Bioresource Technology 153 (2014) 126–130

Contents lists available at ScienceDirect

Bioresource Technology

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

Production of *Sporotrichum thermophile* xylanase by solid state fermentation utilizing deoiled *Jatropha curcas* seed cake and its application in xylooligosachharide synthesis

Ayesha Sadaf, S.K. Khare *

Department of Chemistry, Indian Institute of Technology, New Delhi 110016, India

HIGHLIGHTS

- Utilization of deoiled *Jatropha* seed cake for *Sporotrichum thermophile* xylanase.
- Production of *S. thermophile* xylanase in high titre (1025 U/g dry seed cake).
- *S. thermophile* endoxylanase is effective for xylooligosachharide (XOS) production.
- Xylotetraose rich XOS produced are desirable in prebiotic applications.

ARTICLE INFO

Article history:
Received 23 September 2013
Received in revised form 19 November 2013
Accepted 21 November 2013
Available online 4 December 2013

Keywords:
Xylanase
Sporotrichum thermophile
Xylooligosachharides
Jatropha curcas

ABSTRACT

De-oiled *Jatropha curcas* seed cake, a plentiful by-product of biodiesel industry was used as substrate for the production of a useful xylanase from *Sporotrichum thermophile* in solid state fermentation. Under the optimized conditions, 1025 U xylanase/g (deoiled seed cake) was produced. The xylanase exhibited half life of 4 h at 45 °C and 71.44 min at 50 °C respectively. It was stable in a broad pH range of 7.0–11.0. K_m and V_{max} were 12.54 mg/ml and 454.5 U/ml/min respectively. *S. thermophile* xylanase is an endoxylanase free of exoxylanase activity, hence advantageous for xylan hydrolysis to produce xylooligosachharides. Hydrolysis of oat spelt xylan by *S. thermophile* xylanase yielded 73% xylotetraose, 15.4% xylotriose and 10% xylobiose. The *S. thermophile* endoxylanase thus seem potentially useful in the food industries.

1. Introduction

Xylooligosaccharides (XOS) are the oligomers constituted of xylose units. They exhibit excellent prebiotic effect and health benefits, while consumed as part of diet by supporting the growth of *Bifidobacteria* and *Lactobacillus* (Falck et al., 2013; Moure et al., 2006) Beside their role as prebiotics, these are also used in food, feed and pharmaceuticals industries (Deutschmann and Dekker, 2012). Hence, there is an increasing demand for their production. Generally XOS are produced from xylan rich agro-residues by variety of methods viz. thermal, acid, alkaline or enzymatic hydrolysis (Vázquez et al., 2000). The enzymatic processes employ xylanase and offer advantages of mild reaction conditions, better specificities, higher yields and absence of any by-product. However, the xylanases to be used for XOS production should preferably be (i) free of or have very little exo-xylanase and β-xylosidase activities (ii) stable at high temperature to withstand xylan extraction conditions and (iii) available at low cost (Chapla et al., 2012). These should possess predominant endo β, 1–4 xylanase activity which is required for xylan hydrolysis. Hence, thermophilic endo-xyalnases are best suited for XOS production.

Sporotrichum thermophile is a thermophilic fungus which is reported to produce many interesting enzymes like cellulase (Coutts and Smith, 1976), pectinase (Kaur and Satyanarayana, 2004), esterase (Topakas et al., 2004), phytase (Singh and Satyanarayana, 2006) and an interesting endoxylanase (Badhan et al., 2007; Katapodis et al., 2003; Vafiadi et al., 2010). Xylanase from *S. thermophile* 'belonging to family EX 11' has predominant endo β, 1–4 xylanase activity and is free of exoxylanase activity (Vafiadi et al., 2010). Xylanases produced by *Trichoderma reesei* and *Thermomyces lanuginosus* also belong to this family and have been well studied.

