



## Governing parameters on xylanase production using developed bioreactor

Chee Keong Lee<sup>\*</sup>, Darah Ibrahim<sup>1</sup> and Ibrahim Che Omar<sup>2</sup>

<sup>1</sup>Industrial Biotechnology Research laboratory, School of Biological Sciences,  
Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.

<sup>2</sup>Campus Jeli, Universiti Malaysia Kelantan, Beg Berkunci No 100,  
17600 Jeli, Kelantan, Malaysia.

Email: [cklee1311@yahoo.co.uk](mailto:cklee1311@yahoo.co.uk)

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### ABSTRACT

**Aims:** Chemicals bleaching are common steps in pulp and paper manufacturing process. The reduction of chemical use would result in lower waste treatment costs while minimizes the impact on the environment. This can be achieved using enzyme (xylanase). Compared to submerged fermentation process, the cost for enzyme production is much cheaper using solid state fermentation process (SSF). The present study aimed to examine factors affect xylanase production using developed SSF bioreactor.

**Methodology and results:** The performance of developed bioreactor namely FERMSOSTAT had been investigated in xylanase production studies. In this studies, local isolate *Aspergillus niger* USM AI 1 was used together with sugarcane bagasse and palm kernel cake (ratio 1:1), which serve as substrates for xylanase production. The highest xylanase activity and production of 397 U/g and 103 U/mg<sub>6</sub> glucosamine were obtained, respectively when the fermentation process was carried out under examined enzyme production conditions of 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h.g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min. On the other hand, compared to initial SSF conditions for xylanase production, xylanase activity increased 681% after all factors affect xylanase production have been examined.

**Conclusion, significance and impact of study:** The performance of the newly developed bioreactor is good since it can be used to produce xylanase with high title when compared with previous researcher findings.

**Keywords:** Solid state fermentation, xylanase, *Aspergillus niger*, sugarcane bagasse, palm kernel cake

### INTRODUCTION

Solid state fermentation (SSF) is an alternative for submerged fermentation in the production of biotechnology products such as enzymes, bioactive secondary metabolites, organic acids and so forth. Many researches have been carried out using SSF, in which this contribute to a wide range of applications not only at laboratory scale (Gupte and Madamwar, 1997; Gutierrez-Correa and Tengerdy, 1998; Hang and Woodams, 1998; Kotwal *et al.*, 1998; Sekar and Balaraman, 1998) but also at bigger scale (Durand and Chereau, 1988; Durand *et al.*, 1996; Xue *et al.*, 1992; Fernandez *et al.*, 1996). In addition, comparative studies between SSF and submerged (SmF) have claimed many advantageous for the former (Maldonado *et al.*, 1998), which include superior yields and higher concentration of products, simple fermentation equipment required, less effluent production and so forth (Pandey *et al.*, 2008).

The major factors that affect the SSF process include microorganism and substrate, substrate particle size and pre-treatment, moisture level, temperatures, aeration and

agitation as well as metabolic heat removal during the fermentation process. On the other hand, selection of an appropriate type of bioreactor is also crucial since different bioreactors have been classified into different groups based on different classification criteria. FERMSOSTAT is a horizontal stirrer drum fermenter and according to Mitchell *et al.* (2000; 2006) classification criterions, it was classified into group III, in which the bed in this bioreactor group is either intermittently or continuously mixed and the air is circulated around the bed but does not forcefully blown through the bed. The aim of this present work was to investigate the performance of developed bioreactor namely FERMSOSTAT in xylanase production studies using *Aspergillus niger* USM AI 1 grown on sugarcane bagasse and palm kernel cake as substrates.

### MATERIALS AND METHODS

#### Microorganism

In this study, local isolate, *Aspergillus niger* USM AI 1 was

\*Corresponding author

used and it was obtained from Industrial Biotechnology Research Laboratory (IBRL) culture collection, USM Penang. It was isolated from decayed wood obtained from the Northern Region of Peninsular Malaysia (Lee *et al.*, 2011).

### **Production of xylanase enzyme using bioreactor**

#### *Spore production and inoculums preparation*

Fungus cultivation for spore production was carried out according to Lee *et al.* (2011). In this method, fungus spore was obtained after 4 to 5 days growth on Potato Dextrose Agar under room temperature in medium bottles. The extraction of fungus spore was performed using distilled water containing 0.1% (w/v) Tween 80 (Smits *et al.*, 1996). The spore concentration was estimated by direct microscopic counting using hemocytometer (Raimbault and Alazard, 1980). The spore suspension at  $1 \times 10^8$  spore/mL [20% (v/w)] was mixed with the prior autoclaved growth medium. This medium was used as inoculums for SSF process.

#### *Substrates sterilization*

Palm kernel cake ( $\leq 0.5$  mm) and sugarcane bagasse ( $\leq 2$  mm) at 1:1 ratio were used as substrates for xylanase production. The substrates were manually mixed before being transferred into the FERMSOSTAT through the substrate port located on the top of the fermenter vessel. The substrate was pre-treated cum sterilized *in situ* by hot air sterilization at 130 °C for 3 h (Lonsane *et al.*, 1992a). After the sterilization process, the fermenter and substrates were allowed to cool down before inoculation of the substrates were carried out (Lee *et al.*, 2011).

#### *Medium composition*

The growth medium used in SSF process consisted of mineral salts and trace elements. The composition of the growth medium used was according to Pang *et al.* (2006). The pH of the medium was adjusted to 7.0 prior to autoclave. The pH of the growth medium was not controlled during the course of fermentation.

#### *Inoculation and sampling process*

The inoculation of growth medium containing spore and sampling process during the fermentation process was performed according to Lee *et al.* (2011). Air/water pressure type of spraying nozzles was used to inoculate solid substrate with growth medium and spore. Sampling process was carried out throughout the fermentation process. About 10 g of substrate was sampled out each time from three different sampling ports for every 24 h interval. The substrate was mixed for 5 min before the sampling process. The sample was used to determine the xylanase activity and fungus biomass.

#### *Enzymes extraction*

The enzyme extraction process was carried out according to Aikat and Bhattacharyya (2000), in which the fermented substrates was mixed with distilled water containing 0.1% (w/v) Tween 80. The mixture was stand still for 2 h under room temperature. The solid residue was then separated from the enzymic solution by filtration through Whatman 1 filter paper.

#### *Determination of xylanase activity*

Xylanase activity was determined according to the method described by Gessesse and Gashe (1997) using oat spelt xylan (Fluka, USA) dissolved in 50 mM citric-NaOH buffer as substrate. One unit of enzyme activity was defined as the amount of enzymes, which catalyzes the liberation of 1  $\mu$ mol of reducing sugar per minute under above specified assayed condition. Enzyme activity was expressed as unit (U) per g of fermented substrate, while enzyme production was expressed as unit (U) per mg of glucosamine content of the fungus growth. All assays were carried out in triplicates and the results were presented as the mean of the triplicates experiments.

#### *Determination of fungal growth*

The glucosamine content of fungus was used to determine the biomass of *A. niger* USM AI 1. The method used was as described by Swift (1972). The glucosamine was measured spectrophotometrically at 530 nm using glucosamine as standard.

#### *Xylanase production*

Factors affecting the growth of *A. niger* USM AI 1 and the production of xylanase were examined throughout this present study. The factors examined included amount of substrates, moisture content, incubation temperature, aeration rate and aeration time, mixing speed and mixing interval. The highest xylanase production condition obtained from each experiment was used in the following experiments unless otherwise stated.

