

**Amylase production by Solid State Fermentation of agro-industrial wastes
using *Bacillus* species**

ABSTRACT

This study evaluated amylase production by *Bacillus* species employing the solid state fermentation (SSF) method using five agro-industrial wastes namely corn cobs, potato peel and maize straw, groundnut husk and corn chaff. Five *Bacillus* species were tested for amylase production abilities and *Bacillus subtilis* showed the highest amylase production ability after incubation. Corn chaff gave maximum enzyme production (3.25U/ml) at 30, while the least enzyme was recorded on groundnut husk (2.35U/ml) at 25. Potato peel had maximum enzyme production by *Bacillus subtilis* (3.05U/ml) at pH 7.0 while the least enzyme production was from groundnut husk (2.84U/ml) at pH 4.0. Thus there was an increase in enzyme production with corresponding increase in substrate concentration. The results obtained in this study support the suitability of using agro-industrial wastes as solid state fermentation substrates for high production of amylase. It's also a means of solving pollution problems thus making solid state fermentation an attractive method.

Key words: Agro-industrial wastes, amylase, *Bacillus* species, fermentation, solid state.

1.0 INTRODUCTION

Amylase is one of the most widely used enzymes in the industry. It hydrolyses starch and is used commercially for the production of sugar syrups from starch which consist of glucose, maltose, and higher oligosaccharides (Hagihara *et al.*, 2001). Amylases are of great significance in biotechnological applications ranging from food, fermentation, detergent, pharmaceutical, brewing and textile to paper industries (Kathiresan, and Manivannan, 2006).

To meet the higher demands of these industries, low cost production of amylase is required.

The amylases can be derived from several sources, such as plants, animals and micro-organisms. Because of their short growth period, the enzymes from microbial sources generally meet industrial demands (Odee, *et al.*, 2007). The first enzyme produced

34 industrially was an amylase from a fungal source in 1994, which was used for the treatment
35 of digestive disorders (Crueger, and Crueger, 2009).

36 Amylase is produced in bacteria, fungi, plants and animals. the major bacteria belong to
37 *Bacillus* species and fungi such as *Aspergillus niger*, *Penicillium* sp., *Cephalosporium* and
38 *Rhizopus* are the major α -amylase producing microorganisms (Suganthi *et al.*, 2011).
39 However, due to efficient production strategies, microorganisms have substantial potential to
40 contribute to a number of industrial applications (Sodhi *et al.*, 2005). Such industrially
41 important microorganisms are found within the *Bacillus* species because of their rapid growth
42 rates that lead to short fermentation cycles, their capacity to secrete proteins into extra
43 cellular medium and general handling safety (Pandey *et al.*, 2010).

44 Production of these α amylases has been investigated through submerged (SmF) and solid-
45 state fermentation (SSF) (Perez-Guarre *et al.*, 2003). However, the contents of a synthetic
46 medium are very expensive and uneconomical, so they need to be replaced with more
47 economically available agricultural and industrial byproducts, as they are considered to be
48 good substrates for SSF to produce enzymes (Kunamnen *et al.*, 2005). Therefore this study
49 focused on the production of amylase enzyme by solid state fermentation of different agro-
50 industrial wastes (corn cobs, potato peel and maize straw, groundnut husk and corn chaff)
51 using *Bacillus* species.

52

53 **1.1 MATERIALS AND METHODS**

54 **1.2 COLLECTION OF SUBSTRATE**

55 Five Agro industrial wastes namely corn cobs, potato peel, maize straw, groundnut husk and
56 corn chaff were collected from different locations in Umuahia. They were washed with
57 distilled water 2-3 times and then treated with 1% NaOH for 30 min. The substrates were

58 autoclaved and dried in oven at 80°C for two days. Dried substrates were ground using a
59 grinder to make small particles (Jamieson *et al.*, 2001).

60 **1.3 TEST BACTERIUM**

61 Stock culture plate of *Bacillus* species sourced from National Roots Crops Research Institute,
62 Umudike maintained on Nutrient Agar slant was used as starter culture for the fermentation.

63 **1.4 SCREENING OF TEST BACTERIAL**

64 Primary screening of test bacteria for production of alpha amylase was done by the Starch
65 Agar Plate Method described by Jonnes *et al.*, (2011). Species that showed the widest zone of
66 clearance in starch hydrolysis were selected for use in Solid State Fermentation.