S. thermophile xylanase has been purified and characterized by Katapodis et al., 2003. Its preference for cleaving internal glycosidic bonds of xylan makes it quite promising for xylan hydrolysis and XOS production. However, the low level of xylanase production

* Corresponding author. Address: Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India. Tel.: +91 112659 6533; fax: +91 11 2658 1102.
E-mail addresses: skhare@rocketmail.com, skkhare@chemistry.iitd.ac.in (S.K. Khare).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.11.058

in *S. thermophile* poses a concern towards its industrial applications. This necessitates its cost effective production with high titre.

Solid-state fermentation (SSF) has been a viable technique for production of enzymes and other industrial products (Mienda et al., 2011). Herein, the agro-industrial by-products/biomass are used as substrate as a replacement of synthetic media ingredients, thereby cutting the bulk of the production cost. The agro-residue or biomass act as support and provide nutrients for the growth of microorganisms which in turn produce desirable enzymes. SSF has been used to produce many industrial enzymes viz. proteases from defatted soybean cake (Germano et al., 2003), β-glucosidase from rice bran (Ng et al., 2010), cellulase from wheat bran (Maurya et al., 2012), lipase from gingelly oil cake (Mala et al., 2007), amylase from crude millet (Maktouf et al., 2013), laccase from orange peel (Chairin et al., 2013). Wheat bran, soybean meal and saw dust have been effectively used to produce xylanase from *Colletotrichum graminicola* (Zimbardi et al., 2013), *Aspergillus niger* (Vitcosque et al., 2012) and *Arthrobacter sp.* (Murugan et al., 2011) respectively.

Jatropha curcas is a major source material for producing biodiesel. About 2–3 tons of residual *jatropha* seed cake is generated as by-product out of each ton seed processed into biodiesel (http://www.svlele.com/biogas.htm). This deoiled seed cake is considered quite toxic due to the presence of phorbol esters, curcin and other antinutrients (Joshi et al., 2013; Rakshit et al., 2008). This restricts its use in cattle feed, unlike other oilseed cakes. Left to decay as such, the unutilized deoiled *jatropha* seed cake adds to existing environmental problem. Its use as substrate in SSF addresses its meaningful utilization.

We have previously demonstrated that overall composition of deoiled *jatropha* seed cake containing 60% protein, 0.6% fat, 9% ash, 4% fiber and 26% carbohydrate is quite supportive to microbial growth and efficiently produces solvent stable protease and lipase (Mahanta et al., 2008). SSF is additionally advantageous for thermophilic enzymes, because herein the inherent heat generated in the SSF process (because of low heat transfer capacity of the solid substrate) could be exploited by thermophiles for better growth. Considering the success of SSF in producing high titre of xylanase (1455 U/g) from thermophilic mould *Scytalidium thermophilum*, while using deoiled *jatropha* seed cake as substrate (Joshi and Khare, 2011), it was considered worthwhile to explore SSF for *S. thermophile* xylanase production. The production by SSF was envisaged to be cost effective because the *jatropha* seed cake, a by-product is being explored as substrate. This would replace the costly synthetic media ingredients otherwise used for production of *S. thermophile* xylanase.

Present work describes the high titre production of a xylanase from *S. thermophile* by SSF using deoiled *jatropha* seed cake as substrate. This being an endoxylanase, has been shown to efficiently produce XOS from hydrolysis of xylan. The study also serves as a viable strategy for utilization of toxic *jatropha* seed cake.

2. Methods

The media components and oat spelt xylan were purchased from Hi Media Laboratory, (Mumbai, India). Birchwood and beechwood xylans were sourced from Sigma Chemicals (St. Louis, USA). All other chemicals used were of analytical grade. Deoiled *J. curcas* seed cake (DSC) was obtained from CRDT, Indian Institute of Technology, Delhi, India.

2.1. Culture

The xylanase producer thermophilic *S. thermophile* strain was isolated and provided by Prof. T. Satyanarayana, Department of Microbiology, Delhi University South Campus, New Delhi, India. The strain was maintained on PDA slants at 4 °C and sub-cultured at 15 day interval.