#### *Comparison with Trichoderma reesei*

*Trichoderma reesei*, which is an established culture for cellulases and xylanases production was used as a benchmark for the production of cellulases and xylanases by *A. niger* USM AI 1 using developed bioreactor.

#### **Statistical method**

The significance of difference between each test variable were determined using one way ANOVA analysis and Least Significance Test, computed using SPSS version 11.5 software. All tests were done with a confidence interval of 95%.

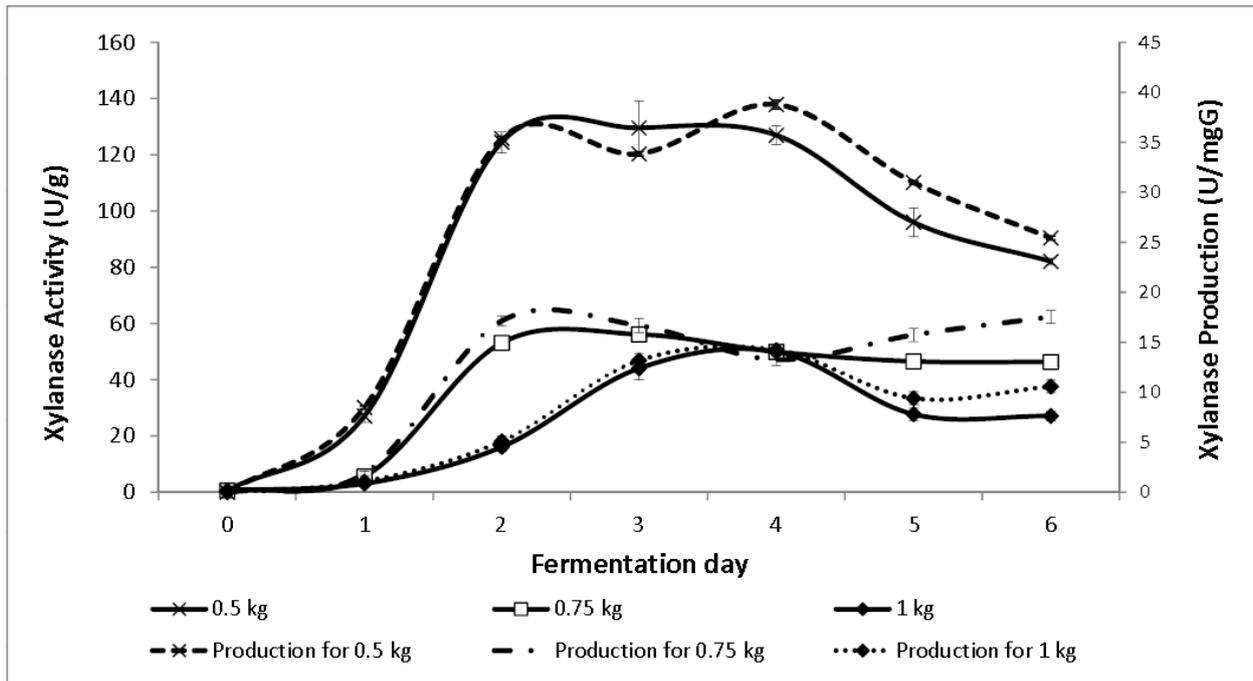
**RESULTS AND DISCUSSION**

**Effect of different amount of substrate on xylanase production**

The fermentation profiles for xylanase activity and production using different amount of substrate are shown in Figure 1. The results indicated that, significant difference ( $p < 0.05$ ) in xylanase activity was detected when 0.5 kg of substrate was used compared with the other amount of substrate used in fermentation process. Xylanase production increased with decreasing the used of different amounts of substrate in the fermentation process. About 61% drops in xylanase activity was detected when the amount of substrate used in the fermentation process was increased by 100% from 0.5 kg to 1.0 kg. The results obtained are in agreement with Muhammad *et al.* (2014) who obtained maximum xylanase activity of 65 U/g when 10 g of substrate was used compared to the lowest activity when 30 g of substrate was used in the study. The maximum level of xylanase activity was detected after 3 days of

fermentation process, when 0.5 kg and 0.75 kg of substrate were used compared with 4 days, when 1.0 kg of substrate was used in the fermentation process. The highest xylanase activity of 130 U/g and 38.7 U/mg<sub>G</sub> glucosamine of xylanase production were gained after 3 and 4 days of fermentation process using 0.5 kg of substrate, respectively. Only 6 U/g higher in xylanase activity was obtained although the amount substrate used in the fermentation process increased about 33% from 0.75 kg to 1.0 kg.

Significant reduction in xylanase production with an increasing used of amount of substrate in the fermentation process were probably due to different substrate depth. During sterilization, the chances of each substrate particle from thinner substrate depth expose to heat will be higher compared to thicker substrate depth. Substrate with higher exposure to heat during sterilization will be more amenable to microbial attack (Lonsane *et al.*, 1985) and consequently more easily to be degraded by *A. Niger* USM AI 1 in this study. Thus, more enzyme was produced per gram of substrate used.



**Note:** The SSF process was carried out at the indicated amount of substrate; 75% (w/w) moisture content; 28 °C; no aeration and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

**Figure 1:** Effect of different amount substrate on xylanase activity and production.

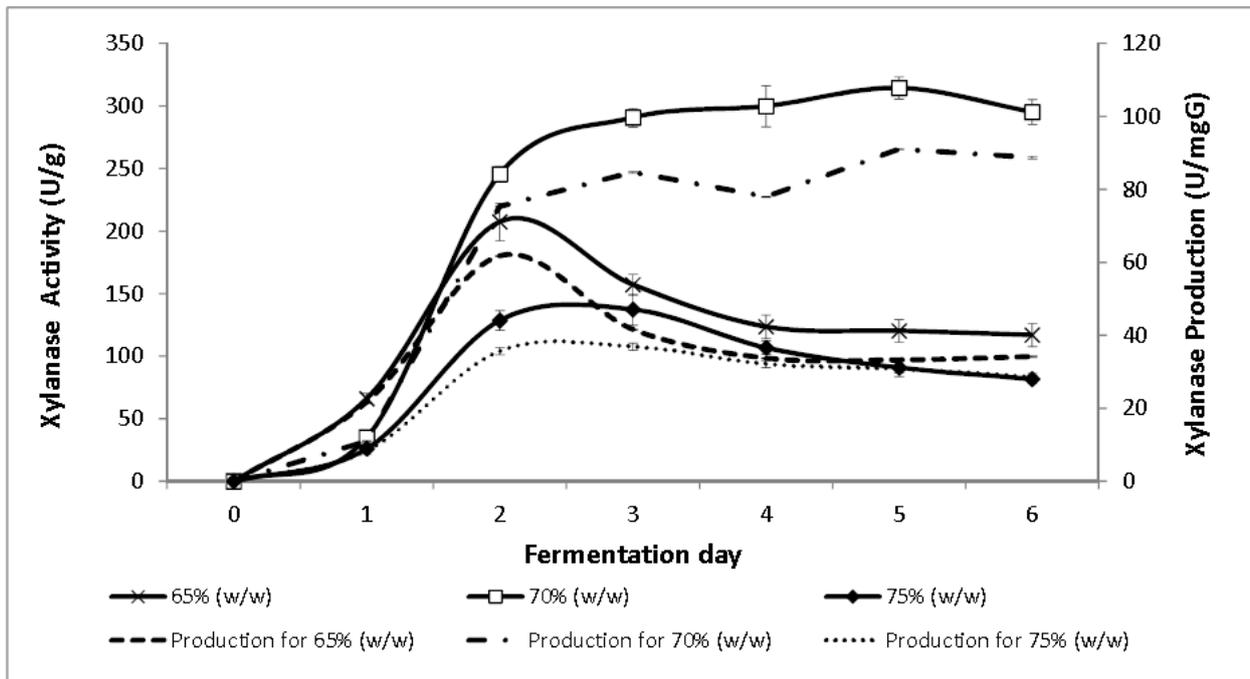
**Effect of different moisture content on xylanase production**