67 **1.5 Solid State Fermentation technique**

68 Solid state fermentation experiments as described by (Rajshree and Rajni, 2011), were
69 conducted in 100ml Erlenmeyer flasks containing 1g of the substrate impregnated with 10ml
70 of sterile liquid nutrient broth. The flasks were autoclaved at 121°C for 15min. and inoculated
71 with 1ml of the prepared inoculum, thoroughly mixed and incubated at 37°C for 5 days.

72

73

74 **1.5.1 Enzyme extraction**

75 The amylase enzyme was extracted from Solid State Fermentation medium by a simple
76 contact method described by Jamieson *et al.*, (2001). After incubation, 100 mL sodium
77 phosphate buffer of pH 6.9 was added into each experimental flask. The flaks were shaken
78 (150 rpm) for half an hour and the material was filtered through a filter paper. The filtrate
79 was centrifuged at 1000 rpm for 10 min at -10°C. The supernatant was carefully collected and
80 used as crude enzyme extract.

81 **1.6 AMYLASE ENZYME ASSAY**

82 For assay, previously inoculated nutrient starch broth was centrifuged at 8000g for 12
83 minutes and the supernatant was used as crude enzyme source. The assay of amylase was
84 conducted following the method of (Jamieson *et al.*, 2001).

85 **1.7 Optimization of fermentation parameters for amylase production and activity**

86 Optimization of agro industrial wastes samples fermentation was for the following
87 parameters for amylase production: incubation period, temperature, medium pH, and
88 substrate concentration (Ramesh, and Lonsane, 2007).

89 **1.8 Statistical Analysis**

90 One-Sample T-Test was used to investigate the significant difference in the effects
91 fermentation parameters of the substrates for amylase activity at 95% confidence interval.
92 The data were analyzed using the program IBM SPSS Version 16.

94 **2.0 RESULTS**

95 Table 1 shows the shows the identification and characterization of *Bacillus* spp

96 Table 2 shows the effect of incubation period on amylase enzyme. The isolate showed
97 highest production of amylase after 35hours of incubation at 2.11U/ml, 2.33U/ml and
98 2.39U/ml respectively.

99 Table 3 shows the effect of Temperature on amylase production. The maximum enzyme
100 production was detected at 40°C (2.52 U/ml), (2.35 U/ml), (2.45 U/ml), (2.30 U/ml) and
101 (2.44 U/ml).

102 Table 4 shows the effect of pH of the medium on amylase production. Maximum enzyme
103 activity was at pH 7.0, enzyme was produced maximally (2.55 U/ml), (2.54 U/ml),
104 (2.34 U/ml), (2.43 U/ml) and (2.49 U/ml) respectively. It was recorded at pH8 that the
105 activity of enzyme were slightly declined (2.35 U/ml), (2.30 U/ml) and (2.25 U/ml) for each
106 substrate at 24hours of incubation.

107 Table 5 shows the effect of substrate concentration on amylase production. There was
 108 increase in enzyme production with increase in substrate concentration up to 5g.

109

Table 1: Identification and characterization of *Bacillus* species

Colonial features	Gram Reaction	Cell Arrangement	Spore stain	Catalase	Oxidase	Coagulase	Indole	Citrate	Motility	Methyl Red	Voges-P	Suspected bacteria
White Moisture	+	Short Rod	+	+	-	-	-	+	+	+	+	<i>Bacillus</i> spp

Key: - = Absent, + = Present

Table 2: Effect of Incubation Period on Amylase Activity (U/ml)

Sample Substrate and Optical Density Reading (540nm)						
Incubation Period (hr)	Corn Cobs	Potato Peels	Maize Straws	Groundnut Husks	Corn chaffs	Standard Values
25	1.46 ^a ± 0.71	1.45 ^a ± 0.71	1.44 ^a ± 0.71	1.32 ^c ± 0.71	1.48 ^a ± 0.71	0.00
30	1.75 ^b ± 0.71	1.79 ^b ± 0.71	1.75 ^c ± 0.71	1.68 ^d ± 0.71	1.81 ^b ± 0.71	0.00
35	2.84 ^c ± 0.71	2.86 ^d ± 0.71	2.83 ^c ± 0.71	2.75 ^b ± 0.71	2.89 ^c ± 0.71	0.00
40	2.61 ^d ± 0.71	2.59 ^d ± 0.71	2.60 ^d ± 0.71	2.55 ^b ± 0.71	2.65 ^d ± 0.71	0.00
45	2.52 ^c ± 0.71	2.55 ^c ± 0.71	2.56 ^c ± 0.71	2.50 ^c ± 0.71	2.02 ^c ± 0.71 ^a	0.00

