International Biodeterioration & Biodegradation 110 (2016) 99–107

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Degradation of poly(butylene succinate) and poly(butylene succinate-co-butylene adipate) by a lipase from yeast *Cryptococcus* sp. grown on agro-industrial residues

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ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form
4 March 2016
Accepted 7 March 2016
Available online 22 March 2016

Keywords:
Cryptococcus sp.
Lipase
Cottonseed oil cake
Poly(butylene succinate)
Poly(butylene succinate-co-butylene adipate)
Degradation

ABSTRACT

The yeast, *Cryptococcus* sp. MTCC 5455 was grown on various agro-industrial residues for the production of lipase. A maximum lipase activity of 753 ± 19 U g dry substrate^{-1} (U gds^{-1}) and a biomass of 103 ± 5 mg gds^{-1} was obtained at 25 °C and 120 h using cottonseed oil cake with 71% moisture content and 30% (v/w) inoculum. The recovery of the enzyme was increased by 14.1%, when the fermented substrate was extracted in 2 mM $CaCl_2.2H_2O$ solution. The crude enzyme partially purified by $(NH_4)_2SO_4$ precipitation showed a major 22 kDa protein on SDS-PAGE. The enzyme has good potential for hydrolysis of poly(butylene succinate) (PBS) and poly(butylene succinate-co-butylene adipate) (PBSA) and complete degradation of the polymeric films were observed at 72 h and 16 h respectively. The degradation was evaluated by Fourier transform infrared (FTIR) and ^{1}H NMR spectroscopy. The complete hydrolysis of polymers by *Cryptococcus* sp. lipase makes the process ideal and also serves as a baseline for its exploitation in polymer degradation.

1. Introduction

In the past decade, persistence of synthetic polymers in the environment poses a major threat to natural ecological systems (Hidayat and Tachibana, 2012). These plastics are not incorporated into the carbon cycle and have diminished landfill capacity owing to their low biodegradability. Degradable polyesters, as green materials are considered as an alternative to the current traditional thermoplastics from the perspective of ecological security and waste management (Li et al., 2011; Novotny et al., 2015; Shah et al., 2015). They are designed to degrade completely under environmental conditions or by microbial attack into water soluble compounds. Accordingly, various biodegradable aliphatic polyesters including poly(β-hydroxybutyrate) (PHB), poly(ε-caprolactone) (PCL), poly(butylene succinate) (PBS), poly(butylene succinate-co-butylene adipate) (PBSA) and poly(lactic acid) (PLA) have been developed. Among them, PBS and PBSA are synthesized from di-acids and diols with relatively low production cost and act as substitute for routine plastics due to their equivalent mechanical properties to that of polyethylene and polypropylene (Zhao et al., 2005). There are reports on microbial degradation of PBSA and PBS using *Leptothrix* sp. TB-7 (Nakajima-Kambe et al., 2009), *Azospirillum brasilense* (Wu, 2012), *Aspergillus versicolor* (Zhao et al., 2005) and *Bacillus pumilus* 1-A (Hayase et al., 2004), *Aspergillus fumigates* (Ishii et al., 2008) respectively. However, enzymatic degradation of these plastics is very much limited (Maeda et al., 2005; Li et al., 2011).

Lipases are currently receiving much attention due to their multifaceted applications in various industrial processes such as hydrolysis of oils and fats, alcoholysis, aminolysis, peroxidations, interesterifications, in synthesis of food ingredients and as additives in detergents (Rodrigues and Fernandez-Lafuente, 2010). In

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http://dx.doi.org/10.1016/j.ibiod.2016.03.005
0964-8305/

particular, microbial lipases are more suitable because of their low cost, simple production, susceptibility to expression in host microorganisms, diverse specificity, wide range of pH and thermal operational optima (Sharma et al., 2001). Extensive studies have been carried out with bacterial and fungal lipases and reports are available on lipase producing yeasts such as *Candida rugosa* (Rao et al., 1993; Benjamin and Pandey, 1997), *Yarrowia lipolytica* (Dominguez et al., 2003), *Aureobasidium pullulans* HN2.3 (Liu et al., 2008) and *Pichia lynferdii* (Kim et al., 2010). However, confined investigation has been directed towards the lipase of *Cryptococcus* (Chen et al., 1997). The yeast, *Cryptococcus* sp. MTCC 5455 (earlier cited as *Cryptococcus* sp. S-2) produces α -amylase, lipase, polygalacturonase and xylanase (Kamini et al., 2000) and could be utilized for wastewater treatments. The efficacy of *Cryptococcus* sp. lipase has been shown in transesterification of rice bran oil (Kamini and Iefuji, 2001), removal of triglyceride soil from fabrics (Thirunavukarasu et al., 2008) and degradation of polymers, polylactic acid (Masaki et al., 2005) and polyurethane (Thirunavukarasu et al., 2015).

The material cost of the medium is one of the important parameters for commercialization of any fermentation processes to make it viable. An intriguing approach to produce industrial enzymes is solid state fermentation (SSF) as it involves low cost substrates, less energy and space prerequisite, simpler equipment, higher productivity and easier downstream processing (Pandey, 2003; Demir and Tari, 2016). Moreover, there is an increasing trend towards effective use of agro-industrial residues for production of value-added products using SSF (Nidheesh et al., 2015). Most of the studies on lipase production by SSF have been carried out with fungi and bacteria, while only few reports are available on synthesis of lipase by yeasts (Rao et al., 1993; Benjamin and Pandey, 1997; Dominguez et al., 2003). This paper describes the production of an extracellular *Cryptococcus* sp. MTCC 5455 lipase, using cottonseed oil cake, for the hydrolysis of PBS and PBSA, since enzymatic degradation is considered to be an ideal plastic waste treatment strategy for polymer recycling.