Figure 2 shows the xylanase activity and production profiles when different moisture contents were used in the fermentation process. The result indicated significant difference ( $p < 0.05$ ) in xylanase activity exhibited at different level of moisture contents used in the fermentation process. Although only 5% (w/w) differences in moisture content were used in the fermentation process, it caused about 56% drop in xylanase activity. This can be seen when 70% and 75% (w/w) of moisture content were used in the fermentation process. Highest xylanase activity of 314 U/g and xylanase production of 91 U/mg<sub>G</sub> glucosamine were obtained after 5 days of fermentation process using selected moisture content of 70% (w/w). However, these findings were different from Pangiotou *et al.* (2003) who reported highest xylanase activity at optimum moisture content of 80% (w/w) using *Fusarium oxysporum* grown on corn Stover. In addition, the highest xylanase enzyme production (64.3 ± 1.57 U/g) was observed when moisture content of 11:10 (liquid:solid) ratio was but further increased in moisture content resultant decreased in xylanase production (Muhammad *et al.*, 2014). On the other hand, according to Ranganathan and Vasanthi (2011), the highest xylanase (147.3 U/g dry medium) production was obtained when the ratio of paddy husk to liquid fermentation medium of 2:9 was used in SSF

process. As reported elsewhere, highest enzymes activities were obtained with initial moisture content of 80% (w/w) (Abdulah *et al.*, 1985; Dechamps *et al.*, 1985; Roussos *et al.*, 1991; Kalogeris *et al.*, 1998).

On the other hand, xylanase activity reaching maximum level of 207 U/g and 129 U/g after 2 days of fermentation process when 65% and 75% (w/w) of moisture content was used, respectively. Figure 2 also revealed that xylanase production dropped drastically after maximum production day when different moisture contents were used except for 65% moisture content. Xylanase production of 61.8 U/mg<sub>G</sub> and 35.7 U/mg<sub>G</sub> glucosamine were gained using 65% and 75% (w/w) moisture content, respectively.

The moisture content of the substrate is one of the critical factors influencing the outcome of SSF and is governed by the water-holding-capacity of the substrate, the type of end-product as well as the requirement of the microorganism. The inter particle mass transfer within the solid phase to the growing microorganism depends on the substrate characteristics and the moisture content (Krishna, 1999). In addition, increasing the moisture level above optimum can cause a reduction in enzyme yield due to steric hindrance of the growth of the producer strain by reduction the substrate porosity and alteration of substrate particle structure, thus limiting oxygen transfer (aeration) (Ramesh and Lonsane, 1990).



**Note:** The SSF process was carried out at the indicated moisture content, 0.5 kg substrate; 28 °C; no aeration and mixing for 5 min at 0.5rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

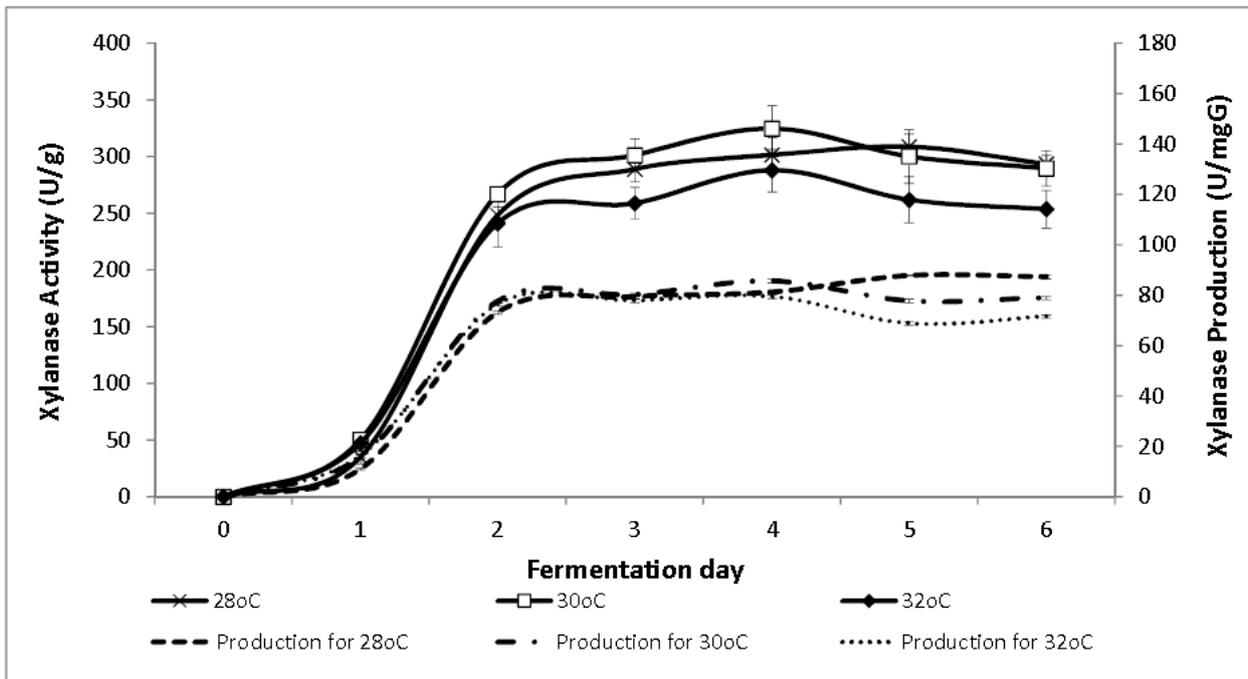
**Figure 2:** Effect of different moisture content on xylanase activity and production.

**Effect of different incubation temperature on xylanase production**

The enzyme production increased sharply after day 1 of fermentation and reaching maximum level after 4 to 5 days of fermentation process (Figure 3). Marginal decrease in xylanase activity detected after the optimum fermentation day showed that xylanase are less stable throughout the fermentation process. The highest xylanase activity of 325 U/g was detected when the fermentation process was carried out for 4 days under selected incubation temperature of 30 °C. However, statistical analysis demonstrated no significant difference ( $p>0.05$ ) in xylanase activity obtained under selected incubation temperature compared with the other incubation temperature used in the fermentation process. Similar result was reported by Shah and Madamwar (2005), who observed maximum xylanase activity when the SSF process was carried out under the optimum temperature of 30 °C for 4 days using *Aspergillus foetidus* MTCC 4898 grown on untreated corncobs moistened with modified Mandels and Strenberg medium. In addition, as reported by Muhammad *et al.* (2014), the highest xylanase activity ( $64.3\pm 1.3$  U/g) was detected when the

SSF process was carried at 30 °C using *Trichoderma viride*-IR05. On the other hand, Panagiotou *et al.* (2003) reported maximum production of xylanase under the optimum temperature of 27 °C but about 13% dropped at in production at 30 °C. While, Ncube *et al.* (2012) obtained optimum xylanase production at when *A. niger* was grown on *Jatropha curcas* seed cake at 25 °C in SSF.