2.2. Inoculum preparation

Five mililitre of sterile saline solution containing 0.1% (v/v) Tween-80 was added to the culture slants of *S. thermophile* and the surface was gently rubbed using a wire loop. Inoculum was prepared by suspending the spores in sterile saline solution. The spore suspension prepared was adjusted to the desired range by sterile saline solution and counted using a hemocytometer (Neubauer-improved, Paul Marienfeld GmbH & Co. KG Germany).

2.3. Solid-state fermentation

Five gram deoiled *jatropha* seed cake moistened with 7.5 ml Tris–HCl buffer (0.1 M, pH 9.5) was seeded with 6% inoculum of *S. thermophile* and incubated at 35 °C. In above protocol, following parameters were varied by one at a time approach:

Effect of pH was recorded by using buffers of different pH viz. acetate (0.1 M, pH 5.0), sodium phosphate (0.1 M, pH 6.0–7.0) and Tris–HCl buffer (0.1 M, pH 8.0, 9.0, 9.5 and 10.0). The moisture level was varied by keeping seed cake: buffer ratio as 1:2, 1:2.5, 1:3, 1:3.5 and 1:4 (w/v). Inoculum size was varied to 3%, 4%, 5%, 6% and 7%. Particle size of 0.2, 0.3, 0.6, 2 and 2.8 mm were used for seeing the effect of size. Incubation temperature was varied to 25, 30, 35, 40, 45 and 50 °C. Glucose, birchwood xylan and cellulose at a concentration of 1% (w/v) were supplemented as carbon source. All the experiments in the study were performed in triplicate and the variation was within ±5%.

2.4. Extraction of xylanase

Separate flasks (in triplicate) were used for each variable. The SSF was carried out for 4 days after which entire content of each flask was harvested. The extraction was done by adding 40 ml sodium phosphate buffer (0.1 M, pH 7.0) followed by constant shaking at 150 rpm for 30 min at 30 °C. Entire content was then squeezed through a muslin cloth and residual matter subjected to two more extraction cycles. All the extracts were finally pooled and centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was used as the crude enzyme.

2.5. Xylanase assay

Xylanase activity was estimated by following the method of Bailey et al. (1992) using oat-spelt xylan as substrate. Briefly 1 ml of a suitable diluted enzyme was mixed with 0.5 ml of oat spelt xylan (1% w/v in 0.1 M, sodium phosphate buffer, pH 7.0). The reaction mixture was incubated at 50 °C for 15 min. The reducing sugar generated as reaction product was quantified by dinitrosalicylic acid (DNSA) method (Miller, 1959). One unit of enzyme activity is defined as the amount of enzyme required to produce 1 μmol of xylose per minute.

2.6. Xylanase characterization

2.6.1. Substrate specificity and kinetic constants

Substrate specificity of the xylanase was determined by assaying it towards different substrates, oat spelt xylan, beechwood xylan and birchwood xylan (1% w/v in 0.1 M sodium phosphate buffer, pH 7.0). Other conditions were kept same as described in assay procedure.

Kinetic parameters were determined towards oat spelt xylan as substrate at concentrations 0.2–0.6 mg/ml in 0.1 M sodium

phosphate buffer pH 7.0. Lineweaver–Burke plot was used for determining K_m and V_{max}.

2.6.2. pH and temperature stability

Xylanase was incubated in buffers of different pH viz. sodium acetate (pH 5.0), sodium phosphate (pH 6.0–7.0) and Tris–HCl (pH 8.0–11.0) for 1 h. The residual xylanase activity was determined under standard assay conditions. For thermal stability, the enzyme in 0.1 M sodium phosphate buffer, pH 7.0 was incubated at different temperatures. The residual activity was determined under standard assay conditions.