Values are mean ± standard deviations from two replicates

Table 3: Effect of Temperature on Amylase Activity (U/ml)

Temperature (°C)					
Substrate	25	30	35	40	45
Corn Cobs	1.75 ^b ± 0.71	2.45 ^a ± 0.71	2.65 ^a ± 0.71	2.94 ^a ± 0.71	2.80 ^c ± 0.71
Potatoes Peel	1.72 ^c ± 0.71	2.35 ^a ± 0.71	2.70 ^b ± 0.71	2.95 ^b ± 0.71	2.65 ^d ± 0.71
Maize Straw	1.85 ^c ± 0.71	2.55 ^a ± 0.71	2.80 ^c ± 0.71	3.02 ^c ± 0.71	2.72 ^e ± 0.71
Groundnut Husk	1.71 ^a ± 0.71	2.35 ^d ± 0.71	2.76 ^d ± 0.71	2.32 ^d ± 0.71	2.62 ^c ± 0.71
Corn chaff	1.70 ^a ± 0.71	3.25 ^e ± 0.71	2.55 ^e ± 0.71	2.75 ^e ± 0.71	2.57 ^b ± 0.71

Values are mean ± standard deviation from two replicates

Table 4: Effect of pH Amylase Activity (U/ml)

pH					
Substrate	4.0	5.0	6.0	7.0	8.0
Corn Cobs	1.90 ^a ± 0.71	2.02 ^b ± 0.71	2.50 ^d ± 0.71	3.04 ^a ± 0.71	2.85 ^a ± 0.71
Potatoes Peel	1.95 ^a ± 0.71	2.05 ^c ± 0.71	2.55 ^d ± 0.71	3.05 ^a ± 0.71	2.80 ^a ± 0.71
Maize Straw	1.92 ^a ± 0.71	2.00 ^d ± 0.71	2.55 ^d ± 0.71	2.99 ^c ± 0.71	2.75 ^b ± 0.71
Groundnut Hust	1.81 ^c ± 0.71	2.45 ^e ± 0.71	2.64 ^a ± 0.71	2.84 ^c ± 0.71	2.65 ^c ± 0.71
Corn chaff	1.85 ^c ± 0.71	1.92 ^e ± 0.71	2.62 ^a ± 0.71	2.93 ^d ± 0.71	2.65 ^c ± 0.71

Values are mean ± standard deviation from two replicates

Table 5: Effect of substrate concentration on Amylase Activity (U/ml)

Substrate	Substrate concentration (g)				
	1	2	3	4	5
Corn Cobs	1.46 ^b ± 0.71	1.94 ^a ± 0.71	2.39 ^a ± 0.71	2.75 ^a ± 0.71	3.32 ^a ± 0.71
Potato Peels	1.55 ^a ± 0.71	1.76 ^b ± 0.71	2.07 ^b ± 0.71	2.89 ^b ± 0.71	3.49 ^b ± 0.71
Maize Straws	1.02 ^c ± 0.71	1.34 ^c ± 0.71	2.70 ^c ± 0.71	3.06 ^c ± 0.71	3.21 ^c ± 0.71
Groundnut Husks	1.52 ^d ± 0.71	1.71 ^b ± 0.71	1.94 ^d ± 0.71	2.82 ^b ± 0.71	3.05 ^d ± 0.71
Corn chaffs	1.34 ^e ± 0.71	1.86 ^e ± 0.71	2.15 ^e ± 0.71	2.69 ^a ± 0.71	2.94 ^e ± 0.71