As shown in the Figure 3 incubation temperatures showed no significant influence on xylanase production. This is probably because the temperatures used were only differences 2 °C that not really can shows relatively high differences in the enzyme produced. The incubation temperatures used in this research work were only in between 28 °C to 32 °C. This was because the fungus (*A. niger* USM AI 1) used in this fermentation process grew well within the temperatures used. Generally the temperature maintained in the SSF system is depends on the growth kinetics of the microorganism employed rather than on the enzyme produced. In addition, similar to this observation is the highest xylanase titers in fungal system have already been reported to happen generally at temperatures, which are optimum for the growth of cultures used in SSF (Biswas *et al.*, 1990).



**Note:** The SSF process was carried out under the indicated incubation temperature, 0.5 kg substrate; 70% (w/w) moisture content; no aeration and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

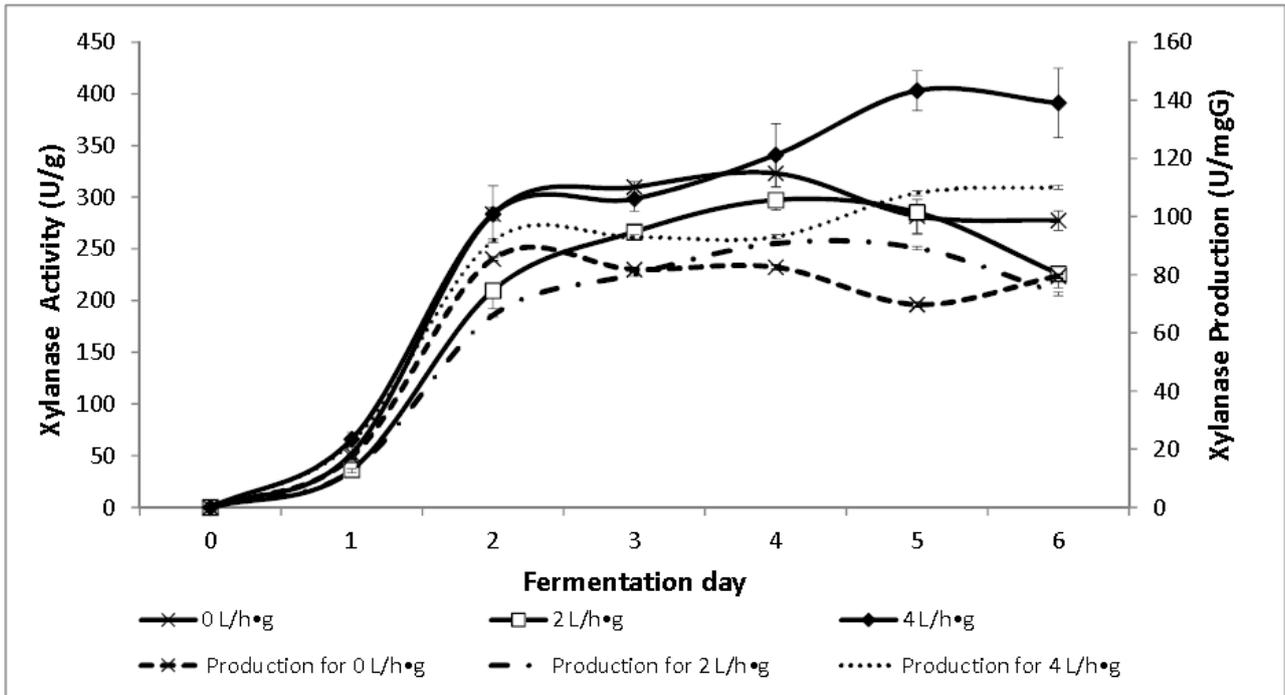
**Figure 3:** Effect of different incubation temperature on xylanase activity and production.

**Effect of different aeration rate on xylanase production**

The xylanase activity and production profiles under different aeration rates are shown in Figure 4. Significant difference ( $p < 0.05$ ) in xylanase activity was detected at aeration rate of 4 L/h.g fermented substrate compared with other aeration rate used during the fermentation process. For aeration at 4 L/h.g fermented substrate, the xylanase activity reaching maximum level after 5 days of fermentation process compared with 4 days when no aeration and aeration at 2 L/h.g fermented substrate were used. When aeration at 0 L/h.g, 2 L/h.g and 4 L/h.g fermented substrate were used during the fermentation process, their respective maximum xylanase activities detected were 323 U/g, 297 U/g and 403 U/g. On the other hand, as reported by Kalogeris *et al.* (2003), who found out that xylanase production was increased with increasing airflow rate up to 0.6 L/h.g. On the other

hand, Rodríguez-Fernández *et al.* (2011) reported that the highest xylanase activity of 65.38 U/g was obtained when the SSF process was carried out in a 2 kg bioreactor using *Aspergillus niger* F3 grown on citrus peel with airflow rate of 1 Vkg M. About 36% higher in enzyme activity was detected when the airflow rate used in the fermentation process increased from 2 L/h.g to 4 L/h.g fermented substrate. On the other hand, aeration at 2 L/h.g fermented substrate exhibited xylanase production of 90.7 U/mg<sub>G</sub> glucosamine after 4 days of fermentation process.

Based on the results obtained, it can be concluded that aeration significantly influence the xylanase production. The amount of aeration (air) required in SSF process are depends on many factors such as the product being produced, the substrate depth, metabolic activity of microorganism used and amount of heat to be removed and etc (Lonsane *et al.*, 1985).



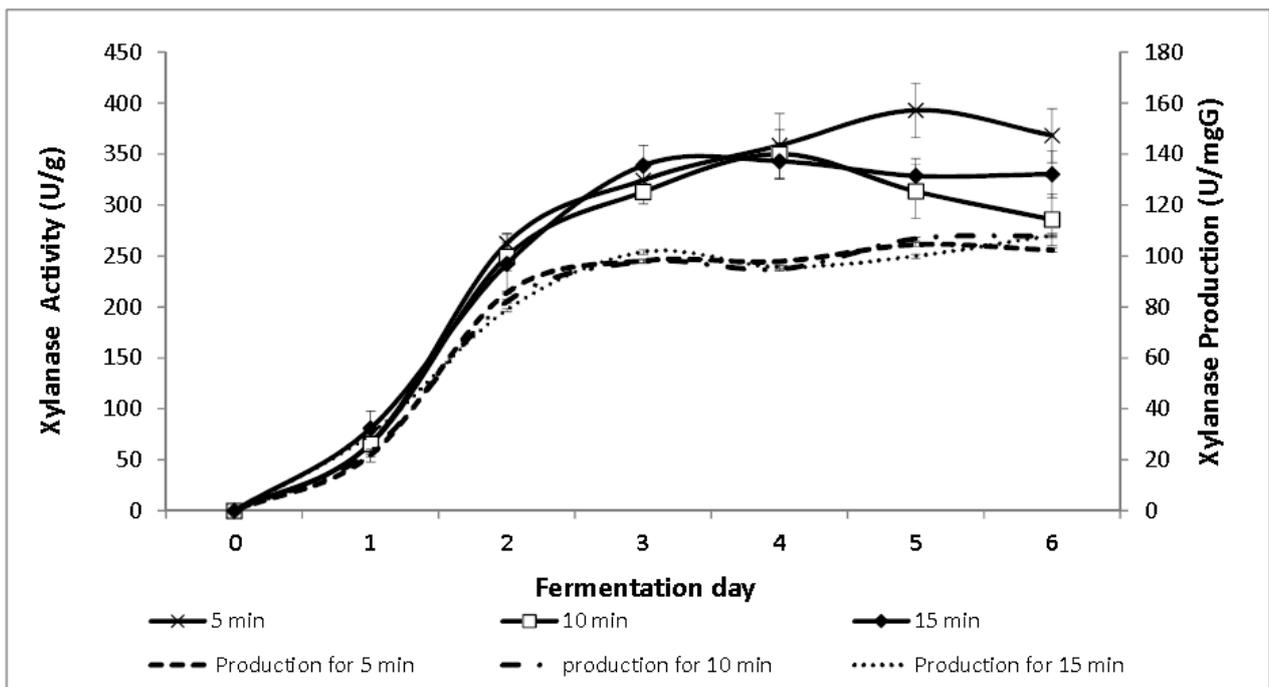
**Note:** The SSF process was carried out under the indicated aeration rate for 5 min; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