2.7. Xylooligosaccharide synthesis from xylan

Xylan solution was prepared by mixing 1% oat spelt xylan in sodium phosphate buffer (0.1 M, pH 7.0). Xylanase (20 U/ml) was added to 5 ml xylan solution and the reaction mixture incubated at 45 °C with constant shaking. Aliquots were withdrawn at various time intervals (0, 1, 2, 4, 6, and 8 h) and boiled to stop the reaction. These were then centrifuged at 14,000 rpm 10 min. The supernatant was filtered through 0.22 μm filter. The filtrate was analyzed by HPLC (Perkin–Elmer series 200 HPLC system, Massachusetts, USA) using aminex analytical column. Acetonitrile: water (70:30) was used as mobile phase at a flow rate of 0.5 ml/min. The peaks were detected by Refractive index detector (Perkin–Elmer, series 200a, Massachusetts, USA).

All the experiments in the study were performed in triplicate and the variation was within ±5%.

3. Results and discussion

3.1. Optimization of SSF conditions

S. thermophile grew well on DSC (deoiled seed cake) and initially 270 U xylanase/g was obtained under unoptimized conditions. Further optimization was carried out to enhance the xylanase production.

Moisture content, initial pH, inoculum, particle size, temperature, carbon supplementation and inducer were varied. The results are summarized in Table 1. It is quite evident that substrate to moisture content, pH, temperature, and above all birchwood xylan as inducer significantly enhanced the xylanase production.

3.1.1. Initial moisture content

Moisture content is an important factor in SSF, since the level of free water in the solid matrix defines the physiological and biological activity of microorganisms (Kamra and Satyanarayana, 2004). Maximum 430 U xylanase/g DSC was reached at 1:1.5 ratio of water:jatropha seed cake (Fig. 1) The same amount of moisture content has been found to be optimum in the case of *Bacillus pumilis* in SSF of wheat bran (Banu and Ingale, 2012). Slighty higher levels of moisture i.e. 1:3–1:4 have been optimum in case of *Thermoascus auranticus* using wheat straw as substrate (Kalogeris et al., 1998).

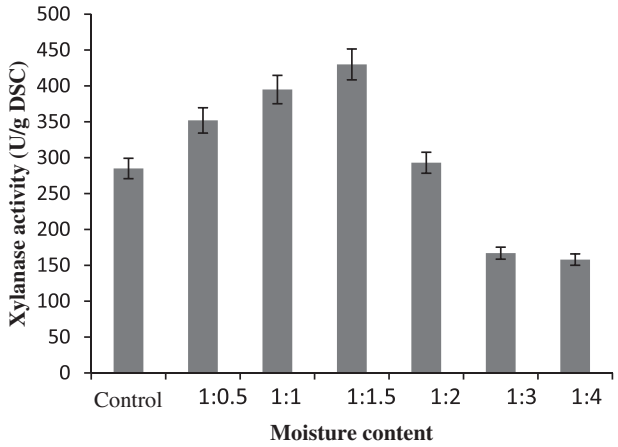

Fig. 1. Effect of moisture content on xylanase production by *S. thermophile*. Five grams deoiled *jatropha* seed cake (DSC) was moistened with different volumes of distilled water so as to fix the ratio at 1:0.5, 1:01, 1:1.5, 1:02, 1:03, 1:04 (w/v). The moistened samples were inoculated with 4% inoculum size and incubated at 35 °C for 4 days. The entire content of flask was harvested and xylanase activity was determined.

3.1.2. pH

pH is an important factor in any fermentation process since most microbial activities are dependent on it (Prakasham et al., 2005). Xylanase production was maximum (681 U/g) when initial pH was adjusted to 9.5. At pH 7.0 and below considerable low xylanase activity was observed (Fig. 2).This can be attributed to the alkaliphilic nature of the enzyme.

3.1.3. Supplementation of carbon sources

Xylanases are inducible enzymes (Sachslehner et al., 1998). Among the carbon sources viz. glucose, birchwood xylan and cellulose supplemented to DSC, birchwood xylan resulted into maximum xylanase yield. Similar effect of xylan induction has also been previously reported in case of *S. thermophilum* (Joshi and Khare, 2012).

