RESEARCH ARTICLE

Effect of moisture content on the production of protease by *Fusarium oxysporum* using agroindustrial waste

Vidhale NN and Deshmukh Rupali R

Department of Microbiology, Shri. Shivaji Science College, Amravati 444604, M.S., India
Email: vidhalenana@gmail.com

Manuscript details:

Received: 23.04.2015
Revised : 12.05.2015
Revised received: 23.06.2015
Accepted: 25.06.2015
Published : 30.06.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Vidhale NN and Deshmukh Rupali R (2015) Effect of moisture content on the production of protease by *Fusarium oxysporum* using agroindustrial waste. *Int. J. of Life Sciences*, 3(2): 162-166

ABSTRACT

The effect of moisture content on the production of protease from *Fusarium oxysporum* was studied using agro industrial waste as substrates such as dal mill waste, oil mill waste, molasses, fruit waste and vegetable garbage under solid state fermentation. Dal mill waste, oil mill waste and vegetable garbage produced maximum protease activity in presence of all the types of moistures (25%, 35%, 45%, 55%, 65%, & 75%) after 96 hrs of incubation. However molasses and fruit waste gave highest protease production in presence of 55 % and 65% moisture content after 7^{th} day of incubation. Among all the substrate dal mill waste and oil mill waste were promising in being utilized faster for the production of protease enzyme.

Keywords: Agoindustrial waste, *Fusarium oxysporum,* protease

INTRODUCTION

Protease is one of the most commercial enzyme used in food processing, detergent industry, dairy industry, silver recovery, medical purpose, leather making, meat processing, and chemical industry as well as in waste water treatment (Negi and Benerjee, 2006). This enzyme occurs widely in plants and animals, but commercially proteases are produced exclusively from microorganisms. Molds of the genera *Aspergillus, Penicillium* and *Rhizopus* are especially used for producing proteases (Sandhya *et al.*, 2005). Solid state fermentation has gained tremendous attention for the low cost production of industrially important enzymes by utilization of various types of waste as agro industrial waste. In search for cheaper fermentation processes with a high enzyme yield, SSF was found to be more attractive (Kota *et al.*, 1999). Different mticroorganisms were utilized for the production of protease fungi such as *Aspergillus ,Penicillium , Rhizopus, Chrysosporium, Mucor, Scedosporium* and bacteria like *Bacillus licheniformis, Bacillus firmus, Bacillus alcalophilus, Bacillus subtilis, Bacillus thuringiensis, Streptomyces spp*. etc.Hence study was carried out to study protease production potentiality of Fungus *Fusarium oxysporum.*

MATERIALS AND METHODS

Substrates preparation

Agro industrial waste such as dal mill waste, oil mill waste, Molasses, fruit waste and vegetable garbage were collected and powdered to size about 2 mm in homogenizer and then sieved through 20-40 mesh screens to obtain a particle having diameter between 0.42 to 0.85 mm. Each of such substrate was supplemented with 0.83 gm K2HPO4 and 0.16gm $MgSO_4$, 1.5% agar-agar and 10 ml distilled water and autoclaved for 15 min. at 15 lb/inch ^{2}. Initial utilization of these substrates for production of protease by *Fusarium oxysporum* strain was studied under Solid state fermentation, incubated for 7 days at room temperature (25 to 30^{0}C). Initially 20 g of 50% moistened substrate was sterilized and thoroughly mixed with 1ml spore suspension of 7 day old culture of *Fusarium oxysporum*. This substrate along with spore suspension was poured in sterilized Petri dish and allowed to incubate at room temperature for 7 days.

Enzyme estimation

After every 24 hrs of interval 1gm fermented substrate was harvested from petri plate and transferred to test tube containing 5ml phosphate buffer. The contents were homogenized and centrifuged at 2000 rpm for 30 min to remove all particulate matter. Protease activity was assayed as suggested by Keay and Wrildi (1970). To 1 ml of culture filtrate, 1ml of 2% casein solution was added and the mixture was incubated at 37^{0}C for 10 min. The reaction was terminated by adding 2ml 0.4 M TCA (Trichloro acetic acid), again incubated at 37^{0}C for 20 min. and filtered through Whatman filter paper no1. One ml of the filterate was added to 5 ml of sodium carbonate (0.4M) and 1ml Folin- Ciocalteus's reagent and incubated at 37^{0}C for 30 min. The Absorbance was measured at 660 nm. in Spectrophotometer .