**Figure 4:** Effect of different aeration rate on xylanase activity and production.

**Effect of different aeration time on xylanase production**

As shown in Figure 5 xylanase activity decreased with increasing of aeration time used in the fermentation process. However, the decreased in xylanase activity was not significant ( $p>0.05$ ). On the other hand, longer aeration time used during the fermentation process resulted in shorter fermentation time required by the enzymes to reach maximum level. These can be seen from 5, 10 and 15 min of aeration times with their respective optimum fermentation days of 5, 4 and 3 days. Only 5 min of aeration time was enough for xylanase to achieve optimum activity of 393 U/g after 5 days of fermentation process. In addition, about 11% and 13.8% drop in the enzyme activity were detected when the aeration time used was increased from 5 min to 10 min and 15 min, respectively. About 38% declined in xylanase activity was detected when the aeration time used in the fermentation process was increased from 10 L/h.g to

15 L/h.g bagasse (Milagres *et al.*, 2004). While, according to Rosangela *et al.* (2013), the highest xylanase activity (746 U/g) was obtained when the SSF process was carried out in Erlenmeyer flask using *Aspergillus oryzae* (P6B2) grown on wheat bran with continuous aeration rate of 20 mL/min. On the other hand, 10 and 15 min of aeration time shared a similar highest xylanase production of 108 U/mg<sub>G</sub> glucosamine compared with 105 U/mg<sub>G</sub> glucosamine when 5 min of aeration time was used. As shown in Figures 4 and 5 higher aeration rate used in the fermentation process resulted in shorter time required for xylanase to achieve maximum level. This is mostly due to rapidly decreased moisture level with higher and longer air supply would occur (Milagres *et al.*, 2004).



**Note:** The SSF process was carried out under the indicated aeration time; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; 4 L/h.g fermented substrate of aeration rate and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

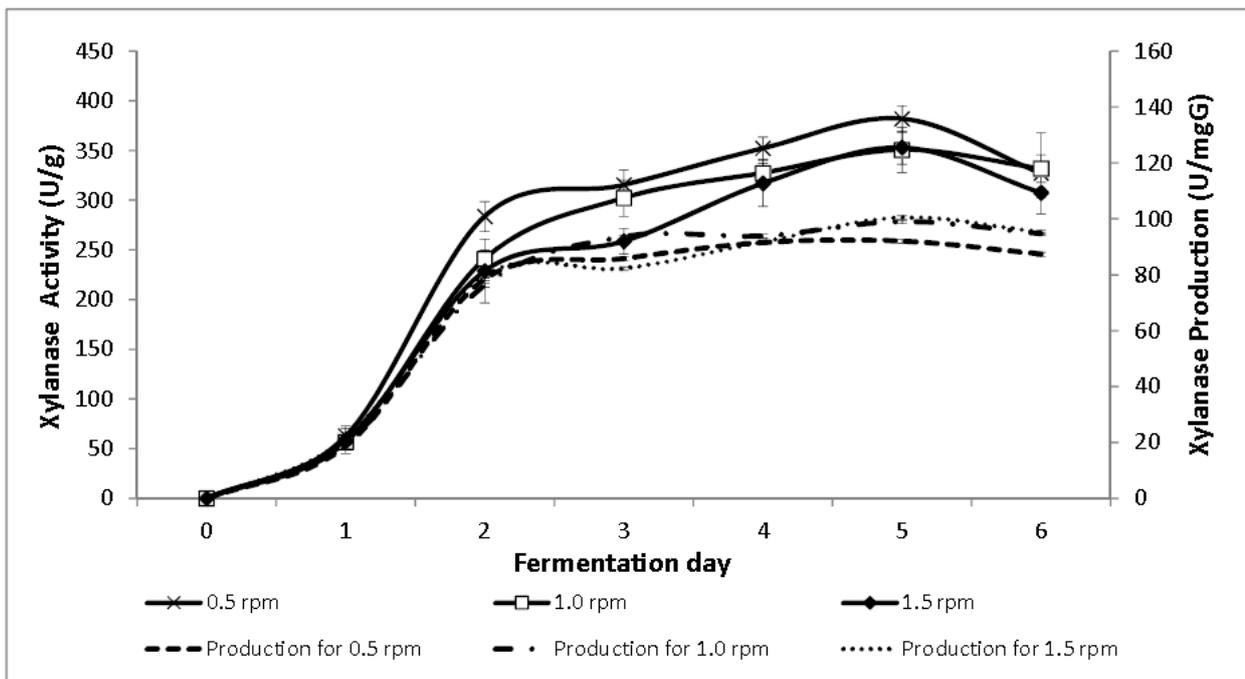
**Figure 5:** Effect of different aeration time on xylanase activity and production.

**Effect of different mixing speed on xylanase production**

Data presented in Figure 6 shows that xylanase activity required 5 days of fermentation time to reach maximum level regardless the difference in mixing speed used in the fermentation process. In addition, mixing at 1.0 and 1.5 rpm resulted no significant difference ( $p>0.05$ ) in the enzyme activity. This can be seen when only 3.6 U/g or 1% difference in xylanase activity was observed when 1.0 and 1.5 rpm of mixing speed were used during the fermentation process. The highest xylanase activity of 382 U/g was obtained when mixing at 0.5 rpm was used in the fermentation process with 103 U/mg<sub>G</sub> glucosamine of enzyme production. However, statistical analysis indicated no significant differences ( $p>0.05$ ). On the other hand, about 2973 U/g of xylanase activity was produced by *Thermoascus aurantiacus* in rotating drum bioreactor mixing at 10 rpm for 1 min at 3 h interval using wheat straw as carbon source (Kalogeris *et al.*, 1999). In addition, about 12% decreased in the enzyme production was

detected after the optimum enzyme production days using 1.5 rpm of mixing speed compared to only about 6% when lower mixing speed was used.

Mixing is important in SSF process as it ensures homogeneity with respect to temperature and gaseous environment (Banks, 1984; Trilli, 1986). In addition, mixing also can promote mass and heat transfers as well as uniform distribution of the nutrients added during the course of fermentation (Lonsane *et al.*, 1992a). The requirement of mixing in SSF system is depend on the type of process, reactor design and the product concerned. Xylanase activity achieved maximum level after 5 days of fermentation process regardless the different mixing rate was used. No great effect of different mixing speed on xylanase production was detected in this study. This probably due to narrow range (0.5 rpm interval) of mixing speed used.



