

Research Paper

Production of plant cell wall degrading enzymes by monoculture and co-culture of *Aspergillus niger* and *Aspergillus terreus* under SSF of banana peels

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Abstract

Filamentous fungi are considered to be the most important group of microorganisms for the production of plant cell wall degrading enzymes (CWDE), in solid state fermentations. In this study, two fungal strains *Aspergillus niger* MS23 and *Aspergillus terreus* MS105 were screened for plant CWDE such as amylase, pectinase, xylanase and cellulases (β -glucosidase, endoglucanase and filterpaperase) using a novel substrate, Banana Peels (BP) for SSF process. This is the first study, to the best of our knowledge, to use BP as SSF substrate for plant CWDE production by co-culture of fungal strains. The titers of pectinase were significantly improved in co-culture compared to mono-culture. Furthermore, the enzyme preparations obtained from monoculture and co-culture were used to study the hydrolysis of BP along with some crude and purified substrates. It was observed that the enzymatic hydrolysis of different crude and purified substrates accomplished after 26 h of incubation, where pectin was maximally hydrolyzed by the enzyme preparations of mono and co-culture. Along with purified substrates, crude materials were also proved to be efficiently degraded by the cocktail of the CWDE. These results demonstrated that banana peels may be a potential substrate in solid-state fermentation for the production of plant cell wall degrading enzymes to be used for improving various biotechnological and industrial processes.

Key words: solid state fermentation, plant cell wall degrading enzymes (CWDE), banana peels, *Aspergillus* strains, enzymatic hydrolysis.

Introduction

The chemical composition of plant cell wall comprises (approximately) 25% lignin and 75% carbohydrates or sugars (Smith, 1971). The carbohydrate fraction consists of cellulose, hemicelluloses (xylan *etc.*) and pectin, whereas the non-fermentable polyphenolic fraction is lignin, which acts as glue, holding together the cellulose fibers. The biomass provides a renewable chemical feedstock, which is degraded using a variety of physico-chemical methods. These treatment processes possess many disadvantages; therefore, biological conversion using microbial enzymes is a topic of intense research around the globe.

Currently, microbial enzymes that attack plant cell wall are widely exploited in the biotechnology sector. Mainly filamentous fungi are reportedly involved in the efficient degradation of plant cell wall as they utilize the com-

plex plant cell wall polysaccharides as a sole source of carbon by producing a wide range of hydrolases such as cellulases, hemicellulases and amylases. Due to the development of large-scale fermentation technologies, the production of microbial enzymes accounts for a significant proportion of the biotechnology industry's total output. Among the processes used for enzyme production, solid state fermentation (SSF) is favored because of a number of advantages. The process of SSF may be defined as the fermentation process of cultivation of microorganisms on a non-soluble material which is used as a source of nutrients as well as a physical support under controlled conditions without free-flowing aqueous phase (Pandey, 1992).

A variety of traditional (agricultural products) and non-traditional (agricultural and food processing wastes) substrates can be employed in SSF. Among such wastes, Banana peel (BP) is an under-utilized substrate. Banana is one of the major and highly consumed fruit, originated

from the tropical region of South Asia (Anhwange *et al.*, 2009). Because of the high use, banana waste *i.e.*, peels are generated in bulk and constitute up to 30-40% of the total fruit weight. Being perishable, its disposal is a matter of concern in order to alleviate environmental pollution. Since, this fruit residue contains carbohydrates, proteins, fibers in a significant amount and lignin content is in less quantity (Essien *et al.*, 2005), it can be used as a supplementary source for the production of industrial enzymes (Oberoi *et al.*, 2011).

The carbohydrates (CHO) which are present in huge quantities in BP include celluloses, hemicelluloses, starch and a major proportion of pectic contents. The highly rich CHO-content (Essien *et al.*, 2005) and some other basic minerals in BP provide the cheap source for the growth of microorganisms. Earlier, *Trichoderma viridae* has been reported to produce cellulase from BP under solid state fermentation (Sun *et al.*, 2011). BP as a solid medium in SSF, not only provides advantage of being cost effective for the biosynthesis of a number of industrial enzymes but also promotes substantial reduction of the agro-industrial pollution to the environment (Ponnuswamy *et al.*, 2011).