Effect of Moisture content

All the five substrates were provided with different moisture percents such as 25%, 35%, 45%, 55%, 65% and 75%. Protease production in SSF in all the fives substrate under above moisture content was studied. Protease production was estimated from second day of incubation up to 7 days.

RESULTS AND DISCUSSIOINS

All the agro industrial waste substrates were provided with moisture 25%, 35%, 45%, 55%, 65% & 75% and incubated for 7 days at room temperature. Dal mill waste, oil mill waste and vegetable garbage produced maximum protease activity in presence of all the types of moistures after 96 hrs of incubation. However molasses and fruit waste gave highest protease production in presence of 55 % and 65% moisture content after 7th day of incubation. Among all the substrate dal mill waste and oil mill waste were promising in being utilized faster for the production of protease enzyme. Results of enzyme estimation are summarized in Table-1 and fig- 1, 2, 3, 4, and 5. The highest enzyme production (121.50 ug^{-1}) was obtained at 60% initial moisture content by *Streptomyces sp*. (N902) (Lazim *et al*. 2009). A similar observation has been reported in case of *Streptomyces sp*. 594 protease production by De Azerodo *et al*. (2005). Study of Germano *et al*. (2003) indicated the requirement of 55 and 63% initial moisture content for maximum proteases production by *Penicillum* LPB- 9 and *A. falvus* (Malathi and Chakroborty (1991) respectively, in SSF. In the study of Chutmanop *et al*. (2008), the optimum initial moisture level was about 50%

Table 1: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a solid state fermentation using agro industrial waste as substrate.

Type of waste	% of moisture	Production of protease (µg/ml)at different Incubation period						Mean ± SD Value
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	
Dal mill waste	25	7.2	7.8	7.9	10.9	10.5	10.3	9.1 ±1.49
	35	6.3	7.4	8	10.9	10.7	10.3	8.93±1.78
	45	6.4	7.6	8.1	10.9	10.8	10.6	9.07±1.78
	55	6.5	7.7	8.4	10.8	10.7	10.4	9.08±1.65
	65	6.4	7.4	8.2	10.8	10.3	9.2	8.72±1.55
	75	6.4	7.3	8.3	10.9	10.4	9.1	8.73±1.60
Oil mill waste	25	6.6	11.1	9	10.9	9.6	9.7	9.48±1.48
	35	5.8	11.1	10.1	10.8	8.9	9.8	9.42±1.77
	45	6.2	10.9	10.3	10.7	8.1	10.3	9.42±1.71
	55	6.7	8.7	10.4	10.8	7.8	10.3	9.12±1.51
	65	6.4	8.7	10.3	11	8	10.1	9.08±1.57
	75	7	8.4	10.3	10.9	6.6	5	8.03±2.07
Molasses	25	2.3	2.8	2.4	4.2	7.4	7.4	4.42 ±2.20
	35	1.9	1.6	2.1	5.1	6.2	6.3	3.87 ±2.04
	45	1.4	1.3	2	4.5	7.4	7.5	4.02 ±2.65
	55	1.3	1.2	2	5.2	7.8	7.8	4.22 ±2.86
	65	1	1.1	1.6	4.6	7.7	7.8	3.97 ±2.93
	75	2.5	1.3	2.2	4	7.6	7.5	4.18 ±2.51
Fruit waste	25	2.2	3.3	3.6	4.9	7.6	7.7	4.88 ±2.11
	35	2.2	3.2	4.2	5.6	7.9	8	5.18 ±2.21
	45	2.4	2.7	3.5	5	7.1	7.4	4.68 ±1.99
	55	2.2	3.4	4.3	5.4	8.5	8.6	5.4 ±2.43
	65	2.1	3	3.7	4.9	8.6	8.6	5.15 ±2.58
	75	2	3.5	3.8	6.1	8.1	8.2	5.28 ±2.35
Vegetable garbage	25	3.7	3.2	6.1	8.1	8.3	9.5	6.48 ±2.37
	35	2.8	4.2	6.9	9.8	9.3	8.9	6.98 ±2.65
	45	3.3	4.1	6.1	9.1	8.6	6.6	6.3 ±2.13
	55	3.5	3.6	7.2	6.9	7.3	7.5	6 ±1.74
	65	3	3.9	5.9	9.7	9.7	8.2	6.73 ±2.66
	75	3.8	3.5	7.1	8.7	8.7	7.6	6.57 ±2.14