**Note:** The SSF process was carried out under the indicated mixing speed; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h-g fermented substrate for 5 min and mixing for 5 min for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

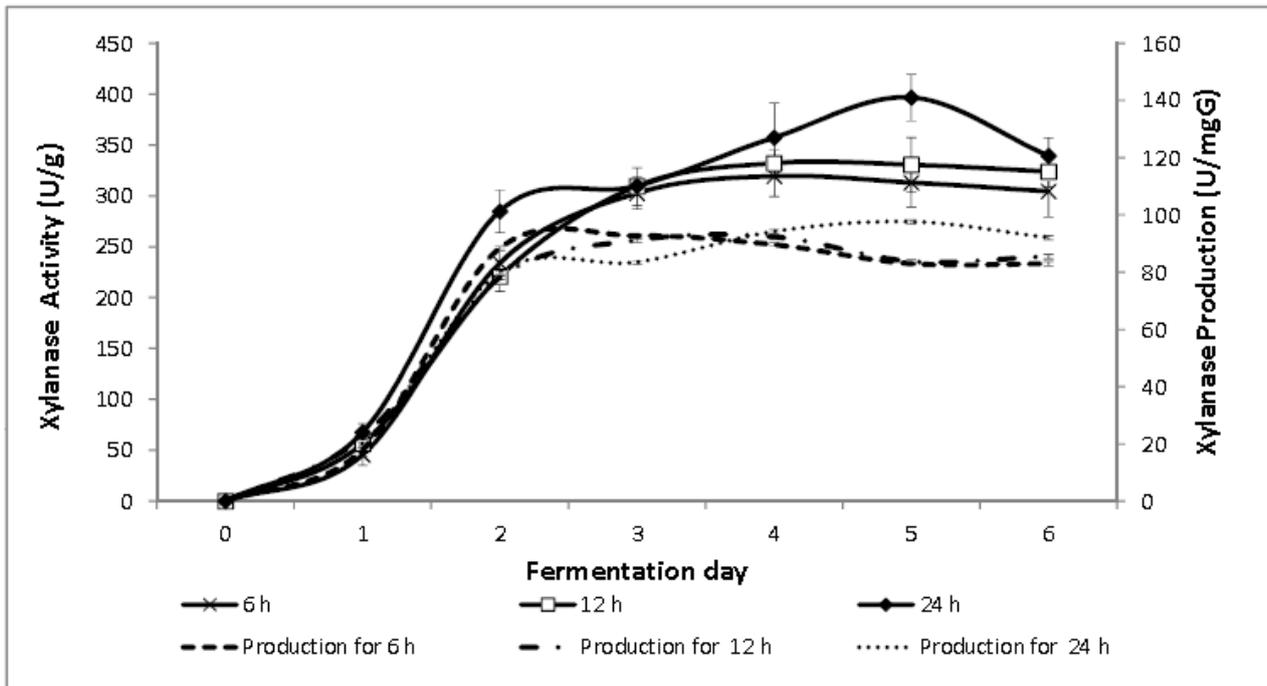
**Figure 6:** Effect of different mixing speed on xylanase activity and production.

**Effect of different mixing interval on xylanase production**

As shown in the Figure 7, xylanase activity increased rapidly from day 1 of fermentation process, reaching the maximum level after 4 days of fermentation process when mixing at every 6 h and 12 h interval were used compared with 5 days when mixing at every 24 h interval was used during the fermentation process. In addition, the enzyme produced decreased with the increased in mixing interval used during the fermentation process. The highest xylanase activity of 397 U/g obtained under selected mixing interval (24 h interval) was significant difference ( $p < 0.05$ ) compared with mixing at every 12 h and 6 h interval. At the same time, about 20% dropped in enzyme activity was detected when the mixing interval used during the fermentation process was increased from 24 h to 6 h interval. On the other hand, xylanase production studies indicated that highest xylanase production of 103 U/mg<sub>G</sub> glucosamine was observed when mixing interval of every 24 h interval was used during the fermentation

process compared with about 97.8 U/mg<sub>G</sub> glucosamine when mixing interval of every 12 h was used.

There has not been any clear principle behind the extent of mixing of the substrate. While most of the researchers have used a low mixing speed (1-15 rpm), some have used high rates of rotation. Different results have been reported by different authors (Silman, 1980; Kargi and Curme, 1985; Kumar and Lonsane, 1987, 1988; Mitchell *et al.*, 1988; Lonsane *et al.*, 1992b). Therefore, it is necessary to study agitation and analyze its effects particularly for a determinate process. The result obtained in this study indicated that higher mixing interval resulted in a declined in enzymes activity. This probably due to problems related to disruption of fungal attachment to the solids substrates and damage to fungal mycelial due to shear forces (Lonsane *et al.*, 1992a).



**Note:** The SSF process was carried out under the indicated mixing interval; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h-g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min. Arrow bars indicate means with standard error of three replicates.

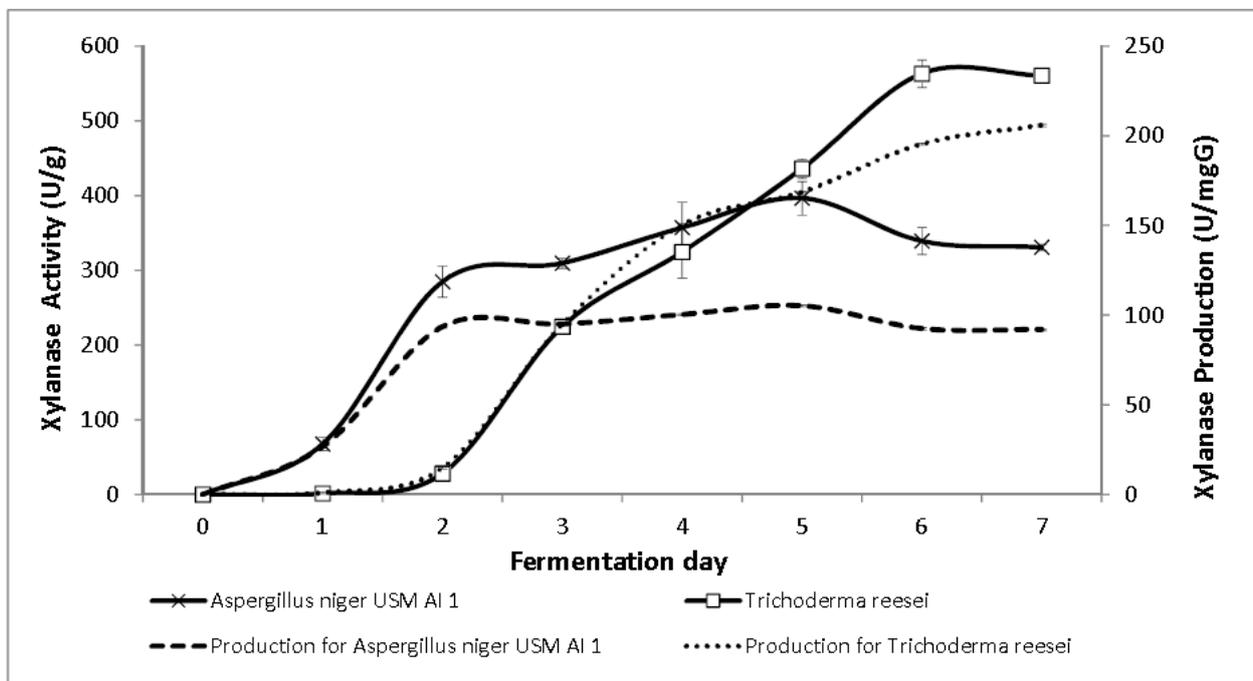
**Figure 7:** Effect of different mixing interval on xylanase activity and production.