In the present work solid-state fermentation of BP is reported for the production of multienzyme preparation by mono- and co-culture of *Aspergillus* strains: *A. niger* and *A. terreus*. The cultures were previously reported for their ability to produce hydrolytic enzymes (Sohail *et al.*, 2009).

Materials and Methods

Organism and culture maintenance

Strains of *Aspergillus terreus* (MS105) and *A. niger* (MS23) were obtained from the culture collection of the Department of Microbiology, University of Karachi. The cultures were maintained on SDA slants, stored at 4 °C and sub-cultured periodically.

Substrate pretreatment

The natural substrate, Banana peels (BP), was collected from a local fruit juice shop and was used after solar drying, ground and sieving through 100 mesh sizes.

Preparation of spore suspension (Inoculum)

Fungal cultures maintained on Sabouraud Dextrose agar (SDA) slants at 4 °C, were transferred to SDA plates and incubated at 37 °C for seven days. The spores on the agar surface were gently scraped and blended in 10 mL sterile saline, vortexed and used as spore suspension (inoculum). The spores were enumerated under microscope suspended in a hemocytometer. The count was finally adjusted to 5×10^7 spores/mL.

Solid state fermentation

Solid-state fermentation (SSF) was carried out according to the modified Kang *et al.* (2004) method. Briefly,

2 g BP were autoclaved in a 100 mL Erlenmeyer flask at 121 °C for 30 min. To moist the solid substrate, an appropriate quantity (3.25 mL) of mineral salt medium (MSM); as prescribed by Mandels and Weber (1969), was added to maintain the desired moisture level (*i.e.* 65%). Each flask was then inoculated with spore suspension to obtain a final concentration of 5×10^7 spores/gm substrate, mixed and incubated at 28 °C or 37 °C and 90% humidity for one week under static condition.

Extraction and storage of crude enzymes

After incubation, 40 mL of 50 mM sodium citrate buffer pH 4.8; containing 0.05% (w/v) tween-80 was added to each flask and kept under agitation at 150 rpm for 2 h. It was followed by the separation of fungal biomass and culture medium residues using four layers of Whatman#1 filter paper. Filtrate was centrifuged at 6000 rpm for 20 min to obtain clear cell-free culture supernatant (CFCS). CFCS was used as crude enzyme preparation and stored at 4 °C until used.

Enzyme assays

Activities of xylanase, pectinase, amylase, β -glucosidase, endoglucanase and filterpaperase in CFCS were determined by protocol described by Sohail *et al.* (2009). Briefly, crude enzyme preparation (25 μ L) was incubated in the presence of 25 μ L substrate; birchwood xylan (0.5% w/v) for xylanase, citrus pectin (1% w/v) for pectinase, starch (0.5% w/v) for amylase, salicin (1% w/v) for β -glucosidase, carboxymethyl cellulose (1% w/v) for endoglucanase and Whatman # 1 filter paper strip (3 cm x 0.5 cm) for filterpaperase, in 50 mM Sodium citrate buffer, pH 4.8, at 50 °C for 30 min. Amount of reducing-sugar was determined by DNS method (Miller, 1969) against standard curves of xylose, galacturonic acid and glucose. One International Unit (IU) of enzyme was defined as the amount of enzyme that releases one micromole of reducing-sugar per minute under standard assay conditions.

Effect of supplementations on enzyme production

To analyze the effect of various inducers, BP was supplemented with xylan (0.5%), pectin (1%), starch (0.5%) and CMC (1%) for multi-enzyme production under the process of solid state fermentation.

Hydrolysis of crude and commercially available substrates

For hydrolysis, different crude substrates (*i.e.*, banana peels, lime peels, potato peels, sugar-cane bagasse, corn leaves) and purified substrates (*i.e.*, xylan, pectin, starch, avicel, CMC) were used. Crude substrates were treated as described for banana peels earlier. To the 100 mL Erlenmeyer flask, 0.5 g of substrate in 50 mM sodium citrate buffer pH 4.8 containing 0.02% sodium azide (antibacterial) and 5 IU of crude enzyme preparation were added,

incubated at 50 °C for 26 h with agitation at 150 rpm in an orbital shaker. Samples were taken periodically and amount of reducing sugars were measured by DNS method (Miller, 1969) against standard curve of glucose.