Values are mean± standard deviation from two replicates

DISCUSSION

This study evaluated amylase production by solid state fermentation of agro-industrial wastes using *Bacillus* spp. The amylase production by *Bacillus subtilis* is influenced by number of fermentation parameters. *The Bacillus subtilis* showed the highest amylase production at 24 hours of incubation with Potatoes Peel having the highest production of amylase (2.36U/ml) at 35°C, followed by Corn Cobs which also recorded high amylase production (2.34U/ml) at 35°C. Hence Potatoes Peel is the best substrate for enzyme activity when compared to other agro-industrial wastes in this study. Similar result was reported by (Ikram-ul-Haq *et al.*, 2003), who found out that wheat bran was a better substrate for α -amylase production by *Bacillus licheniformis*. Gangadharan *et al.* (2008) have reported that maximum amylase production was achieved at 24-48 h incubation period. *Bacillus subtilis* has shorter period of incubation for the production of α -amylase when compared to earlier reports. Chandrasekhar *et al.*, (2012) has evaluated the production of amylase at 12, 24, 36, 48 and 60 hours using *B. subtilis* cultured on banana waste and found more production at 24 hours, which corroborate with the present study. Above this incubation period, the amylase enzyme activity started to decrease. This may be due to the decrease in growth of the isolate. Most of the studies reported the highest enzyme production between 35 hours and 48 hours (Raju *et al.*, 2013) on

the contrary, (B5) showed optimum production after 25 hours, thus proving early harvesting time for industrial use.

Temperature is one of the important physical factors influencing the enzyme production (Ritesh *et al.*, 2011). Corn chaff produced the maximum enzyme production at 30°C (2.75 U/ml). This could be due to the mesophilic nature of the organism. The finding of this present study supports the finding of Asgher *et al.*, (2007) who found that amylase produced by *Bacillus subtilis* JS2004 gave the best activity at 40°C.

The result of the effect of temperature on enzyme production by *Bacillus subtilis* was almost identical to that reported for *B. lichemiformis* growing on wheat bran (Ramesh, and Lonsane, 2009), for *Bacillus subtilis* growing on banana stalk (Krishna, and Chandrasekaran, 1996), for *Bacillus megaterium* isolated from cassava waste (Mukesh-kumara *et al.*, 2012). Whereas, Vipul-Verma *et al.*, (2011) reported that the optimum temperature of enzyme activity was 40°C. These results indicate the independent nature of the temperature effect irrespective of the type of solid substrate used. It was also observed in this study that the enzyme production declined below and above 40°C temperature and this was due to lesser growth of the bacteria (Khurshid *et al.*, 2001). Vasantha and Hemashenpagam, (2012) have also evaluated the influence of temperature on amylase production.

Among the physicochemical parameters, pH of the growth medium plays an important role by inducing morphological changes in the organism and in enzyme secretion. Variation of pH results due to substrate consumption (eg: protein hydrolysis) and metabolite production like organic acids. Increase in pH from 4 to 6 increases enzyme activity, further increase in pH up to 9 decreases activity. *Bacillus subtilis* could grow and produce α -amylase over a wide range of pH (4-11). Potatoes peel had maximum enzyme production (2.55 U/ml) at pH 7.0. Similarly, Tanyildizi *et al.*, (2007) observed pH 7 as optimum for amylase production by *Bacillus amyloliquefaciens*. For the amylase production, most of the *Bacillus* sp. reported to

have optimum pH between 7-10 (Saxena *et al.*, 2007). Krishna and Chandrasekaran, (1996) reported production of α -amylase by *Bacillus subtilis* on banana fruit stalk and got optimum activity at pH 7.0. Shaista Kokab *et al.*, (2003) reported production of α -amylase by *Bacillus subtilis* utilizing banana peel and got optimum activity at pH 7.0. It was recorded that at pH 8 the activity of enzyme slightly declined to (2.35 U/ml), (2.30 U/ml) and (2.25 U/ml) for each substrate at 24 hours of incubation. When pH is altered below or above the optimum the activity it appears to be decreased or becomes denatured (Basabrani *et al.*, 2012). Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth (Radley, 2006). Terui, (2003) went on to report 6.8 as an optimum pH for the production of amylase by *B. subtilis*.

It has been suggested that the metabolic activity of bacteria is very sensitive to pH level of media. Kim *et al.*, (2004) have reported that the initial pH of solid substrate was found to have an impact on α -amylase production by *Bacillus subtilis* grown on Peat Moss (PM). Further, the type of buffer used in nutrient solution is a key factor in governing α -amylase production by the *Bacillus subtilis*.