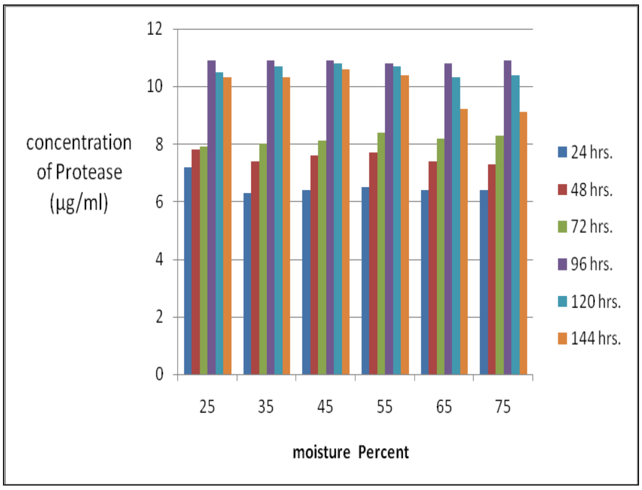

Fig 1: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using dal mill waste as substrate.

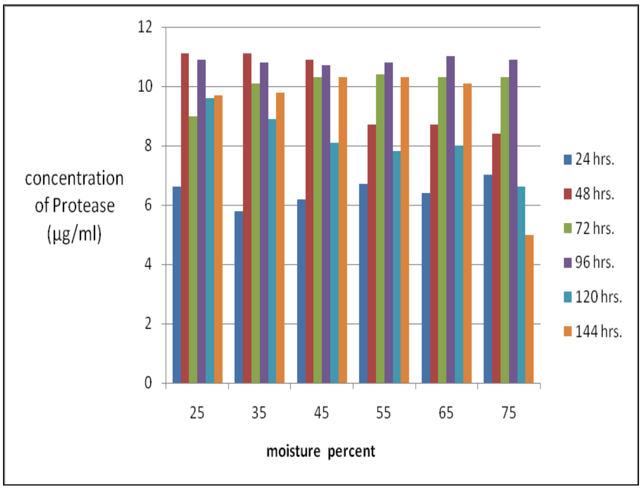

Fig 2: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using oil mill waste as substrate.

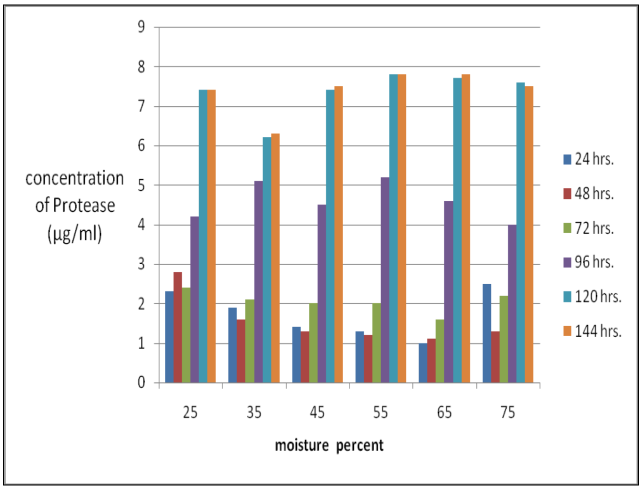

Fig 3: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using molasses as a substrate.

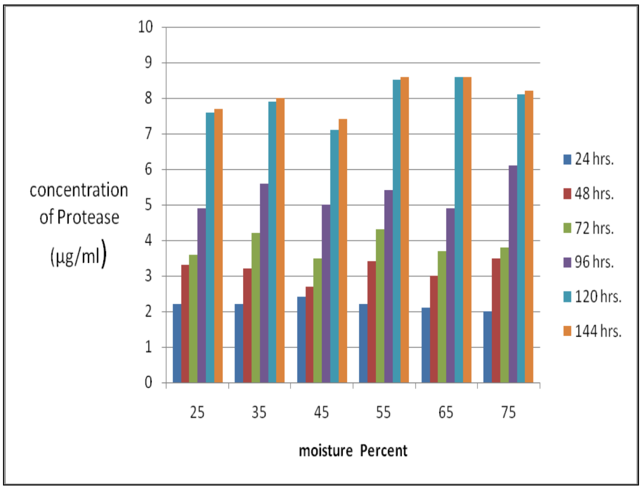

Fig 4: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using fruit waste as a substrate.

