AR, Canellas LP (2010) Humic substances isolated from residues of sugar cane industry as root growth promoter. Sci Agric 67:206–212. https://doi.org/10.1590/S0103-90162010000200012

Couto SR, Sanromán MA (2006) Application of solid-state fermentation to food industry—a review. J Food Eng 76:291–302. https://doi.org/10.1016/j.jfoodeng.2005.05.022

Deraman M, Saad SKM, Ishak MM (2010) Carbon/carbon nanotubes (CNTs) composites from green pellets contain CNTs and self-adhesive carbon grains from fibres of oil palm empty fruit bunch. Aip Confe Proc 1284:179–186. https://doi.org/10.1063/1.3515546

Hassan A, Salema AA, Ani FN, Bakar AA (2010) A review on oil palm empty fruit bunch fiber-reinforced polymer composite materials. Polim Compos 31:2079–2171. https://doi.org/10.1002/pc.21006

Kredics L, Antal Z, Manczinger L, Szekeres A, Kevei F, Nagy E (2003) Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. Food Technol Biotechnol 41:37–42 **(ISSN 1330-9862)**

Kupski L (2012) Produção de celulase a partir da indústria arrozeira empregando Rhizopus oryzae e *Trichoderma reesei*. Dissertação apresentada ao programa de Engenharia de Alimentos. http://www.ppgalimentos.furg.br/images/stories/Dissertacoes/2012/larine%20kupisk-%20produo%20de%20celulases%20a%20partir%20de%20resduo%20da%20indstria%20arrozeira%20empregando%20rhizopus%20oryzae%20e%20trichoderma%20reesei%20.pdf. Accessed 3 Sept 2017

Motta FL, Santana MHA (2012) Biomass production from *Trichoderma viride* in nonconventional oat medium. Biotechnol Progr 28:1245–1250. https://doi.org/10.1002/btpr.1578

Motta FL, Santana MHA (2013) Production of humic acids from oil palm empty fruit bunch by submerged fermentation with *Trichoderma viride*: cellulosic substrates and nitrogen sources. Biotechnol Progr 29:631–637. https://doi.org/10.1002/btpr.1715

Motta FL, Santana MHA (2014a) Comparison of humic acids production by *Trichoderma viride* and *Trichoderma reesei* using the submerged fermentation of oil palm empty fruit bunch. J Microbiol Biotechnol 13:1067–1074. https://doi.org/10.5897/AJB2013.13414

Motta FL, Santana MHA (2014b) Solid-state fermentation for humic acids production by a *Trichoderma reesei* strain using an oil palm empty fruit bunch as the substrate. Appl Biochem Biotechnol 172:2205–2217. https://doi.org/10.1007/s12010-013-0668-2

Pandey JK, Kumar AP, Singh R (2003) Biodegradation of packaging materials: composting of polyolefins. Macromol Sympos 197:411–420. https://doi.org/10.1002/masy.200350735

Rajagopalan S, Modak JM (1995) Evaluation of relative growth limitation due to depletion of glucose and oxygen during fungal growth on a spherical solid particle. Chem Eng Sci 50:803–811. https://doi.org/10.1016/0009-2509(94)00452-W

Rozman HD, Mohd Ishak ZA, Ishiaku US (2005) In: Mohanty A, Misra M, Drzal LT (eds) Oil palm fiber–thermoplastic composites. Taylor and Francis, Boca Raton, p 407

Schuster A, Schmoll M (2010) Biology and biotechnology of *Trichoderma*. Appl Microbiol Biotechnol 87:787–799. https://doi.org/10.1007/s00253-010-2632-1

Senesi N, Loffredo E (2001) Soil humic substances. In: Hofrichter M, Steinbuchel A (eds) Biopolymers Lignin, humic substances and coal. Wiley, Weineim, pp 247–299

Thibault J, Pouliot K, Agosin E, Perez-Correa R (2000) Reassessment of the estimation of dissolved oxygen concentration profile and KLa in solid-state fermentation. Process Biochem 36:9–18. https://doi.org/10.1016/S0032-9592(00)00156-4

Volpi MPC, Santos VS, Badan APR, Santana MHA, Bastos RG (2018) The role of lignocellulosic composition and residual lipids in empty fruit bunches on the production of humic acids in submerged fermentations. Appl Biochem Biotechnol 3:957–964. https://doi.org/10.1007/s12010-018-2850-z

Volpi MPC, Badan APR, Santana MHA, Bastos RG, Santos VS (2019) Characterization of lignocellulosic composition and residual lipids in empty fruit bunches from palm oil processing. Gras Aceites 70:314–322. https://doi.org/10.3989/gya.0818182