Results and Discussions

The present study was carried out using banana peels (BP) as a novel solid support for microbial anchorage for higher levels of enzyme production in SSF. Previously, BP was reported as a substrate for the production of cellulase by *Trichoderma* (Sun *et al.*, 2011), amylase by *Penicillium sp.* (Ponnuswamy *et al.*, 2011), laccase by *A. fumigatus* (Vivekanand *et al.*, 2011) under SSF processes. Since BP is enriched with carbohydrates, proteins, fats, dietary fibers and other basic nutrients (Essien *et al.*, 2005) that can support fungal growth, therefore, the potential of *Aspergillus* strains was evaluated for the production of plant cell wall degrading enzymes using this substrate as growth medium. To our knowledge this is the first report describing the production of multi-enzyme preparation by mono- and co-culture under SSF of BP.

Temperature is one of the important factors which strongly affect the SSF process (Pandey *et al.*, 2000). The cultivation temperature does not only affect the growth rate of organisms but it also has marked effects on the level of enzyme production. Therefore, this study was carried out at two different cultivation temperatures (*i.e.* 28 °C and 37 °C) in order to determine the optimum growth and enzyme production.

The strain MS23 gave maximum productivity of almost all the investigated enzymes in this work at 28 °C which decreased upon increasing the temperature up to 37 °C (Table 1). Haltrich *et al.* (1996) in their study on fungal xylanases reported that *A. niger* grew well at 28 °C and xylanase production was significantly decreased at higher temperature (40 °C). Sun *et al.* (2011) also reported about higher production of cellulases under SSF of banana peels at 30 °C compared to at 40 °C. It might be due to that at temperatures higher than optimum, the growth of the fungus was inhibited and hence the enzyme production (Yaun and Rungyu, 1999; Rahman *et al.*, 2003).

Whereas, the optimum temperature for the enzyme production by *A. terreus* was found to be 37 °C (Table 1)

that was in line with Naseeb (2007). Gawande *et al.* (1999) also reported enhanced productivity of xylanase at 35 °C by *A. terreus*.

The heat generated from the metabolic activities of the fungi, growing on the specific solid substrate, also influence the SSF process, together with the environmental temperature. Mrudula and Anitharaj (2011) reported maximum pectinase production on orange peels under SSF by *A. niger* at the cultivation temperature 50 °C. Moretti *et al.* (2012) also recorded that the maximum activities were observed at 60 °C for xylanase and 70 °C for CMCase from *A. fumigatus* M.7.1 using sugar-cane bagasse and wheat bran (1:1 w/w) as SSF substrate. But in our study, enhanced production of pectinase by *A. niger* was obtained at 28 °C using banana peels. This can be attributed towards different substrate composition used for SSF processes and different strains used for plant cell wall degradation.

Various workers have used another strategy to increase the industrially important enzymes under SSF; they supplemented the solid substrate with one or more nutritional component that may act as an enzyme inducer. For instance, Wang *et al.* (2012) used α -cellulose as a model substrate on corn cob to induce the cellulase production by *A. fumigatus* ECU0811. Enhanced production of amylase and xylanase was observed by Singh *et al.* (2012) using *Streptomyces sp.* MSC702, when the production medium was mixed with wheat bran and rice bran. Dhillon *et al.* (2012) also studied the effect of various inducers (lactose, CuSO₄, veratryl alcohol) on the rapid bioproduction of cellulase and hemicellulase using apple pomace as substrate. In the current study, BP was also supplemented with different carbon sources such as starch, birchwood xylan, citrus pectin and carboxymethyl cellulose (CMC) which resulted in the induction and repression of enzyme production in SSF. Almost all the carbon sources tested in this study positively enhanced the enzyme synthesis, proving BP a promising substrate for CWDE production.

The supplementation of xylan to BP enhanced the production of xylanase, pectinase and FPase by strain MS23 compared to the BP without supplementation (Table 2). Nonetheless, production of xylanase, amylase and β -glucosidase decreased upon similar supplementation by the strain MS105 (Table 3). Kang *et al.* (2004) has reported

Table 1 - Effect of temperature on enzyme production (IU/mL).