2. Materials and methods

2.1. Materials

In the present study, all the chemicals used were of AR grade and acquired from Hi-Media Limited, Sigma–Aldrich and S.D. Fine Chemicals Limited, Mumbai, India. The industrial residues, coconut oil cake (COC), cottonseed oil cake (CtOC), gingelly oil cake (GOC), wheat bran (WB) and rice bran (RB) and the inducers were purchased from local market, Chennai, India. Crude protein content of the substrates were analysed according to the ISO 5983–1979 and the oil content was determined in a Soxhlet apparatus according to the ISO 659–1988. The poly(butylene succinate)-co-(butylene adipate) (PBSA- 58,000) and poly(butylene succinate) (PBS-60,000) used in this study were provided by Showa Highpolymer Co. Ltd., Tokyo, Japan.

2.2. Inoculum and fermentation conditions

The yeast strain, *Cryptococcus* sp. MTCC 5455 grown in the YM medium (3 g l^{-1} yeast extract, 5 g l^{-1} malt extract, 5 g l^{-1} peptone and 10 g l^{-1} dextrose, pH 6.2 ± 0.2) for 40 h at 25 °C (5.4–5.8 × 10^8 cells ml^{-1}) was used as the inoculum. Lipase production was carried out in 250 ml Erlenmeyer flasks, each containing 10 g of various agro-residues with an initial moisture content altered to 60% with distilled water. The flasks were inoculated with 30% (v/w) of the inoculum and the contents were well-mixed and incubated at 25 °C for 5 days. Samples were taken at 24 h intervals and assayed for enzyme activity from 48 h, since lipase activity was low at 24 h. Results are expressed as the mean ± SD from repetition of all the experiments in triplicates.

2.3. Lipase production by SSF

Consecutive optimization studies were carried out by varying the initial moisture (50%–75%) and inoculum concentration (15–60%, v/w) for 10 g of substrate. The effect of addition of various organic nitrogen sources (beef extract, meat extract, peptone, tryptone and yeast extract, 5%, w/w), inorganic nitrogen sources (NH_4NO_3, $(NH_4)_2SO_4$, $NH_4H_2PO_4$ and $NaNO_3$, 5%, w/w), carbon sources (fructose, glucose, lactose, maltose, sucrose and starch, 5%, w/w) and inducers (olive oil, sardine oil, sesame oil, soybean oil and sunflower oil, 5%, w/w) to the substrate was studied for optimal lipase production. Time course studies were carried out using optimized parameters to estimate biomass and lipase production in 2.8 L Fernbach flasks with 100 g substrate. For this, 10 g of substrate inoculum grown for 48 h was transferred into Fernbach flask containing 90 g of substrate and incubated for a period of 120 h and assayed for lipase activity.

2.4. Enzyme assay and biomass estimation

At the end of fermentation period, 1 g of the fermented substrate was homogenized with 10 ml of phosphate buffer (0.1 M, pH 7.0) using a pestle and mortar and centrifuged at 8000 rpm for 5 min. The resultant supernatant was used as the enzyme source. The lipase activity was estimated by the spectrophotometric method using *p*-nitrophenyl laurate (*p*-NPL) as substrate at 410 nm and a molar extinction coefficient of 1.68 (Isobe et al., 1988). One unit of lipase activity was defined as the amount of enzyme that liberates 1 μmol of *p*-nitrophenol per minute under the standard assay conditions and the lipase activity was expressed as U g of dry substrate^{-1} (U gds^{-1}). Enzyme from the fermented substrate was extracted with tap water, distilled water, phosphate buffer (0.1 M, pH 7.0), salts (NaCl, $(NH_4)_2SO_4$, $CaCl_2.2H_2O$) and surfactants (Tween 80 and Triton X-100), respectively. The specific activity was determined by estimating the protein content of the enzyme as per the method Lowry et al. (1951). The biomass estimation was carried out by adding 10 ml of distilled water to 1 g of fermented substrate, vortexed for 5 min and centrifuged at 1000 rpm for 5 min to separate the substrate particles. The supernatant thus obtained was again centrifuged at 8000 rpm for 5 min and the biomass was dried at 70 °C until constant weight was achieved.

2.5. Partial purification of Cryptococcus sp. lipase

The crude enzyme produced under the optimum conditions was partially purified by ammonium sulphate precipitation (80% saturation). The precipitate collected by centrifugation was dissolved in 0.1 M phosphate buffer (pH 7.0), dialysed against the same buffer and lyophilized. The molecular weight of the protein was analysed using SDS-PAGE and the proteins were stained with coomassie brilliant blue R-250.

2.6. Scanning electron microscopic (SEM) studies

Fermented substrate samples taken at different time intervals were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 1 h at 30 °C and washed three times with the same buffer. The samples were then impregnated with 1.25% OsO_4 in 0.1 M phosphate buffer (pH 7.4) for 1 h and dehydrated through a series of 10–100% ascending series of ethanol for 15 min at each stage and finally dried in liquid nitrogen. The dried samples were coated with

gold in argon medium and the surface was scanned to view the yeast growth and budding cells using JEOL JM – 5600 electron microscopic unit at 20 kV accelerating voltage.

2.7. Enzymatic degradation of PBS and PBSA

The crude extract obtained from SSF was partially purified by ammonium sulphate precipitation (80% saturation). The precipitate collected by centrifugation was dissolved in 0.1 M phosphate buffer (pH 7.0) and dialysed against the same buffer, lyophilized and used for the degradation of PBS and PBSA films. For preparation of polymeric film, polymer pellet weighing 25–27 mg were placed between two aluminium plates with an aluminium spacer of 200 μm in thickness and heat pressed at 55 °C and 140 °C for PBSA and PBS respectively, for 7 min. The plates were then quenched in ice water to detach the film from the plates. The film, thus obtained was opaque, circular in shape and with a thickness of ca. 200 μm. Enzymatic degradation studies were carried out in series of 100 ml screw capped Erlenmeyer flasks containing a polymeric film (25–27 mg of PBS or PBSA), 10 ml of 0.1 M phosphate buffer (pH 7.0) and 1000 U of lipase (61.3 U mg of protein^{-1}) and incubated at 30 °C for 96 h with shaking at 120 rpm. Polymer degradation was monitored at 6 h intervals by measuring the weight of film before and after incubation. Films were taken out carefully from the flasks, washed twice with distilled water, air dried and weighed. The degradation products in the reaction mixture were extracted with chloroform, evaporated to dryness using a rotary evaporator and analysed by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectrometer.