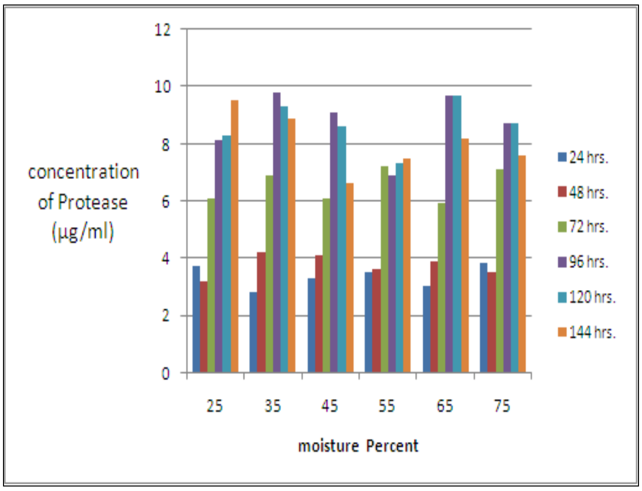

Fig5: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using vegetable garbage as substrate.

which afforded a high protease activity value.. In the study carried out by Sajeed and Vidhale (2013) the effect of initial moisture on protease production was maximum at initial moisture level of 50% (w/w).

REFERENCES

Chutmanop Jarun, Sinsupha Chuichulcherm, Yusuf Chisti and Penjit Srinophakun (2008) Protease production by *Aspergillus oryzea* in solid state fermentation using agroindustrial substrates. *Chemical Technology*, 1018: 1012- 1018.

De Azeredo, L.A.I., Lima, M.B., de-Coleho, R.R.R., Freire, D.M.G.: Thermophilic protease production by *Streptomyces* sp. 594 in submerged and solid state fermentation using feather meal. *J. Appl. Microbiol*. 100, 641–647 (2006)

Germano S, Pandey A, Osaka CA, Rocha SN, Soccol CR (2003) Characterisation and stability of proteases from Penicillium sp. produced by solid-state fermentation. *Enzyme Microb Technol*., 32:246–251

Hadeer Lazim Hadeer. Houda Mankai. Nedra Slama.Insaf Barkallah. Ferid Limam(2009): Production and optimization of thermophilic alkaline protease in Solid state state fermentation by *Streptomyces* sp. CN902. *J Ind. microbial Biotechnol*. vol.36: pp 531-537.

Keay L and Wildi BS (1970) Proteases of the genus *Bacillus*. I. Neutral proteases. *Biotechnol. Bioeng.*, 12: 179–212. doi: 10.1002/bit.260120205

Kota KP. Sridhar P (1999) Solid state cultivation of *Streptomyces clavuligerus* for cephamycin C production. *Process Biochem*., 34:325-328.

Malathi S, Chakraborty R (1991;) Production of Alkaline Protease by a New*Aspergillus flavus* Isolate under Solid-Substrate Fermentation Conditions for Use as a Depilation Agent. *Applied and Environmental Microbiology*, 57(3):712-716..

Negi, S. and Benergee, R. (2006): Optimization of amylase and protease production from *Aspergillus awamori* in single bioreactor through EVOP factorial design technique. *Food Technol Biotechnol.,* 44:257-261).

Sajeed Ali Syed and Vidhale NN (2013) Protease Production by *Fusarium oxysporum* in Solid-State Fermentation Using Rice Bran. *American journal of microbiological research*,1,(3): 45-47.

Sandhya, C., Sumantha, A., Szakacs, G and Pandey, A. (2005) Comparative evaluation of neutral protease production by *Aspergillus oryzea* in submerged and solid-state Fermentation. *Biochemical process*., 40: 2689-2694.