It was observed in this study that after 24 hours of incubation at 35°C, broth slightly increased from 1g to 5g, having maximum enzyme production at 2.99U/ml, 2.82U/ml and 2.71U/ml from the various substrates. Thus, the ability of enzyme production means the more substrate concentration the more the enzyme production. This could be attributed to the fact that bacteria might have utilized medium faster and has undergone decline phase due to nutrient depletion. The difference in enzyme production could be attributed to certain factors which are associated either with the structure of the substrate or with the composition of individual substrates. These results support the suitability of using agro-industrial wastes as solid substrate for high production of α -amylase (Sidhu *et al.*, 1997).

The contents of synthetic media are very expensive and these contents might be replaced with more economically available agricultural by-products to reduce the cost of the media (Ikram-ul-Haq *et al.*, 2003). Therefore, agro-industrial wastes and by-products such as starchy materials had been used for Biosynthesis of amylases to solve the pollution problems and obtain a low cost media (Mukherjee *et al.*, 2009). The use of agricultural wastes makes solid-state fermentation (SSF) an attractive alternative method (Ellaiah *et al.*, 2002).

CONCLUSION

Among the cheap sources tested, potatoes peel was best for maximum amylase production at 35°C. The optimum activity of enzyme was obtained at 40°C incubation temperature and 35 hours incubation period.

REFERENCES

- Agosin, D., Jarpa, S., Rojas, E. and Espejo, E. (2009). Solid state fermentation of pine sawdust by brown-rot fungi. *Enzyme and Microbial Technology*, 11:511-517.
- Alazard, D. and Rimbault, M. (2001). Comparative study of amylolytic enzymes production by *Aspergillus niger* in liquid and solid state cultivation. *European Journal of Applied Microbiology and Biotechnology*, 12:113-117.
- Aneja, K.R. (2002). Experiments in Microbiology, Plant Pathology, Tissue culture and Mushroom Production Technology. New Age International (P), New Delhi, India, 169-171.
- Asgher, M., Asad, M.J., Rahman, S.U. and Legge, R.L. (2007). A thermostable α -amylase from a moderately thermophilic *Bacillus subtilis* strain for starch processing. *Journal of Food Engineering*, 79(3): 950-955.
- Ashfaq, M., Sushil, S. and Neelam, P. (2014). Optimization of pretreatment and fermentation conditions for production of extracellular cellulase complex using sugarcane bagasse. *Bio-information*, 10(10): 606-610.
- Auria, R., Hernandez, S., Rimbault, M. and Revah, S. (2010). Ion exchange resin: a model support for solid state growth fermentation of *Aspergillus niger*. *Biotechnology Techniques* 4:391-396.
- Basabrani, B.G., Devi, S.B. Unni, W. and Samanta R. (2012). Immobilization of Partially Purified Alpha-amylase Enzyme Produced by a Soil born *Bacillus spp.* *Advances in Applied Science Research*, 3(5): 2739-2744.

- Baysal, Z., Uyar, F., and Aytakin, C. (2003). Solid state fermentation for production of α amylase by a thermotolerant *B. subtilis* from hot-spring water. *Process Biochemistry*, 38: 1665-1668.
- Bertrand, T.F., Frederic, T. and Robert, N. (2004). Production and partial characterization of a thermostable amylase from ascomycetes yeast strain isolated from starchy soil. McGraw Hill Inc, New York, 53-55.
- Bradford, M.M. (2006). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248–254.
- Chakraborty, S., Khopade, A., Kokare, C., Mahadik, K., and Chopade, B. (2009). Isolation and characterization of novel alpha amylase from marine *Streptomyces* sp. *Journal of Molecular Catalogues*, 58: 17–23.
- Chandrashekhar, U., Radha, I. K. and Basappa, B. K. (2012). Production of α -amylase Using Banana Waste by *Bacillus subtilis* Under Solid State Fermentation. *European Journal of Experimental Biology*, 2(4): 1044-1052.
- Crueger, W., and Crueger, A. (2009). Industrial Microbiology. Sinauer Associates, Sunderland, 40: 225-234.
- De-Azeredo, L., Leite, S., Freire, D., Benchetrit, L., and Coelho, R. (2001). Proteases from actinomycetes interfere in solid media plate assays of hyaluronidase activity. *Journal of Microbiology Methods*, 45: 207–212.
- Ellaiah, P., K., Adinarayana, Y., Bhavani, P., Padmaja, and Srinivasulu, B., (2002). Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species. *Process Biochemistry*, 38: 615-620.
- Gangadharan, D., Sivaramakrishnan, S., Nampoothiri, K.M. and Pandey, A. (2006). Solid Culturing of *Bacillus amyloliquefaciens* for Alpha Amylase Production., *Food Technology and Biotechnology*, 44: 269-274.
- Gervais, P., Molin, P., Grajek, W. and Bensoussan, M. (2008). Influence of the water activity of a solid substrate on the growth rate and sporogenesis of filamentous fungi. *Biotechnology and Bioengineering* 31:457-463.
- Griffin, D.M. (2001). Water and microbial stress. *Advances in Microbial Ecology*, 5:91-136.
- Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K., and Chouhan, B. (2003). Strategies for large scale inoculum development for solid state fermentation system: Conidiospores of *Trichoderma harzianum*. *Biotechnology Techniques*, 5:415-420.
- Hagihara, H., Igarashi, K., Hayashi, Y., Endo, K., Ikawa-Kitayama, K., Ozaki, K., Kawai, S., Ho, S. (2001). Novel α amylase that is highly resistant to chelating reagents and