2.8. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of the degraded products were measured in the spectral region between 4000 and 500 cm^{-1} using FTIR spectrometer ABB MB3000 (Canada) by thoroughly mixing the sample with potassium bromide at a ratio of 1:5.

2.9. Nuclear magnetic resonance (NMR)

^{1}H NMR spectra of the extract was obtained using a JEOL (Tokyo) ECA spectrometer, operating at 500 MHz using deuterated DMSO and deuterated chloroform as solvents and tetramethylsilane (TMS) as an internal standard.

3. Results and discussion

3.1. Lipase production by SSF

Selection of suitable substrate is a key aspect in SSF process, since the substrate acts as support matrix, nutrient source and as inducer for growth of the organism and for production of the product. Different agro-industrial residues including coconut oil cake (COC), cottonseed oil cake (CtOC), gingelly oil cake (GOC), rice bran (RB) and wheat bran (WB) were evaluated for lipase production by *Cryptococcus* sp. MTCC 5455 (Table 1). Among the substrates, CtOC was found to be suitable because the activity was 543 ± 20 U gds^{-1} at 120 h, which was 14.68 and 7.87 times higher than the lipase activities of *C. rugosa* with RB (Rao et al., 1993) and *Y. lipolytica* with barely bran (Dominguez et al., 2003), respectively. Earlier CtOC has been used as one of the substrates for production of phytase (Ramachandran et al., 2005) and xylanase (Pal and Khanum, 2010), while the production of lipase was reported to be low with *Aspergillus niger* (Kamini et al., 1998). The lipase activity obtained from *Cryptococcus* sp. with other substrates ranged from 143 to 365 U gds^{-1} at 120 h, which was substantially low, compared to the maximal activity obtained with CtOC. Moreover, CtOC constitutes the largest share in terms of total availability of meal followed by soy cake, rape seed and rice bran in India and considering the oilseed tonnage, cotton is the second largest field crop and available in large quantities throughout the year. Hence, further studies were carried out using CtOC as substrate for production of lipase and this is the first report showing the growth of a yeast, *Cryptococcus* sp. on CtOC.

Table 1
Effect of different substrates on lipase production by *Cryptococcus* sp.

Substrates	Lipase activity (U g dry substrate^{-1})				
	48 h	72 h	96 h	120 h	144 h
COC	68 ± 5	127 ± 10	148 ± 12	256 ± 14	149 ± 11
CtOC	312 ± 17	462 ± 15	498 ± 17	543 ± 20	512 ± 18
GOC	83 ± 6	155 ± 11	181 ± 19	209 ± 15	187 ± 10
RB	128 ± 11	168 ± 13	245 ± 18	143 ± 11	129 ± 12
WB	310 ± 16	384 ± 19	367 ± 19	365 ± 14	349 ± 13

3.2. Optimization studies

Lipase production was found to increase gradually with increase in moisture content from 50 to 71% and a maximum yield of 753 ± 19 U gds^{-1} was obtained with 71% moisture and 30% inoculum at 120 h. Further increase in moisture content caused 37% decrease in activity (Table 2). This could be due to agglomeration of the substrate, decrease in porosity of the medium, thereby limiting the supply of oxygen and decreasing the inner pinholes for the vent of CO_2, transfer of heat and oxygen (Weng and Sun, 2006). The lipase activity was optimum with inoculum concentration of 30% and further increase in inoculum size (40%) reduced the lipase production by 26.4% (data not shown). Enzyme production attains its peak, when the supplements accessible to the biomass are balanced. Under conditions of imbalance between nutrients and proliferating biomass, there was decreased enzyme synthesis as reported by Ramachandran et al. (2005). Supplementation of carbon sources such as glucose, fructose, starch and sucrose to the substrate did not show stimulatory effect, while there was slight increase in lipase production with lactose (779 ± 18 U gds^{-1}) and maltose (781 ± 19 U gds^{-1}) compared to the control (753 ± 19 U gds^{-1}). Similar results were reported for production of lipase by *C. rugosa*, where addition of maltose showed a marginal and significant effect with rice bran (Rao et al., 1993) and COC (Benjamin and Pandey, 1997), respectively. Among the nitrogen sources, peptone supplementation alone increased the lipase activity to 4.2% than control. As the increase in lipase production was minimal and the supplementation of additives could increase the cost of production, the control system with the substrate, CtOC was used for further studies. Moreover, CtOC alone was sufficient to produce higher lipase yield as it contained essential nutrients (37.3 ± 0.5% crude protein and 30.7 ± 0.3% oil) and supported the growth of *Cryptococcus* sp.

Table 2
Effect of moisture content on lipase production by *Cryptococcus* sp.

Moisture (%)	Lipase activity (U g dry substrate^{-1})				
	48 h	72 h	96 h	120 h	144 h
50	243 ± 12	329 ± 17	459 ± 18	529 ± 17	506 ± 19
60	300 ± 16	344 ± 15	502 ± 20	576 ± 19	513 ± 17
66	312 ± 17	380 ± 19	573 ± 20	676 ± 16	662 ± 20
71	348 ± 19	452 ± 17	630 ± 19	753 ± 19	699 ± 20
75	246 ± 13	441 ± 19	458 ± 15	471 ± 18	455 ± 17

The time course of lipase production from *Cryptococcus* sp. under optimum conditions by SSF is shown in Fig. 1. The increase in lipase activity was approximately equivalent to that of the biomass concentration indicating that the lipase production is a growth associated process and maximum biomass (103 ± 5 mg gds^{-1}) was attained at 96 h of incubation. The optimized process conditions at 10 g level (71% moisture content and 30% inoculum concentration) was applied for production of lipase at 100 g level in 2.8 L Fernbach flask. A lipase activity of 713 ± 10 U gds^{-1} was obtained at 120 h, which was approximately 94.7% of the optimum activity obtained at 10 g level.