**Comparison of xylanase production with *T. reesei***

The result indicated that xylanase produced by *T. reesei* increased rapidly after 2 days of fermentation process and reaching maximum level after 6 days of fermentation process (Figure 8). While, xylanase produced by *A. niger* USM AI 1 increased marginally after 1 day of fermentation process and achieved maximum level after 5 days of fermentation process. However, the result obtained was in disagreement to Shah and Madamwar (2005), who reported maximum xylanase production after 4 days of fermentation process under optimized conditions. In addition, significant differences ( $p < 0.05$ ) in xylanase activity produced by both fungi were observed. This can be seen that highest xylanase activity of 563 U/g was produced by *T. reesei*, which was about 44% higher

compared with xylanase produced by *A. niger* USM AI 1 (391 U/g). On the other hand, the enzyme production of about 204 U/mg<sub>G</sub> glucosamine was obtained by *T. reesei*, which was about 2 fold higher compared with xylanase production (102 U/mg<sub>G</sub> glucosamine) obtained by *A. niger* USM AI 1.

It can be concluded that, enzyme produced by *A. niger* USM AI 1 required shorter fermentation days to achieve maximum level compared to enzymes produced by *T. reesei*. These can be seen when enzymes produced by *T. reesei* reaching maximum level after 6 days of fermentation process compared to 4-5 days when the same enzymes were produced by *A. niger* USM AI 1. This may probably due to slow growth of *T. reesei* compared to *A. niger* USM AI 1.



**Note:** The SSF process was carried out under the indicated fungus; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h-g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min. Arrow bars indicate means with standard error of three replicates.

**Figure 8:** Production of xylanase enzyme by *A. niger* USM AI 1 and *T. reesei* under optimized fermentation conditions.

**CONCLUSION**

It can be concluded that about 50 U/g of xylanase activity was detected at the initial stage of SSF process and the enzyme production increased 681% to 390.8 U/g after all the factors affect the enzyme production had been examined. On the other hand, xylanase produced by *T. reesei* was about 44% higher compared with xylanase produced by *A. niger* USM AI 1. Furthermore, this shows that this newly designed solid state bioreactor

FERMSOSTAT, can be used to produce xylanase with high titer and the results obtained were comparable with previous researcher findings reported elsewhere.

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## REFERENCES

- Abdulah, A. L., Tengerdy, R. P. and Murphy, V. G. (1985). Optimization of solid substrate fermentation of wheat straw. *Biotechnology and Bioengineering*, **5**(27), 20-27.
- Aikat, K. and Bhattacharyya, B. C. (2000). Protease extraction in solid state fermentation of wheat bran by a local strain of *Rhizopus oryzae* and growth studies by the soft gel technique. *Process Biochemistry* **35**, 907-914.
- Banks, G. T. (1984). Scale up of fermentation processes. In: Topics in Enzyme and Fermentation Biotechnology. Wiseman, A. (ed). Ellis Horwood Limited, Chichester. pp. 170-266.
- Biswas, S. R., Jana, S. C., Mishra, A. K. and Nanda, G. (1990). Production, purification and characterization of xylanase from a hyperxylanolytic mutant of *Aspergillus ochraceus*. *Biotechnology and Bioengineering* **35**, 244-251.
- Dechamps, F., Giuliano, C., Asther, M., Huet, M. C. and Roussos, S. (1985). Cellulase production by *Trichoderma harzianum* in static and mixed solid state fermentation reactors under nonaseptic conditions. *Biotechnology and Bioengineering* **27**, 1385-1388.
- Durand, A. and Chereau, D. (1988). A new pilot reactor for solid-state fermentation: Application to the protein enrichment of sugar beet pulp. *Biotechnology and Bioengineering* **31**, 476-487.
- Durand, A., Reynaud, R., Maratray, J., Almanza, S. and Diez, M. (1996). INRA-Dijon reactors for solid-state fermentation: Designs and applications. *Journal of Scientific Industrial Research* **55**, 317-332.
- Fernandez, M., Perez-Correa, J. R., Solar, I. and Agosin, E. (1996). Automation of a solid substrate cultivation pilot reactor. *Bioprocess Engineering* **16**, 1-4.
- Gessesse, A. and Gashe, B. A. (1997). Production of alkaline xylanases by an alkaliphilic *Bacillus* sp. isolated from an alkaline soda lake. *Journal of Applied Microbiology* **83**, 402-406.
- Gupte, A. and Madamwar, D. (1997). Solid state fermentation of lignocellulosic waste for cellulase and beta-glucosidase production by co-cultivation of *Aspergillus ellipticus* and *Aspergillus fumigatus*. *Biotechnology Progress* **13**, 166-169.
- Gutierrez-Correa, M. and Tengerdy, R. P. (1998). Xylanase production by fungal mixed culture solid substrate fermentation on sugar cane bagasse. *Biotechnology Letter* **20**, 45-47.
- Hang, Y. D. and Woodams, E. E. (1998). Production of citric acid from corncobs by *Aspergillus niger*. *Bioresource Technology* **65**, 251-253.
- Kalogeris, E., Christakopoulos, P., Kekos, D., Macris, B. J. (1998). Studies on the solid state production of the most stable endoxylanases from *Thermoascus aurantiacus*. Characterization of two isoenzymes. *Journal of Biotechnology* **60**, 155-163.
- Kalogeris, E., Fountoukides, G., Kekos, D. and Macris, B. J. (1999). Design of a solid state bioreactor for thermophilic microorganisms. *Bioresource Technology* **67**, 313-315.
- Kalogeris, E., Iniotaki, F., Topakas, E., Christakopoulos, P., Kekos, D. and Macris, B. J. (2003). Performance of an intermittent agitation rotating drum type bioreactor for solid state fermentation of wheat straw. *Bioresource Technology* **86**, 207-213.
- Kapilan, R. and Arasaratnam, V. (2011). Paddy husk as support for solid state fermentation to produce xylanase from *Bacillus pumilus*. *Rice Science* **18**(1), 36-45.
- Kargi, F. and Curme, J. A. (1985). Solid state fermentation of sweet sorghum to ethanol in a rotary drum fermenter. *Biotechnology and Bioengineering Journal* **27**, 1122-1125.
- Kotwal, S. M., Gote, M. M., Sainkar, S. R., Khan, M. I. and Khire, J. M. (1998). Production of alpha-galactosidase by thermophilic fungus *Humicola* sp. in solid state fermentation and its application in soya milk hydrolysis. *Process Biochemistry* **33**, 337-343.
- Krishna, C. (1999). Production of bacterial cellulases by solid state bioprocessing of banana wastes. *Bioresource Technology* **69**, 231-239.
- Kumar, P. K. R. and Lonsane, B. K. (1987). Potential of fed batch culture in solid state fermentation for production of gibberellic acid. *Biotechnology Letter* **9**, 179-182.
- Kumar, P. K. R. and Lonsane, B. K. (1988). Batch and fed batch solid state fermentation: Kinetics of cell growth, hydrolytic enzymes production and gibberellic acid production. *Process Biochemistry* **23**, 43-47.
- Lee C. K., Darah, I. and Ibrahim, C. O. (2011). Production and optimization of cellulase enzyme using *Aspergillus niger* USM AI 1 and comparison with *Trichoderma reesei* via solid state fermentation system. *Biotechnology Research International: Volume 2011. Article ID 658493. doi:10.4061/2011/658493.*
- Lonsane, B. K., Durand, A., Renaud, R., Almanza, S., Maratray, J., Desgranges, C., Crooke, P. S., Hong, K., Maloney, C. W. and Tanner, R. D. (1992a). Product leaching and downstream processing. In: Solid Substrate Cultivation. Doelle, H. W., Mitchell, D. A. and Rolz, C. W. (eds). Elsevier Science Publishers, London. pp. 147-171.
- Lonsane, B. K., Saucedo-Castaneda, G., Raimbault, M., Roussos, S., Viniestra-Gonzalez, G., Ghildyal, N. P., Ramakrishna, M. and Krishnaiah, M. M. (1992b). Scale up strategies for solid state fermentation systems. *Process Biochemistry* **27**, 259-273.
- Lonsane, B. K., Ghildyal, N. P., Budiatman, S. and Ramakrishna, S. V. (1985). Engineering aspects of solid state fermentation. *Enzyme and Microbial Technology* **7**, 258-265.
- Maldonado, de Saad., Maldonado, M. C. and de Saad, A. M. (1998). Production of pectinesterase and polygalacturonase by *Aspergillus niger* in submerged