Culture	Temperature	Enzyme production (IU/mL)					
		Xylanase	Pectinase	Amylase	β -glucosidase	Endoglucanase	Filterpaperase
MS23	28 °C	0.702 ± 0.376	0.397 ± 0.198	0.226 ± 0.083	7.951 ± 3.740	3.91 ± 0.279	0 ± 0.00
	37 °C	0.688 ± 0.046	0.230 ± 0.020	0.148 ± 0.00	5.281 ± 0.730	2.32 ± 0.99	0.394 ± 0.041
MS105	28 °C	1.065 ± 0.493	0.512 ± 0.089	0.100 ± 0.140	1.470 ± 0.230	0.440 ± 0.120	0.093 ± 0.01
	37 °C	1.251 ± 0.042	0.482 ± 0.136	0.335 ± 0.230	1.186 ± 0.292	0.187 ± 0.153	0.144 ± 0.03

[Data represent the average of three independent experiments; each enzyme productivity was observed in triplicate ± SD].

the nature of the substrate used in fermentation affects the inducibility of cellulases and hemicellulases. Gupta and Kar (2008) reported that 0.5% increment of xylan to corn cob and wheat bran induced xylanase production by *Bacillus* sp.

In this study, the addition of CMC to BP resulted in enhanced production of endoglucanase and FPase by MS105 (Table 3). This finding is in agreement with Sohail *et al.* (2011) where presence of 1% (w/v) CMC in the medium increased the titers of FPase and endoglucanase by *Alternaria* sp. Aslam *et al.* (2010) has also mentioned the increase in the levels of endoglucanase when *Trichoderma* sp. grew in the presence of CMC indicating that it is the substrate of choice for endoglucanase production.

It is generally agreed that the optimum production of pectinase can be achieved in the medium containing pectic materials as an inducer (Hang and Woodanms, 1994; Solis-Pereira *et al.*, 1993; Naidu and Panda, 1998). Our study affirms this fact as the supplementation of citrus pectin to the banana peels resulted in substantial increase in pectinase production by both the strains tested (Tables 1 and 2). Furthermore, this substrate favored the production of other polysaccharide-degrading enzymes, as well, when supplemented.

On the other hand, starch supplementation to BP under SSF, decreased the yield of CWDE particularly pectinase by MS105. It could be attributed to the repression mechanism brought about by the degradation of starch into glucose by the activity of amylase in the medium. Similar results of catabolite repression of enzyme production by glucose, has been reported by Nandakumar *et al.* (1999) for *A. niger* CFTRI 1105 and Alva *et al.* (2007) for *Aspergillus* sp. JGI 12. In contrast to the finding for MS105, our studies on MS23 showed that starch increment significantly increased the enzyme production by this strain (Table 2), indicating a different genetic regulatory mechanism.

Co-culturing is another strategy to improve enzyme production by industrially important strains. Since the two strains investigated in this study showed variation in the

titers and proportion of different enzymes in their preparations, so these were co-cultured under SSF of BP. As the higher production of enzymes from MS23 was noted at 28 °C and from MS105 at 37 °C, therefore, the strains were co-cultured at these two temperatures and the productivity was compared with monocultures.

Results of co-culture studies demonstrated that pectinase was over-produced at 28 °C compared to the mono culture. The highest production of this enzyme by *Aspergillus* strains was observed when BP was supplemented with xylan at 28 °C (Table 4). Wang *et al.* (2006) also reported the increased production of cellulase, hemicellulase, glucoamylase and pectinase when mixed culture of *Aspergillus niger* was used under SSF process using bran as the main substrate. Elevated production of pectinases in the mixed culture is quite economical and of great commercial importance for various food industry applications; to increase the juice yield and its clarity, preventing haziness of wine in brewing industries, extraction of oil, fermentation of coffee and tea and in the preparation of animal feed (Jayani *et al.*, 2005; Gummadi *et al.*, 2003; Aguilar and Huitron, 1986). This mixed culture strategy not so effective in the production of other hydrolytic enzymes when compared with the findings of single strains production (Table 4 and 5), showing lower synergism among the two molds. Similar results were obtained by Massadeh *et al.* (2001) that the addition of *A. terreus* to *T. reesei* did not increase the reducing sugar concentration compared to single culture using sugarcane bagasse in solid substrate fermentation, showed that mixed culture resulted in a decrease in substrate degradation.