Leaching of products from fermented solids and efficient downstream processing play a critical role in determining the overall economics of the SSF process (Edwinoliver et al., 2010). The extractants used for leaching of lipase from the fermented CtOC had profound effect on the enzyme yield. The data (Table 3) revealed that the enzyme recovery was maximum with 2 mM $CaCl_2.2H_2O$ (859 ± 23 U gds^{-1}), which was 14.1% higher than the enzyme extracted with phosphate buffer (753 ± 19 U gds^{-1}). This could be due to the high dielectric constant of $CaCl_2$, that might reduce the interactive forces between lipase and solid substrate, thereby resulting in high recovery of enzyme from the fermented substrate. However, maximal recovery of the enzyme was reported using Triton X-100 with *Rhizopus chinensis* (Sun et al., 2009) and a combination of NaCl and Triton X-100 with *A. niger* (Edwinoliver et al., 2010), respectively. Similarly, supplementation of NaCl (1%) and Triton X-100 (1%) helped in increasing the recovery of *Cryptococcus* sp. lipase from 753 ± 19 U gds^{-1} to 818 ± 17 U gds^{-1} (Table 3). Furthermore, the reported lipase activity (859 ± 23 U gds^{-1}) and productivity (7.16 U gds^{-1} h^{-1}) obtained with *Cryptococcus* sp. was comparatively higher than that of Rao et al. (1993) (0.77 U gds^{-1} h^{-1}), Benjamin and Pandey (1997) (0.91 U gds^{-1} h^{-1}) and Dominguez et al. (2003) (0.26 U gds^{-1} h^{-1}).

3.3. SDS-PAGE analysis

The apparent molecular mass of the partially purified enzyme obtained from the fermentation of CtOC was found to be 22 kDa (Fig. 2). The result was in concurrence with the report of Kamini et al. (2000), wherein the purified lipase was obtained from *Cryptococcus* sp. in a liquid medium using triolein as an inducer. The production of lipase from CtOC without addition of inducers indicated the constitutive expression of the enzyme.

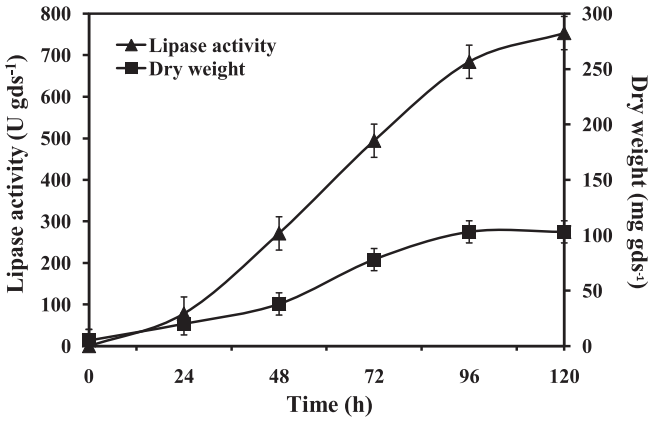

Fig. 1. Time course of lipase production from *Cryptococcus* sp. by SSF.

Table 3
Extraction of lipase using various salts and surfactants.

Salt solutions	Lipase activity (U g dry substrate^{-1})
Tap water	613 ± 12
Distilled water	504 ± 9
Phosphate buffer (0.1 M, pH 7.0)	753 ± 19
NaCl (1%)	758 ± 16
NaCl (2%)	708 ± 11
$(NH_4)_2SO_4$ (1%)	779 ± 21
$CaCl_2.2H_2O$ (2 mM)	859 ± 23
Tween 80 (0.5%)	624 ± 13
Triton X-100 (0.5%)	691 ± 15
Triton X-100 (1%)	723 ± 19
NaCl (1%) + Tween 80 (0.5%)	641 ± 11
NaCl (1%) + Triton X-100 (0.5%)	761 ± 14
NaCl (1%) + Triton X-100 (1%)	818 ± 17

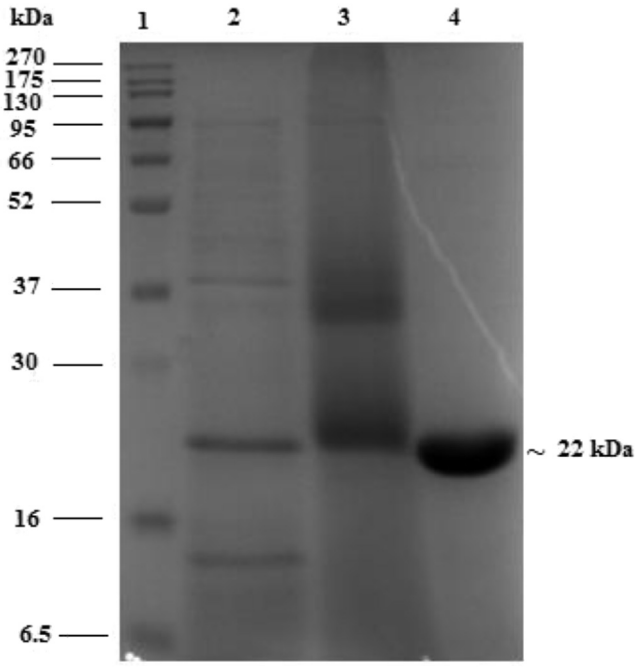

Fig. 2. SDS-PAGE analysis of lipase from *Cryptococcus* sp. Lane 1, molecular marker; lane 2, crude lipase; lane 3, partially purified lipase (ammonium sulphate precipitation); lane 4, partially purified lipase (after dialysis).