- and solid-state systems. *Journal of Industrial Microbiology and Biotechnology* **20**, 34–38.
- Milagres, A. M. F., Santos, E., Piovan, T. and Roberto, I. C. (2004).** Production of xylanase by *Thermoascus aurantiacus* from sugar cane bagasse in an aerated growth fermentor. *Process Biochemistry* **39**, 1387-1391.
- Mitchell, D. A., Doelle, H. W. and Greenfield, P. F. (1988).** Improvement of growth of *Rhizopus oligosporus* on a model solid substrate. *Biotechnology Letter* **18**, 497-501.
- Mitchell, D. A., Krieger, N., Stuart, D. M. and Pandey, A. (2000).** New developments in solid state fermentation II. Rational approaches to the design, operation and scale up of bioreactors. *Process Biochemistry* **35**, 1211-1225.
- Mitchell, D. A., Meien, O. F. V., Luz, Jr. L. F. L. and Berovic, M. (2006).** The scale up challenge for SSF Bioreactors. In: Solid State Bioreactors: Fundamentals of Design and Operation. Mitchell, D. A., Krieger, N. and Berovi, M. (eds). Springer-Verlag, Berlin Heidelberg, Germany. pp. 57-64.
- Muhammad, I., Muhammad, N. and Quratulain, S. (2014).** One-factor-at-a-time (OFAT) optimization of xylanase production from *Trichoderma viride*-IR05 in solid-state fermentation. *Journal of Radiation Research and Applied Sciences* **7**, 317-326.
- Ncube, T., Howard, R. L., Abotsi, E. K., Jansen van Rensburg, E. L. and Ncube, I. (2012).** Jatropha curcas seed cake as substrate for production of xylanase and cellulase by *Aspergillus niger* FGSCA733 in solid-state fermentation. *Industrial Crops and Products* **37**, 118-123.
- Panagiotou, G., Kekos, D., Macris, B. J. and Christakopoulos, P. (2003).** Production of cellulolytic and xylanolytic enzymes by *Fusarium oxysporum* grown on corn stover in solid state fermentation. *Industrial Crops and Products* **18**, 37-45.
- Pandey, A., Soccol, C. R. and Larroche, C. (2008).** General consideration about solid state fermentation process. In: Current Developments in Solid State Fermentation. Pandey, A., Soccol, C.R. and Larroche, C. (eds). Springer Science Business Media, New York, USA. pp. 13-25.
- Pang, P. K., Darah, I., Poppe, L. Szakacs, G. and Ibrahim, C. O. (2006).** Xylanase production by local isolate, *Trichoderma* sp. FETL C3-2 via solid-state fermentation using agricultural wastes as substrates. *Malaysian Journal of Microbiology* **2**, 7-14.
- Pirota, R. D. P. B., Tonelotto, M., Delabona, P. D. S., Fonseca, R. F., Paixão, D. A. A., Baleeiro, F. C. F., Neto, V. B. and Farinas, C.S. (2013).** Enhancing xylanases production by a new Amazon forest strain of *Aspergillus oryzae* using solid-state fermentation under controlled operation conditions. *Industrial Crops and Products* **45**, 465- 471.
- Raimbault, M. and Alazard, D. (1980).** Culture method to study fungal growth in solid fermentation. *European Journal of Applied Microbiology and Biotechnology* **9**, 199-209.
- Ramesh, M. V. and Lonsane, B. K. (1990).** Critical importance of moisture content of the medium in alpha-amylase production by *Bacillus licheniformis* M27 in a solid state fermentation system. *Applied Microbiology and Biotechnology* **3**, 201-205.
- Rodríguez-Fernández, D. E., Rodríguez-León, J. A. de Carvalho, J. C., Sturm, W. and Soccol, C. R. (2011).** The behavior of kinetic parameters in production of pectinase and xylanase by solid-state fermentation. *Bioresource Technology* **102**, 10657-10662.
- Roussos, S., Raimbault, M., Saucedo-Castaneda, G., Viniegra-Gonzalez, G. and Lonsane, B. K. (1991).** Kinetics and ratios of carboxymethyl cellulase and filter paper activities of the cellulolytic enzymes produced by *Trichoderma harzianum* on different substrates in solid state fermentation. *Micologia Neotropical Aplicada* **4**, 19-40.
- Sekar, C. and Balaraman, K. (1998).** Optimization studies on the production of cyclosporin A by solid state fermentation. *Bioprocess Engineering* **18**, 293-296.
- Shah, A. R. and Madamwar, D. (2005).** Xylanase production under solid state fermentation and its characterization by an isolated strain *Aspergillus foetidus* in India. *World Journal of Microbiology and Biotechnology* **21**, 233-243.
- Silman, R. W. (1980).** Enzyme formation during solid substrate fermentation in rotating vessels. *Biotechnology and Bioengineering Journal* **22**, 411-420.
- Smits, J. P., Rinzema, A., Tramper, J., van Sonsbeek, H. M. and Knol, W. (1996).** Solid-state fermentation of wheat bran by *Trichoderma reesei* QM9414: Substrate composition changes, C balance, enzyme production, growth and kinetics. *Applied Microbiology and Biotechnology* **46**, 489-496.
- Swift, J. (1972).** The estimation of mycelial biomass by determination of the hexosamine content of wood tissues decayed by fungi. *Soil Biological and Biochemistry* **5**, 321-322.
- Trilli, A. (1986).** Scale up of fermentation. In: Industrial Microbiology and Biotechnology. Demain, A. L. and Solomon, N. A. (eds). American Society of Microbiology, Washington, USA. pp. 227-307.
- Xue, M., Liu, D., Zhang, H., Hongyan, Q. and Lei, Z. (1992).** A pilot process of solid state fermentation from sugar beet pulp for the production of microbial protein. *Journal of Fermentation and Bioengineering* **73**, 203-205.