The fungal CWDE are frequently applied for the saccharification and hydrolysis of crude lignocellulosic substrates. The simple sugars released by saccharification process can then be utilized in a number of industrial and commercial processes. In the present study, the enzyme preparations obtained by SSF of BP were studied for their potential to degrade BP and other crude substrates along with some purified substrates.

Table 2 - Effect of supplementation of substrates in Banana peels containing mineral salt medium on enzyme production (IU/mL/min) by the strain MS23.

Substrate*	Enzyme production (IU/mL)					
	Xylanase	Pectinase	Amylase	β -glucosidase	Endoglucanase	Filterpaperase
Control	0.702 \pm 0.376	0.397 \pm 0.198	0.226 \pm 0.083	7.951 \pm 3.740	3.91 \pm 0.279	0 \pm 0.00
Xylan	1.909 \pm 0.330	3.828 \pm 0.720	0.358 ^a	3.89 ^a	4.042 \pm 0.638	0.822 \pm 0.05
Pectin	0.745 \pm 0.133	11.75 \pm 1.090	0.792 \pm 0.028	1.168 \pm 0.07	1.043 \pm 0.165	0.358 \pm 0.290
CMC	1.01 \pm 0.133	1.634 \pm 0.02	0.700 \pm 0.064	2.649 \pm 0.035	1.793 \pm 0.173	0.435 \pm 0.086
Starch	0.761 \pm 0.206	1.222 0.146	0.634 \pm 0.143	1.810 \pm 0.173	1.049 \pm 0.286	0.911 \pm 0.466

[Data represent the average of three independent experiments; each enzyme productivity was observed in triplicate \pm SD].

*The substrates were added at 0.5% xylan, 1% pectin, 1% CMC and 0.5% starch separately.

[^a]SD was insignificant.

Table 3 - Effect of supplementation of substrates in Banana peels containing mineral salt medium on enzyme production (IU/mL/min) by the strain MS105.

Substrate*	Enzyme production (IU/mL)					
	Xylanase	Pectinase	Amylase	β -glucosidase	Endoglucanase	Filterpaperase
Control	1.251 \pm 0.042	0.482 \pm 0.136	0.335 \pm 0.230	1.186 \pm 0.292	0.187 \pm 0.153	0.144 \pm 0.03
Xylan	0.378 \pm 0.226	0.464 \pm 0.09	0.093 \pm 0.062	0.588 \pm 0.253	0.526 \pm 0.102	0.306 \pm 0.00
Pectin	0.094 \pm 0.00	1.258 \pm 0.493	0.463 \pm 0.109	0.772 \pm 0.357	1.110 \pm 0.103	0.372 \pm 0.079
CMC	0.773 \pm 0.373	0.836 \pm 0.146	0.131 \pm 0.020	1.0 \pm 0.178	0.954 \pm 0.570	0.328 \pm 0.325
Starch	0.272 \pm 0.136	0 \pm 0.00	0.049 \pm 0.00	0.394 \pm 0.139	0.3813 \pm 0.237	0.259 \pm 0.100

[Data represent the average of three independent experiments; each enzyme productivity was observed in triplicate \pm SD].

*The substrates were added at 0.5% xylan, 1% pectin, 1% CMC and 0.5% starch separately.

Table 4 - Effect of supplementation of substrates in Banana peels containing mineral salt medium on enzyme production (IU/mL/min) by the strain MS23 and MS105 as co-culture at 28 °C.

Substrate*	Enzyme production (IU/mL)					
	Amylase	Xylanase	Pectinase [#]	Endoglucanase	β -glucosidase	Filterpaperase
Control	0.2166 \pm 0.186	0.335 \pm 0.167	0.69 \pm 0.12	0.748 \pm 0.396	0.318 \pm 0.184	8.35 \pm 0.444
Xylan	0.424 \pm 0.793	1.7 \pm 0.151	16.5 \pm 0.286	1.086 \pm 0.793	1.46 \pm 0.833	0.126 \pm 0.16
Pectin	0.039 \pm 0.686	1.06 \pm 0.626	0.37 \pm 0.196	1.5 \pm 0.825	1.226 \pm 0.65	0.0093 \pm 0.012
Starch	0.225 \pm 0.137	0.24 \pm 0.864	0.29 \pm 0.05	0.145 \pm 0.12	0.103 \pm 0.089	0.1133 \pm 0.10
CMC	0.305 \pm 0.300	0.586 \pm 0.524	0.484 \pm 0.352	0.97 \pm 0.586	0.945 \pm 0.549	0.1133 \pm 0.123

[Data represent the average of three independent experiments; each enzyme productivity was observed in triplicate \pm SD].