3.4. Scanning electron microscopic (SEM) studies

The scanning electron micrograph of the CtOC substrate matrix at 0 h is shown in Fig. 3a. Fig. 3b shows the uniform growth of the cells throughout the matrix at 24 h followed by multiplication of the yeast cells by budding at 48 h Fig. 3c, clearly shows the budding yeast (black arrow) and a large matured yeast cell with scars (white arrow) characterized by slightly raised surface with thick edges. Similar results of scar formation on larger and older yeast cells were reported by Northcote and Horne (1952), due to the detaching of budding yeast cells from the matured cells. At 72 h (Fig. 3d), dense population of yeast cells was observed and they were ovoid to ellipsoidal in shape. The growth pattern of *Cryptococcus* sp. on solid substrate, CtOC was shown for the first time, in this study.

3.5. Enzymatic hydrolysis of PBS and PBSA

PBS and PBSA are considered as an alternate choice to the present conventional thermoplastics and have wide applications in

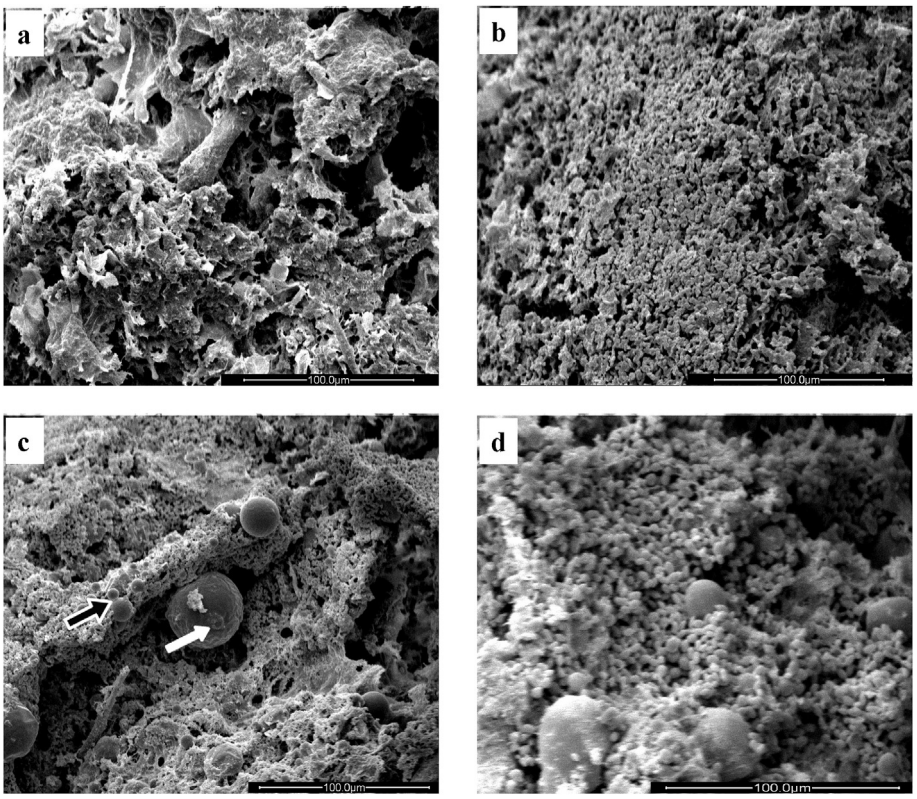

Fig. 3. Scanning electron micrographs of *Cryptococcus* sp. grown on CtOC at (a) 0 h, 500× (b) 24 h, 500× (c) 48 h, 500× (d) 72 h, 700×. Fig. 1c, shows the budding yeast (black arrow) and a large matured yeast cell with scar (white arrow).

industries like biomedical, food, manure and waste composting and agricultural packing (Wu, 2012). Both are degradable in natural environments such as, compost, damp soil, fresh water with activated sludge and seawater. However, degradation rate of these plastics are considerably low and compared to PBS degradation, PBSA degrades in 2–14 days (Hayase et al., 2004; Nakajima-Kambe et al., 2009), while PBS takes several weeks for degradation (Ishii et al., 2008; Wu, 2012) and likely to be accumulated in natural environments. In this study, an attempt was made to degrade the polymeric films of PBS and PBSA with the partially purified lipase of *Cryptococcus* sp. and the degraded films at different time intervals were collected, arranged and shown in Fig. 4 (a–f). The degradation of the films were linear and after 6 h of incubation, the surface of PBSA film appeared rough and translucent, while similar changes were observed in PBS film after 12–14 h of incubation. The PBSA film became very fragile and broken into several pieces at the end of 8 h (Fig. 4b), followed by its papery appearance at 12 h (Fig. 4c) and complete degradation at 16 h. In the case of PBS, the film

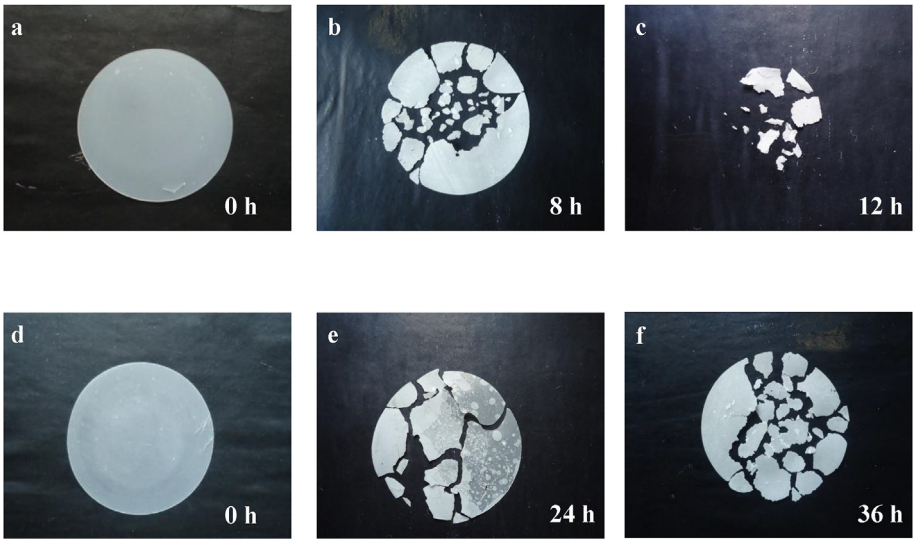

Fig. 4. Enzymatic degradation of PBSA (a–c) and PBS (d–f) films.