Under all optimized conditions viz. 5 g deoiled *jatropha* seed cake supplemented with 1% birchwood xylan, moistened with 7.5 ml of Tris–HCl buffer (0.1 M pH 9.5), seeded with 6% *S. thermophile* inoculum and incubated at 35 °C led to the production of maximum 1025 U xylanase/g DSC (Table 1). Overall 3.7-fold increase was observed over unoptimized 270 U/g initial production level (Fig. 3) This strain has been reported to produce similar titre when wheat bran and citrus pectin was used as substrate in SSF (Kaur and Satyanarayana, 2004). It is thus confirmed that SSF is a good strategy for producing xylanase from *S. thermophile*.

3.2. Kinetic parameters and substrate specificity

The xylanase was characterized to confirm its properties, which are important for its application. The temperature stability of the crude enzyme indicated a half life of 71.44 min at 50 °C. This is in agreement with the temperature stability reported earlier

Table 1
Effect of various SSF parameters on xylanase production from *S. thermophile*.

S.No	Parameter	Optimized condition	Xylanase activity(U/g)
1	Duration of SSF	96 h	270
2	Moisture content (deoiled DSC: water ratio)	1:1.5	430
3	pH	9.5	681
4	Inoculum size	6% (v/w)	698
5	Particle Size	0.6 mm	700
6	Temperature	35 °C	910
7	Inducer	1% (v/w) Birchwood Xylan	1025

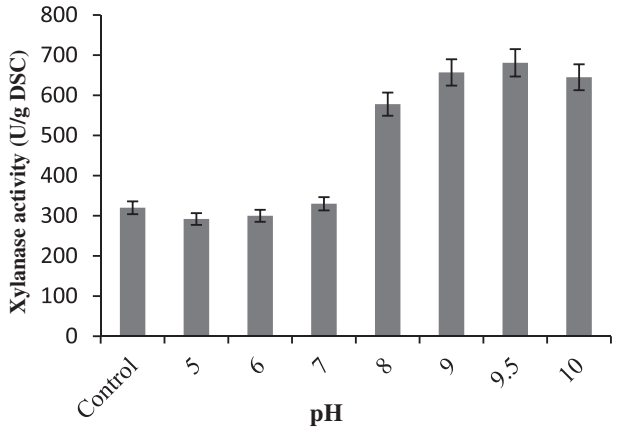

Fig. 2. Effect of initial pH on xylanase production by *S. thermophile*. Five grams DSC moistened to 1:1.5 (w/v) by buffer systems (0.1 M), sodium acetate (pH 5.0), sodium phosphate (pH 6.0–7.0), and Tris–HCl (pH 8.0–11.0), was inoculated with 4% inoculum size. The incubation was done at 35 °C for 4 days. The entire content of flask was harvested and xylanase activity was determined.

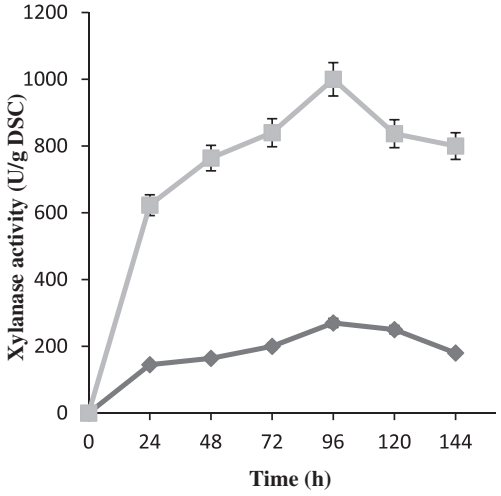

Fig. 3. Solid-state fermentation of deoiled jatropha seed cake (DSC) for xylanase production by *S. thermophile S. thermophile* xylanase production under (◆) unoptimized and (▭) optimized conditions (as summarized in Table 1).

Fig. 4. Substrate specificity of *S. thermophile* xylanase. Xylanase (20 U) was assayed with different xylans (1% w/v in 0.1 M sodium phosphate buffer, pH 7.0) under standard assay conditions as described in method section.