- chemical oxidants from the alkaliphilic *Bacillus* isolate KSM.K.38. *Applied Environmental Microbiology*, 67: 1744–1750.
- Haq, I., Khurshid, S., Ali, K., Ashraf, H., Qadeer, M.A. and Rajoka, I. (2001). Optimization of agro-residual medium for α -amylase production from a hyper-producing *Bacillus subtilis* KCC103 in submerged fermentation *Microbiology and Biotechnology*, 17: 35-37
- Ikram-ul-Haq, H., Ashraf, J. and IqbalQadeer, M.A. (2003). Production of alpha amylase by *Bacillus licheniformis* using an economical medium, *Bio-resources Technology*, 87: 57-61.
- Jagadeeswari S. and Santhi R. (2016). Optimization of Agroresidues for α -Amylase Production by *Bacillus subtilis* PS03 and its Application in Detergent Industry, *Journal of Academia and Industrial Research*, 5(7): 2278-5213
- Joanne, M.W., Lind, M.S and Christopher, J.W. (2011). Dyes and Simple Staining. The study of microbial structures. Microscopy and specimen preparation. *Prescott's Microbiology* 8th Edition. Mc Graw-Hill, New-York, 36-37.
- Jamieson, A. D., Pruitt, K. M. and Caldwell, R. C. (2001). An improved amylase assay. *Journal of Dental Research*, 48(3): 483-496.
- Jianlong, W. and Ping, L. (1998). Biochemical and molecular characterization of a detergent stable alkaline serine-protease from a newly isolated *Bacillus licheniformis* NH1 *Proceedings of Biochemistry*, 33; 313-316
- Kathiresan, K., and Manivannan, S. (2006). α Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. *African Journal of Biotechnology*, 5(10): 829-832.
- Kim, J.H., Hosobuchi, M., Kishimoto, M., Seki, T., Yoshida, T., Taguchi, H. and Ryu, D.D.Y. (2005). Cellulase production by a solid state culture system. *Biotechnology and Bioengineering*, 27:1445-1450.
- Kim, J.W., Barrington, S., Sheppard, J. and Lee, B. (2004). Amylase production by *Aspergillus niger* under solid state fermentation using agroindustrial wastes. *Bio-resources Technology*, 3(5): 2739-2744
- Kokab, S., Ashgar, M., Rehman, K., Asad, M.J., and Aedeyo, O. (2003). Bio-Processing of banana peel for α -Amylase production by *B. subtilis*. *International Journal of Agricultural Biology*, 36–39.
- Konsula, Z. and Liakopoulous, M. (2004). Hydrolysis of starches by the action of a α -amylase from *Bacillus subtilis*. *Proceedings of Biochemistry*, 39: 1745-1749.
- Krishna C., Chandrasekaran, M. (2006). Selection of microorganisms which produce raw-starch degrading enzymes, *Applied Microbiology and Biotechnology*, 27: 443-446.