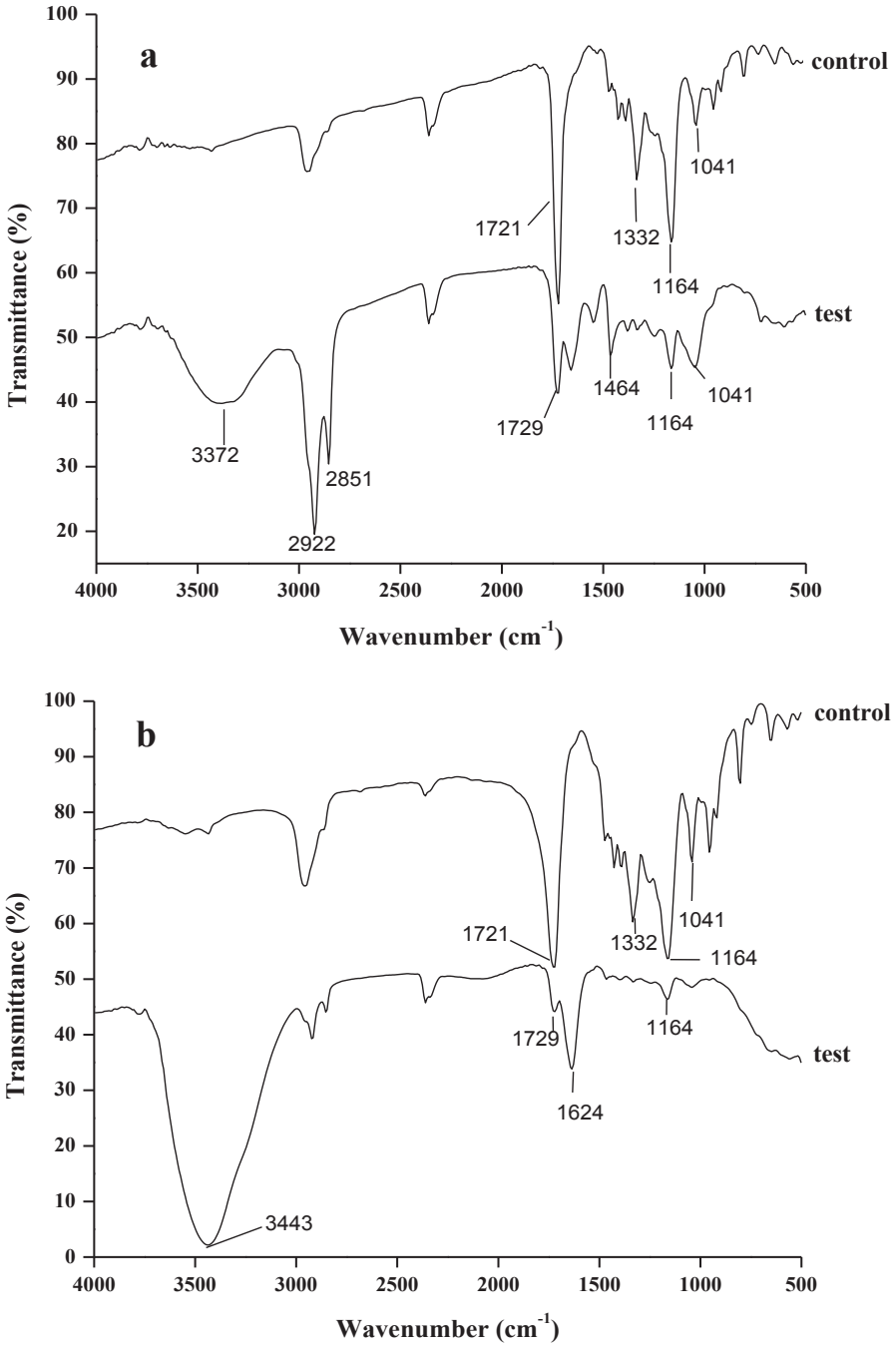

Fig. 5. FTIR spectra of (a) PBS and (b) PBSA.

turned papery only after 24 h (Fig. 4f) incubation and complete degradation of the film was observed at 72 h. The biodegradability of PBS at 72 h could be due to the high crystallinity and glass transition temperature than PBSA as reported by Maeda et al. (2005), where the purified esterase of *Aspergillus oryzae* could degrade PBSA completely and PBS partially with surface erosion. The time taken for the complete degradation of PBSA film by *Cryptococcus* sp. was only 16 h, while it was 4, 6 and 22 days for commercial lipases of *Candida viscosum*, *R. niveus* and *R. orizae* (Hoshino and Isono, 2002), respectively. With reference to PBS, the film was degraded completely in 3 days by the lipase of *Cryptococcus* sp., but lipases of *Pseudomonas cepacia* (Taniguchi et al., 2002) and *C. viscosum* (Hoshino and Isono, 2002) took 3 and 17 days for 58 and 100% degradation, respectively.

