

RESEARCH ARTICLE

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

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| Manuscript details: | ABSTRACT |
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| <p>Received: 23.04.2015 Revised : 12.05.2015 Revised received: 23.06.2015 Accepted: 25.06.2015 Published : 30.06.2015</p> <p>Editor: Dr. Arvind Chavhan</p> <p>Cite this article as: Vidhale NN and Deshmukh Rupali R (2015) Effect of moisture content on the production of protease by <i>Fusarium oxysporum</i> using agroindustrial waste. <i>Int. J. of Life Sciences</i>, 3(2): 162-166</p> <p>Copyright: © 2015 Author(s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p> | <p>The effect of moisture content on the production of protease from <i>Fusarium oxysporum</i> 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 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.</p> <p>Keywords: Agoindustrial waste, <i>Fusarium oxysporum</i>, protease</p> <p>INTRODUCTION</p> <p>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 <i>Aspergillus</i>, <i>Penicillium</i> and <i>Rhizopus</i> are especially used for producing proteases (Sandhya <i>et al.</i>, 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 <i>et al.</i>, 1999). Different mticroorganisms were utilized for</p> |

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 MgSO₄, 1.5% agar-agar and 10 ml distilled water and autoclaved for 15 min. at 15 lb/inch². 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°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°C for 10 min. The reaction was terminated by adding

2ml 0.4 M TCA (Trichloro acetic acid), again incubated at 37°C for 20 min. and filtered through Whatman filter paper no1. One ml of the filtrate was added to 5 ml of sodium carbonate (0.4M) and 1ml Folin- Ciocalteus's reagent and incubated at 37°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 five substrate under above moisture content was studied. Protease production was estimated from second day of incubation up to 7 days.

RESULTS AND DISCUSSIONS

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

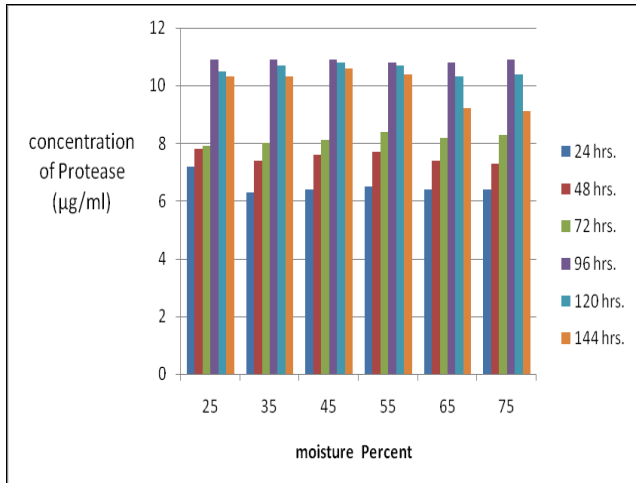


Fig 1: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using dal mill waste as substrate.

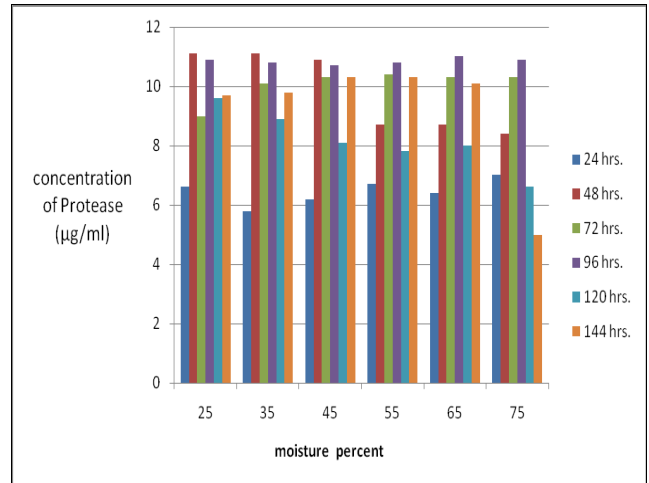


Fig 2: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using oil mill waste as substrate.

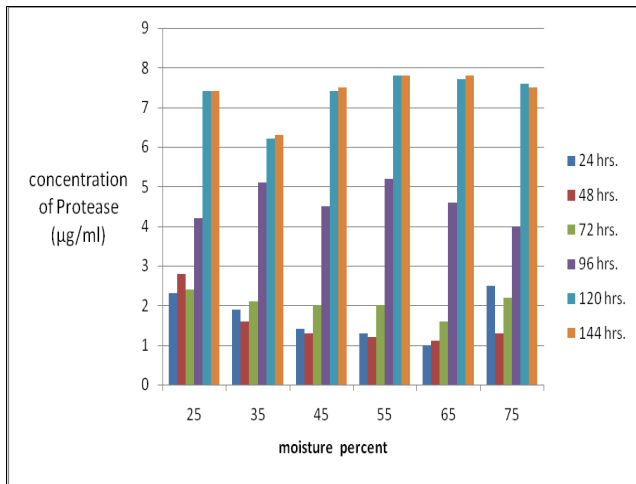


Fig 3: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using molasses as a substrate.

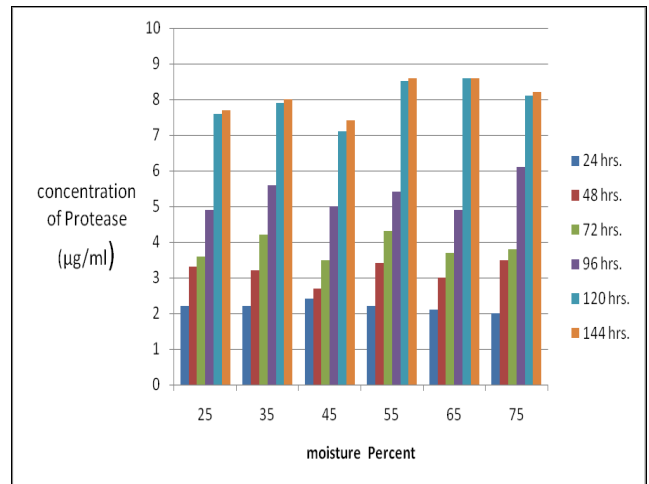


Fig 4: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using fruit waste as a substrate.

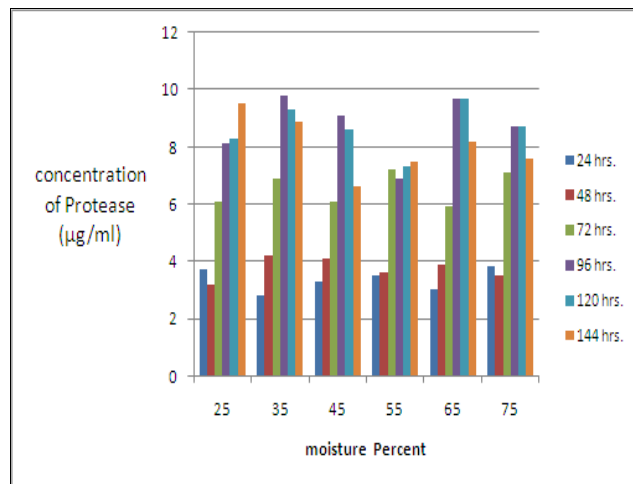


Fig5: Effect of moisture percent on production of protease by *Fusarium oxysporum* in a SSF using vegetable garbage as substrate.

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).

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