- Krishna, C. and Chandrasekaran, M. (1996). Effect of Inorganic Salts and Surfactants on the Production of α -Amylase by a Mangrove Isolate of *Bacillus subtilis* using Solid-State Fermentation. *Applied Microbiology and Biotechnology*, 46: 106–111
- Kunamneni, A., Perumal, K., and Singh, S. (2005). Amylase production in solid state fermentation by the thermophilic fungus *Thermomyces Lanuginosus*. *Journal of Bioscience and Bioengineering*, 100(2): 168 – 171
- Laukevics, J.J., Apsite, A.F., Viesturs, U.E. and Tengerdy, R.P. (2004). Solid substrate fermentation of wheat straw to fungal protein. *Biotechnology and Bioengineering*, 26: 1465-1474.
- Le Dividich J., Seve B., and Geoffroy F., (2006). Protein enrichment of cassava by solid state fermentation using molds isolated from traditional foods. *Journal of Fermentation Technology*, 63:395-399
- Mudgett, R.E. (2006). Solid state fermentations. In: Manual of Industrial Microbiology and Biotechnology, *American Society for Microbiology*, Washington D.C. 66-83.
- Mukesh, K. D. J., Silambarasanb, T., Renugac, R., Ravi, K.M., Karthigai, D.S., Dhandapani, R. and Kalaichelvana, P. T. (2012). Production of a thermostable α -amylase from *Bacillus* sp. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production.. *European Journal of Experimental Biology*, 2(3):590-595
- Mukherjee, A.K., Adhikari, H. and Rai, S.K. (2008). Production of alkaline protease by a thermophilic *Bacillus subtilis* under solid-state fermentation (SSF) condition using Imperata cylindrica grass and potato peel as low-cost medium: characterization and application of enzyme in detergent formulation. *Biochemical Engineering Journal*, 39(2): 353-361.
- Navarro, J.M., Roussos, S. and Raimbault, M. (2002). Maintenance of heat and water balances as a scale-up criterion for the production of ethanol by *Schwanniomyces castellii* in a solid state fermentation system. *Process Biochemistry*, 27:97-107.
- Odee, D.W., Sutherland, J.M., Makatiani, E.T., Mc Inory, S.G., Sprent, J.I. (2007). Plant Soil, International training course on solid state fermentation. Document ORSTOM, Montpellier France, 188: 65-75.
- Oriol, E., Schettino, B., Viniegra-Gonzalez, G. and Raimbault, M. (2008). Solid state culture of *Aspergillus niger* on support. *Journal of Fermentation Technology*, 66:57-62.
- Palki S.K., Sukhjeet K., Hardish K., Arpit S., Pushap R. and Shaily P. (2015). Solid Substrate Fermentation using Agro Industrial Waste: New Approach for Amylase Production by *Bacillus licheniformis* *International Journal of Current Microbiology and Applied Science*, 4(12): 712-717
- Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D. and Mohan, R. (2000). Advances in microbial amylases. *Biotechnology and Applied Biochemistry*, 31(2): 135-152.

- Perez-Guarre, N., Torrado-Agrasar, A., Lopez-Macias, C., and Pastrana, L. (2003). Main characteristics and application of solid substrate fermentation, *Electron. Journal of Environmental Agriculture and Food Chemistry*, 2: 243–350.
- Radley, J.A. (2006). Industrial Uses of Starch and Its Derivatives. Applied Science Publishers Ltd, London, 51-85.
- Raimbault, M. and Alazard, D. (2010). Culture method to study fungal growth in solid fermentation. *European Journal of Applied Microbiology and Biotechnology* 9:199-209.
- Rajshree S. and Rajni S.(2011). Amylase production by solid-state fermentation of agro-industrial wastes using *Bacillus sp.* *Brazilian Journal of Microbiology*, 42: 1334-1342.
- Raju, E. V. N. and Divakar, G. (2013). Production of amylase by using *Pseudomonas aeruginosa* isolated from garden soil. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 2(1):50–56.
- Ramesh, B., Reddy, P.R.M., Seenayya, G., and Reddy, G. (2001). Heat transfert simulation in solid substrate fermentation. *Biotechnology and Bioengineering*, 35:802-808.
- Ramesh, M.V. and Lonsane, B.K. (2007). Effect of cultivation conditions on growth and α -amylase production by a thermophilic *Bacillus sp.* *Biotechnology Letters*, 7: 501–504.
- Ramesh, M.V. and Lonsane, B.K., (2009). Optimization, production and partial purification of extracellular –amylase from *Bacillus spp.* *Biotechnology Letters*, 11: 49–52.
- Ritesh, P., Arbat, T. and Barkha, S, (2011). Production of Glucoamylase by *Aspergillus oryzae* Under Solid State Fermentation Using Agro Industrial Products. *International Journal of Microbiological Research*, 2(3): 204-207.
- Saucedo-Castañeda, G., Gutierrez-Rojas, M., Bacquet, G., Raimbault, M. and Viniegra-Gonzalez, G. (1990). Heat transfert simulation in solid substrate fermentation. *Biotechnology and Bioengineering*, 35:802-808.
- Saucedo-Castañeda, G., Lonsane, B.K., and Krishnaiah, M.M., (2002). Solid state fermentation in the development of agro-food by-products. *Industry and Environment*, 5:27-30.
- Saxena, R. and Singh, R. (2011). Amylase production by solid-state fermentation of agro-industrial wastes using *Bacillus sp.* *Brazilian Journal of Microbiology*, 42(4): 1334-1342.
- Senez, J.C., Raimbault, M. and Deschamps, F. (2010). Protein enrichment of starchy substrates for animal feeds by solid state fermentation. *World Animal Review*, 35: 36-40.