3.6. FTIR analysis

Fig. 5a shows the FTIR spectra of the control film and degraded products of PBS. As a result of hydrolysis of the ester bonds in degraded PBS film, there was significant decrease in transmittance intensity of C═O band at 1721 cm^{-1} (slightly shifted to 1729 cm^{-1}) and −C−O−C− band at 1164 cm^{-1} compared to control. The broad peak at 3372 cm^{-1} was assigned to the stretching vibration of the −OH group corresponding to terminal alcohol as a result of main chain scission at ester linkages as reported for the hydrolysis of polybutylene adipate co-terephthalate (PBAT) by Kijchavengkul

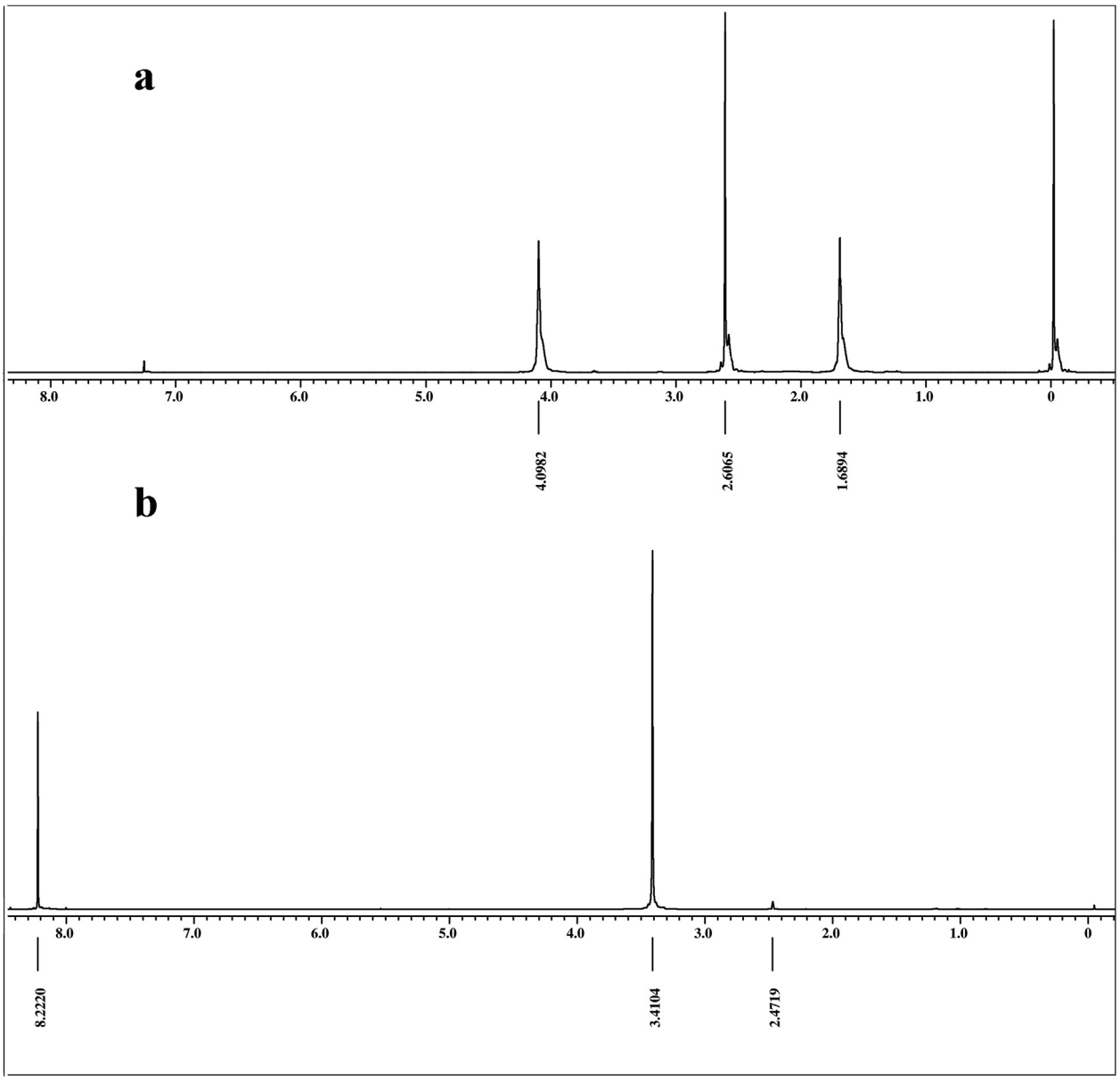

Fig. 6. ^{1}H NMR spectra of (a) PBS film control and (b) degraded product.

et al. (2010). The peaks at 2922 and 2851 cm^{-1} were assigned to methylene groups [$-CH_2-C$ (C–H stretch)] and the peak at 1464 cm^{-1} corresponds to $-CH_2-C$ (C–H bend), respectively. These results confirmed the changes in the chemical structure of PBS due to enzymatic degradation by the hydrolysis of the ester bonds, C=O and –C–O–C–, in the main chain (Umare et al., 2007).

Relatively, the FTIR spectra (Fig. 5b) of degraded PBSA products showed significant reduction in transmittance intensity of C=O and –C–O–C– band and increased OH group stretching vibration, as shown in the spectra of degraded PBS film. The specific transmittance peaks at 1041 cm^{-1} and 1332 cm^{-1} assigned to O–C–C and C–H stretching vibrations of control PBSA film were not detected and a peak appeared at 1624 cm^{-1} could be due to carboxyl acid C=O stretching of the hydrolytic products of PBSA.