(Vafiadi et al., 2010). The birchwood and oat spelt xylans were found to be better substrates for this enzyme (Fig. 4). The K_m and V_{max} towards oat spelt xylan were 12.54 mg/ml and 454.5 U/ml/min respectively.

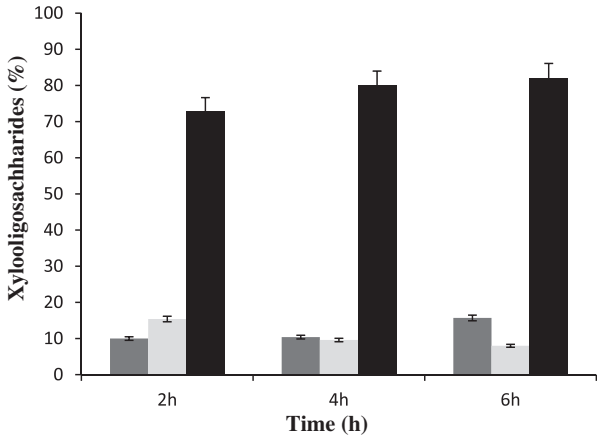

Fig. 5. Xylooligosaccharides content obtained by xylan hydrolysis using *S. thermophile* xylanase. Oat spelt xylan (1% w/v in 0.1 M sodium phosphate buffer pH 7.0) was incubated with 20 U xylanase at 45 °C. The samples were withdrawn at different time intervals. XOS and HPLC analysis were carried out as described in method section. ▭ xylobiose, ▭ xylotriose, ▭ xylotetraose.

3.3. Application in xylooligosaccharide (XOS) synthesis

Since *S. thermophile* xylanase has been reported to contain exclusive endoxylanase activity, it was applied for xylooligosaccharide production by xylan hydrolyisis at pH 7.0 and 45 °C. The products were analysed by HPLC. The results are shown in Supplementary Fig. S1. Evidently, xylobiose, xylotriose and xylotetraose were formed as initial products in 2 h, xylotetraose was the major one. With time course of hydrolysis, amount of xylotetraose increased further reaching to about 80–82% in 6 h. Hydrolysis was completed in 8 h and xylotetraose was the predominant XOS (Fig. 5).

Since no xylose was formed, it confirmed that xylanase possesses only endoxylanase activity. Absence of xylose in XOS is highly desirable for applications as prebiotics and food additives (Aachary and Prapulla, 2009). The production of xylose free and xylotetraose rich XOS makes *S. thermophile* an attractive enzyme.

4. Conclusion

The study optimized a solid state fermentation process, by using deoiled *jatropha* seed cake (DSC) as substrate, for xylanase production from *S. thermophile*. A high titre 1025 U/g (DSC) was attained. The xylanase was predominantly an endoxylanase which was used for the production of xylooligosaccharides (XOS). It efficiently hydrolyzed xylan and yielded XOS rich in xylotetraose. XOS having high proportion of xylotetraose are desirable for prebiotics applications.

The study also led to a viable approach for utilization of DSC which poses a disposal problem due to the presence of toxic phorbol ester.

Acknowledgements

The authors are grateful to Prof. T. Satyanarayana, Department of Microbiology, Delhi University South Campus, New Delhi for providing the *S. thermophile* culture for this study. The financial support for the study provided by Defence Research & Development Organisation (DRDO), New Delhi is greatfully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.11.058.

References

Aachary, A.A., Prapulla, S.G., 2009. Value addition to corncob: production and characterization of xylooligosaccharides from alkali pretreated lignin-sachharide complex using *Aspergillus oryzae* MTCC 5154. Bioresour. Technol. 100, 991–995.

Badhan, A.K., Chadha, B.S., Kaur, J., Saini, H.S., Bhat, M.K., 2007. Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099. Bioresour. Technol. 98, 504–510.

Bailey, M.J., Biely, P., Poutanen, K., 1992. Interlaboratory testing of methods for assay of xylanase activity. J. Biotechnol. 23, 257–270.

Banu, A., Ingale, S., 2012. Xylanase production by *Bacillus pumilus* AB-1 under solid state fermentation and its application. Bull. Environ. Sci. Res. 1, 35–43.