*The substrates were added at 0.5% xylan, 1% pectin, 1% CMC and 0.5% starch separately.

[#]The actual values of pectin will be obtained after multiplying it with 15.

Table 5 - Effect of supplementation of substrates in Banana peels containing mineral salt medium on enzyme production (IU/mL/min) by the strain MS23 and MS 105 as co-culture at 37 °C.

Substrate*	Enzyme production (IU/mL)					
	Amylase	Xylanase	Pectinase [#]	Endoglucanase	β -glucosidase	Filterpaperase
Control	0.786 \pm 0.46	0.29 \pm 0.148	5.15 \pm 0.138	0.6906 \pm 0.348	0.246 \pm 0.311	0.766 \pm 0.473
Xylan	0.186 \pm 0.166	0.24 \pm 0.175	0.28 \pm 0.03	0.273 \pm 0.168	0.42 \pm 0.384	0.394 \pm 0.106
Pectin	0.0713 \pm 0.044	0.1 \pm 0.085	0.12 \pm 0.102	0.146 \pm 0.084	0.0493 \pm 0.024	0.073 \pm 0.044
Starch	0.098 \pm 0.103	0.307 \pm 0.153	0.05 \pm 0.046	0.114 \pm 0.127	0.172 \pm 0.086	0.4126 \pm 0.211
CMC	0.1046 \pm 0.05	0.333 \pm 0.168	0.1 \pm 0.014	0.48 \pm 0.272	0.46 \pm 0.348	1.2 \pm 0.633

[Data represent the average of three independent experiments; each enzyme productivity was observed in triplicate \pm SD].

*The substrates were added at 0.5% xylan, 1% pectin, 1% CMC and 0.5% starch separately.

[#]The actual values of pectin will be obtained after multiplying it with 15.

Our studies revealed that pectin was maximally hydrolyzed after 26 h and produced a higher quantity of reducing sugars (Table 6) in the enzymatic preparation of both the fungal strains (mono and co-culture), attributing the highest activity of pectinase in these loadings.

Crude substrates took more time compared to purified substrates for efficient enzymatic hydrolysis. Although MS23 produced large amount of cellulases, but its enzyme preparation could not degrade xylan and CMC (Figure 1A)

to a greater extent. It may be due to the complex nature of their structures that prevent easy access of enzymes or the enzyme preparation did not contain the optimum ratio of cellulolytic and xylanolytic components to degrade these substrates.

It was also found that the enzyme produced by the strain MS23 was unable to act upon lime peels as no reducing activity was detected in the reaction mixture containing lime peels (Figure 1B). This inability of enzymes can be ex-

Table 6 - Enzymatic hydrolysis of crude and purified substrates.

Substrates		Rate of reducing sugar (g/L.h)			Total reducing sugar (g)		
		Coculture	MS105	MS23	Coculture	MS105	MS23
Purified substrates	Xylan	0.217	1.07	0	0.007	1.40	0
	Pectin	2.190	3.41	6	0.074	4.44	2.08
	Starch	0.296	0.5	0.93	0.010	0.64	1.22
	CMC	0.271	0.8	0	0.01	1.04	0
	Avicel	0.315	0.8	0.75	0.010	1.04	0.98
Crude substrates	Banana peels	0.907	1.9	0.8	0.38	2.46	1.04
	Sugar-cane bagasse	0.074	1.12	1.0	0.002	1.46	1.30
	Corn leaves	0.227	1.53	1.0	0.007	2.0	1.30
	Potato peels	1.118	1.93	0.5	0.1	2.52	0.64
	Lime Peels	1.151	1.63	0	0.28	2.12	0