- Shaista, K., Asghar, M., Rehman, K. and Asad, M.J. (2003). Bioprocessing of Banana Peel for Alpha-amylase production by *Bacillus Subtilis*, *International Journal of Agriculture and Biology*, 6: 56-78
- Sharanappa A., Wani K.S., and Pallavi P. (2011). Bioprocessing of food industrial waste for α -amylase production by solid state fermentation, *International Journal of Advanced Biotechnology and Research*, 2(4): 473-480
- Sidhu, G.S., Sharma, P., Chakrabarti, T. and Gupta, J.K. (1997). Strain improvement for the production of a thermostable α -amylase. *Enzyme and Microbiology Technology*, 21(7):525-530.
- Soccol, C.R. and Vandenberghe, L.P. (2003). Overview of applied solid-state fermentation in Brazil. *Biochemical and Engineering Journal*, 13(2): 205-218.
- Sodhi, H.K., Sharma, K., Gupta, J.K., and Soni, S.K. (2005). Production of a thermostable α amylase from *Bacillus* sp. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. *Process Biochemistry*, 40: 525–534
- Suganthi, R., Benazir, J.F., Santhi, R., Ramesh Kumar, V., Hari, A., Meenakshi, N., Nidhiya, K.A., Kavitha, G. and Lakshmi, R. (2011). Amylase production by *Aspergillus niger* under solid state fermentation using agroindustrial wastes. *International Journal of Engineering Science and Technology*, 3(2): 1756-1763.
- Tanyildizi, M.S., Özer, D. and Elibol, M. (2007). Optimization of α -amylase production by *Bacillus* sp. using response surface methodology. *Proceedings of Biochemistry*, 40(7): 2291-2296.
- Terui, G., (2003). In: *Kinetics of Hydrolase Microorganisms*, (Ed: Sterbackk, U.), Microbial Engineering, 2: 377-395
- Trejo-Hernandez, M.R., Raimbault, M., Roussos, S. and Lonsane, B.K. (2002). Potencial of solid state fermentation for production of ergot alkaloids. *Letters in Applied Microbiology*, 15:156-159.
- Under Solid State Fermentation Using Agro Industrial Products. *International Journal of Microbiological Research*, 2(3): 204-207.
- Vasanth, R. and Hemashenpagam, N. (2012). Production and Medium Optimization of Amylase by *Bacillus* using Fermentation Methods. *Journal of Microbiology and Biotechnology Research*, 2(4): 481-484.
- Ventosa, A. and Nieto, J. (2005). Biotechnological applications and potentialities of halophilic microorganisms. *World Journal of Microbiology and Biotechnology*, 11: 85–94.
- Vijayaraghavan, P. and Vincent, S.G.P. (2012). Cow dung as a novel, inexpensive substrate for the production of a halo-tolerant alkaline protease by *Halomonas* sp. PV1 for eco-friendly applications. *Biochemical England Journal*, 69: 57-60.

Vipul, V., Mrigank, S. A., Abhishek, R. G., Monika, S. and Akhilesh, K. (2011). Identification and Characterization of α -Amylase from Yemeni Bean seeds *Euro. Journal of Experimental Biology*, 1(3):90-96

UNDER PEER REVIEW