Chairin, T., Nitheranont, T., Watanabe, A., Asada, Y., Khanongnuch, C., Lumyong, S., 2013. Purification and characterization of the extracellular laccase produced by *Trametes polyzona* WR710-1 under solid-state fermentation. J. Basic Microbiol.. http://dx.doi.org/10.1002/jobm.201200456.

Chapla, D., Pandit, P., Shah, A., 2012. Production of xylooligosaccharides from corncob xylan by fungal xylanase and their utilization by probiotics. Bioresour. Technol. 115, 215–221.

Coutts, A.D., Smith, R.E., 1976. Factors influencing the production of cellulases by *Sporotrichum thermophile*. Appl. Environ. Microbiol. 31, 819–825.

Deutschmann, R., Dekker, R.F., 2012. From plant biomass to bio-based chemicals: latest developments in xylan research. Biotechnol. Adv. 30, 1627–1640.

Falck, P., Precha-Atsawanan, S., Grey, C., Immerzeel, P., Stålbrand, H., Adlercreutz, P., Karlsson, E.N., 2013. Xylooligosaccharides from hardwood and cereal xylans produced by a thermostable xylanase as carbon sources for *Lactobacillus brevis* and *Bifidobacterium adolescentis*. J. Agric. Food. Chem. 61, 7333–7340.

Germano, S., Pandey, A., Osaku, C.A., Rocha, S.N., Soccol, C.R., 2003. Characterization and stability of proteases from *Penicillium* sp. produced by solid-state fermentation. Enzyme Microb. Technol. 32, 246–251.

Joshi, C., Khare, S.K., 2011. Utilization of deoiled *Jatropha curcas* seed cake for production of xylanase from thermophilic *Scytalidium thermophilum*. Bioresour. Technol. 102, 1722–1726.

Joshi, C., Khare, S.K., 2012. Induction of xylanase in thermophilic fungi *Scytalidium thermophilum* and *Sporotrichum thermophile*. Braz. Arch. Biol. Technol. 55, 21–27.

Joshi, C., Khare, S.K., Gupta, M.N., 2013. Applications of solid state fermentation process in biological detoxification of industrial wastes. Curr. Biochem. Eng. 1, 1–15.

Kalogeris, E., Christakopoulos, P., Kekos, D., Macris, B.J., 1998. Studies on the solid state production of thermostable endoxylanases from *Thermoascus aurantiacus*: characterization of two isoenzymes. J. Biotechnol. 60, 155–163.

Kamra, P., Satyanarayana, T., 2004. Xylanase production by the thermophilic mold *Humicola lanuginosa* in solid-state fermentation. Appl. Biochem. Biotechnol. 119, 145–157.

Katapodis, P., Vrsanská, M., Kekos, D., Nerinckx, W., Biely, P., Claeyssens, M., Macris, B.J., Christakopoulos, P., 2003. Biochemical and catalytic properties of an endoxylanase purified from the culture filtrate of *Sporotrichum thermophile*. Carbohydr. Res. 338, 1881–1890.

Kaur, G., Satyanarayana, T., 2004. Production of extracellular pectinolytic, cellulolytic and xylanolytic enzymes by thermophilic mould *Sporotrichum thermophile* Apinis in solid state fermentation. Indian J. Biotechnol. 3, 552–557.

Mahanta, N., Gupta, A., Khare, S.K., 2008. Production of protease and lipase by solvent tolerant *Pseudomonas aeruginosa* PseA in solid-state fermentation using *Jatropha curcas* seed cake as substrate. Bioresour. Technol. 99, 1729–1735.

Maktouf, S., Kamoun, A., Moulis, C., Remaud-Simeon, M., Ghribi, D., Chaabouni, S.E., 2013. A new raw-starch-digesting α-amylase: production under solid-state fermentation on crude millet and biochemical characterization. J. Microbiol. Biotechnol. 23, 489–498.