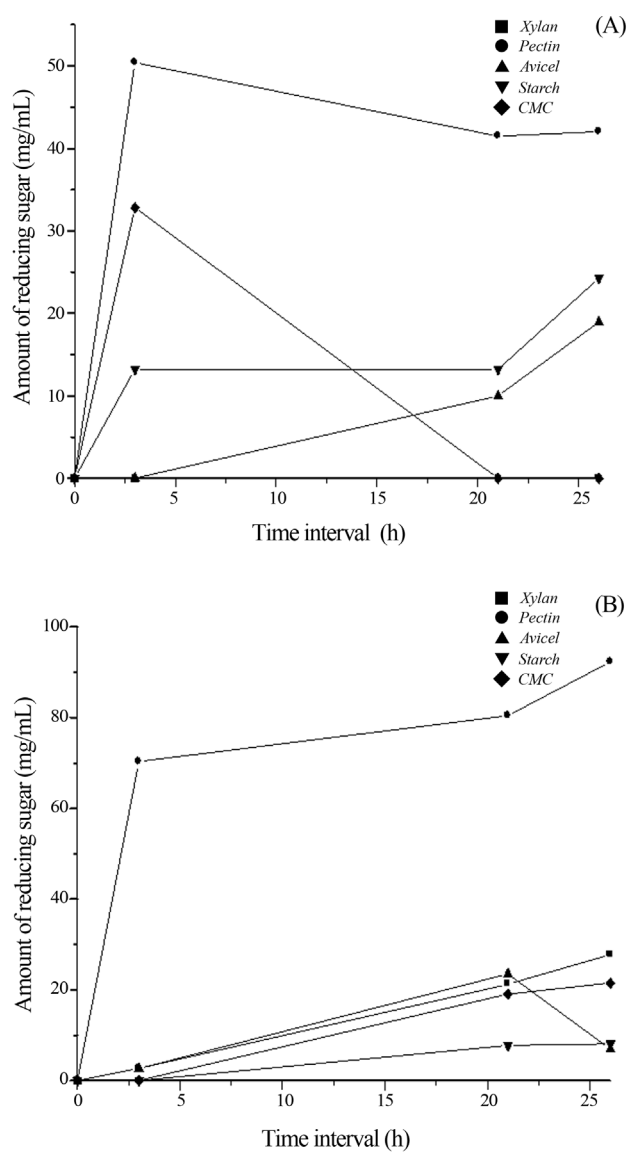


Figure 1 - Enzymatic hydrolysis of purified substrates by monocultures (A) MS23, (B) MS105.

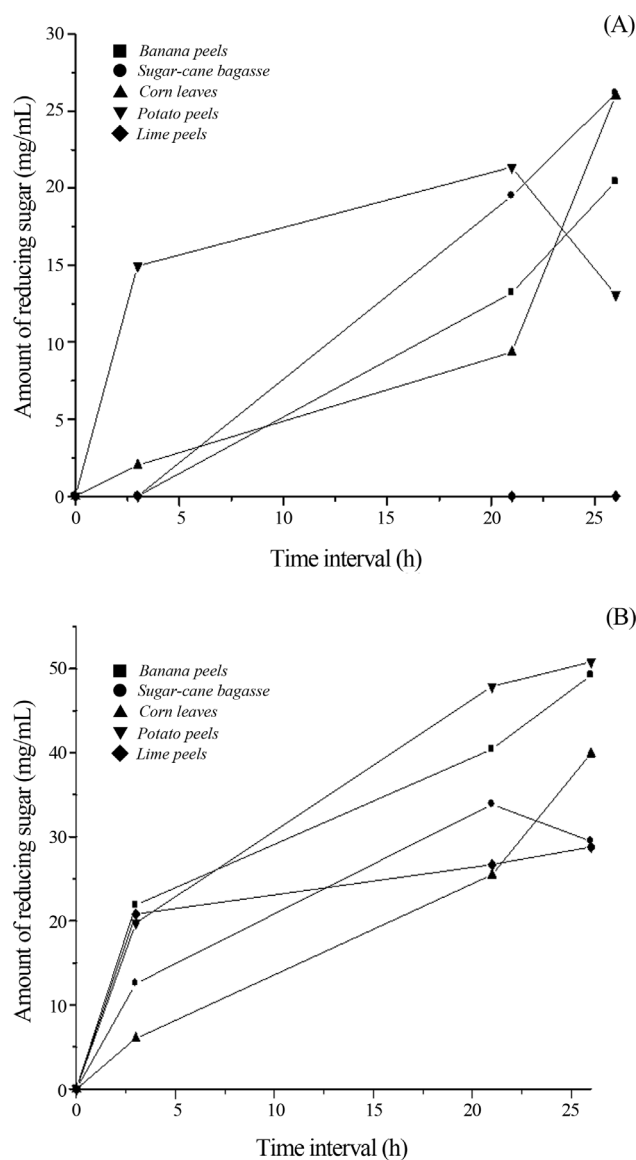


Figure 2 - Enzymatic hydrolysis of crude substrates by monocultures. (A) MS23, (B) MS105.