3.7. NMR analysis

The degraded products of PBS and PBSA were also analysed by NMR. Fig. 6a shows typical proton resonances of PBS at 2.606 ppm for $-C=OCH_2-$ assigned to succinate unit, 4.0982 ppm for $-O-CH_2-$ assigned to 1,4-butanediol unit, respectively, while the PBSA film (Fig. 7a) showed similar chemical shifts at 2.626 ppm (succinate), 4.118 ppm (1,4-butanediol) and an additional peak at 2.325 ppm corresponding to $-C=OCH_2-$ for the adipate unit. The ^{1}H NMR analysis of the water soluble products of PBS (Fig. 6b) and PBSA (Fig. 7b) showed a peak at 8.222 and 8.238 ppm, respectively, assignable to the proton of formic acid, a product formed by the hydrolysis of the anhydride end groups which are highly susceptible to hydrolytic reactions (Rizzarelli and Carroccio, 2009). The absence of 1,4-butanediol peak after enzymatic degradation of PBS and PBSA indicated that the lipase could have hydrolysed the polymer chain from the carboxyl end as reported by Li et al. (2011). The new chemical shift at 3.41 and 3.40 ppm observed in PBS and PBSA, respectively, after enzymatic degradation (Figs. 6b and 7b) could be assigned to the hydroxyl group corresponding to terminal alcohol due to main chain scission at ester linkages, as confirmed by

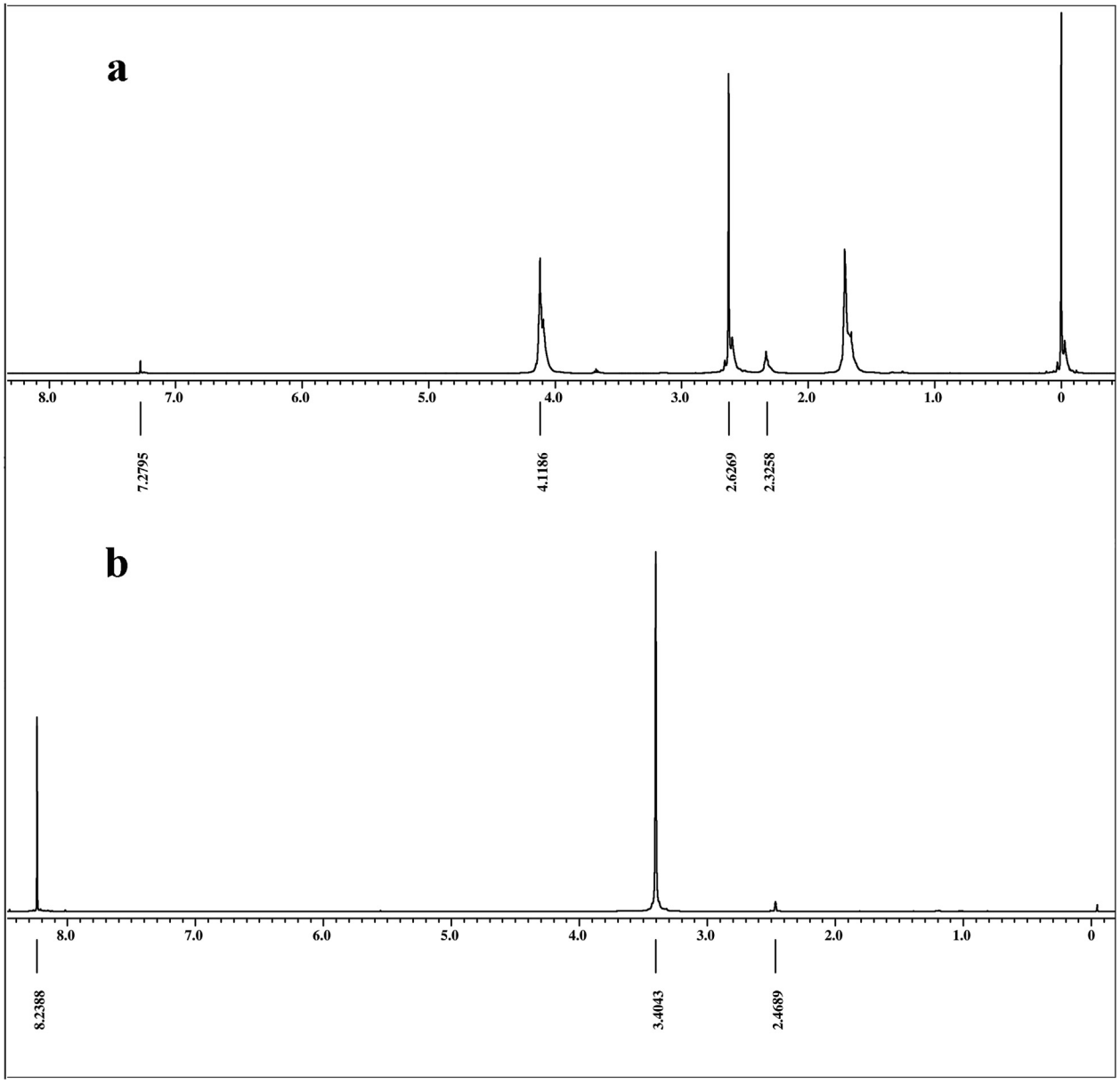

Fig. 7. ^{1}H NMR spectra of (a) PBSA film control and (b) degraded product.

the −OH stretching vibrations in the FTIR spectral regions of PBS and PBSA degraded samples at 3300–3500 cm^{-1}. These results clearly implied that *Cryptococcus* sp. lipase hydrolyses the polymers by cleaving the ester bonds and not mineralize PBS and PBSA.

4. Conclusions

The significance of the present study is complete degradation of PBS and PBSA within a short period of 72 h and 16 h by a lipase produced from *Cryptococcus* sp. The lipase was produced constitutively using agro-industrial residue CtOC, thus reducing the cost of the process and making it viable. Though the results indicate that the enzyme is ideal for treatment of polymer waste, further work is needed to confirm its potential to act on aliphatic-aromatic polymers, which would help in commercial exploitation of the process.

Acknowledgements

The authors thank Director, CSIR-Central Leather Research Institute, Chennai, India for the kind permission to publish this work. We also acknowledge for the grants received from Department of Biotechnology (No. BT/PR13798/PID/06/543/2010), India and the financial assistance extended by Council of Scientific and Industrial Research, New Delhi, India to K. Thirunavukarasu, S. Purushothaman and M. Aarthy.

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