Mala, J.G., Edwinoliver, N.G., Kamini, N.R., Puvanakrishnan, R., 2007. Mixed substrate solid state fermentation for production and extraction of lipase from *Aspergillus niger* MTCC 2594. J. Gen. Appl. Microbiol. 53, 247–253.

Maurya, D.P., Singh, D., Pratap, D., Maurya, J.P., 2012. Optimization of solid state fermentation conditions for the production of cellulase by *Trichoderma reesei*. J. Environ. Biol. 33, 5–8.

Mienda, B.S., Ahmad, I., Umar, I., 2011. Microbiological features of solid state fermentation and its applications – an overview. Res. Biotechnol. 2, 21–26.

Miller, L.G., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.

Moure, A., Gullon, P., Dominguez, H., Parajo, J.C., 2006. Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals: review. Process. Biochem. 41, 1913–1923.

Murugan, S., Arnold, D., Pongiya, U.D., Narayanan, P.M., 2011. Production of xylanase from *Arthrobacter* sp. MTCC 6915 Using Saw Dust as substrate under solid state fermentation. Enzyme Res. 2011, 1–9.

Ng, I.S., Li, C.W., Chan, S.P., Chir, J.L., Chen, P.T., Tong, C.G., Yu, S.M., Ho, T.H., 2010. High-level production of a thermoacidophilic beta-glucosidase from *Penicillium citrinum* YS40-5 by solid-state fermentation with rice bran. Bioresour. Technol. 101, 1310–1317.

Prakasham, R.S., Rao, Ch.S., Rao, R.S., Rajesham, S., Sarma, P.N., 2005. Optimization of alkaline protease production by *Bacillus* sp. using Taguchi methodology. Appl. Biochem. Biotechnol. 120, 133–144.

Rakshit, K.D., Darukeshwara, J., Rathina, R.K., Narasimhamurthy, K., Saibaba, P., Bhagya, S., 2008. Toxicity studies of detoxified Jatropha meal (*Jatropha curcas*) in rats. FoodChem. Toxicol. 46, 3621–3625.

Sachslehner, A., Nidetzky, B., Kulbe, K.D., Haltric, D., 1998. Induction of mannase, xylanase and endoglucanase activities in *Sclerotium rolfsii*. Appl. Environ. Microbiol. 64, 594–600.

Singh, B., Satyanarayana, T., 2006. Phytase production by thermophilic mold *Sporotrichum thermophile* in solid-state fermentation and its application in dephytinization of sesame oil cake. Appl. Biochem. Biotechnol. 133, 239–250.

Topakas, E., Stamatis, H., Biely, P., Christakopoulos, P., 2004. Purification and characterization of a type B feruloyl esterase (StFAE-A) from the thermophilic fungus *Sporotrichum thermophile*. Appl. Microbiol. Biotechnol. 63, 686–690.

Vafiadi, C., Christakopoulos, P., Topakas, E., 2010. Purification, characterization and mass spectrometric identification of two thermophilic xylanases from *Sporotrichum thermophile*. Process. Biochem. 45, 419–424.

Vázquez, M.J., Alonso, J.L., Domínguez, H., Parajó, J.C., 2000. Xylooligosaccharides: manufacture and applications. Trends. Food. Sci. Tech. 11, 387–393.

Vitcosque, G.L., Fonseca, R.F., Rodríguez-Zúñiga, U.F., Bertucci Neto, V., Couri, S., Farinas, C.S., 2012. Production of biomass-degrading multienzyme complexes under solid-state fermentation of soybean meal using a bioreactor. Enzyme Res. 2012, 1–9.

Zimbardi, A.L., Sehn, C., Meleiro, L.P., Souza, F.H., Masui, D.C., Nozawa, M.S., Guimarães, L.H., Jorge, J.A., Furriel, R.P., 2013. Optimization of β-glucosidase, β-xylosidase and xylanase production by *Colletotrichum graminicola* under solid-state fermentation and application in raw sugarcane trash saccharification. Int. J. Mol. Sci. 14, 2875–2902.