plained by taking the complex and heterogeneous structure of plant cell walls in consideration. The literature survey revealed that enzymes extracted from *A. niger* is much more sensitive towards the substrate structure in comparison to that extracted from *T. reesei* (Zuhair, 2008).

Contrary to above findings, the *Aspergillus sp.* MS105 hydrolyzed both types of substrate *i.e.* crude and purified, to a greater extent (Figures 2A and 2B). Although, crude substrates took longer time but produced more amount of reducing sugars than purified substrates (Table 6). It implies that the synergistic action of the plant cell wall degrading enzymes has been found to be most effective for hydrolysis of crude substrates but not for purified substrates.

Our co-culture data showed that the enzyme cocktail of MS23 and MS105 did not hydrolyze crude substrates efficiently in comparison to the enzyme preparation from the monocultures (Figures 3A and 3B). Amongst purified substrates, the hydrolysis of pectin yielded the maximum reducing sugars (0.074 g) while the hydrolysis of xylan contributed the least (0.007 g) (Table 6). As explained ear-

lier, it may be due to higher pectinolytic and low xylanolytic activities in enzyme loadings.

Conclusion

Production of plant cell wall degrading enzymes by the *Aspergillus* strains MS23 and MS105 under SSF of BP, suggests an extensive amount of CWDE in monocultures and higher degree of pectinases in co-culture. To strengthen the biotechnological industries, this could be an effective approach by ultimately purifying the increased enzymes from this cocktail. Overall, these results support the use of BP as an excellent low-cost, promising substrate for the induction of multi-enzymes production under SSF processes.

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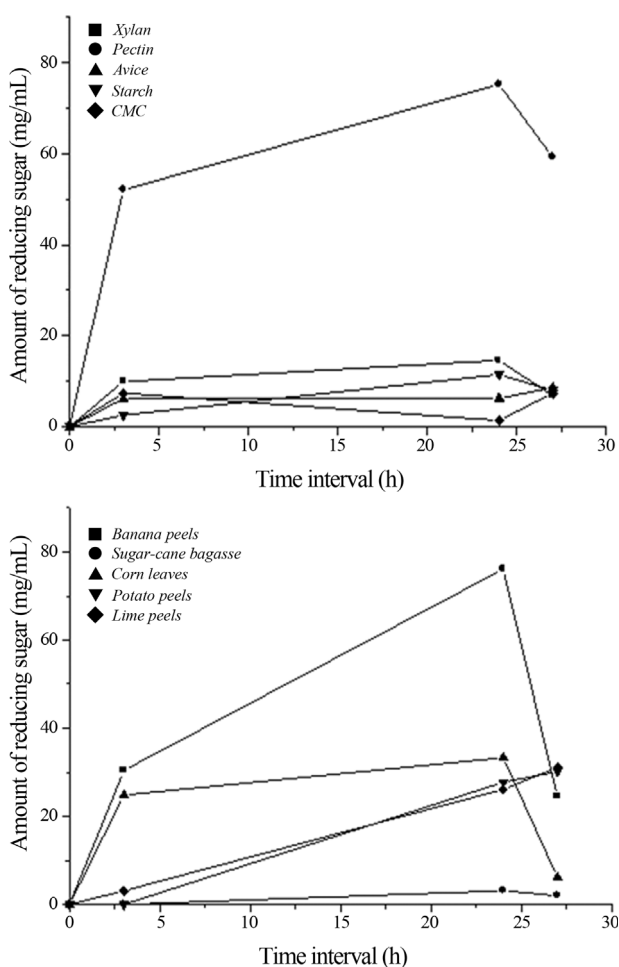


Figure 3 - Enzymatic hydrolysis by co-cultures (MS23+MS105). (A) Purified substrates, (B) Crude substrates.

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