



EFFECT OF TEMPERATURE ON THE PRODUCTION OF PROTEASE BY *FUSARIUM OXYSPORUM* USING AGROINDUSTRIAL WASTE.

Microbiology

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ABSTRACT

Agro industrial waste constitute valuable source for cultivation of microorganisms and production of enzyme. An attempt was made to study protease production at various temperatures by *Fusarium oxysporum* on agroindustrial waste such as dal mill waste, oil mill waste, molasses, fruit waste and vegetable garbage. Dal mill waste and vegetable garbage showed maximum protease production (12.7 µg /ml and 19.9 µg /ml respectively) was at 40 °C after 6 days of incubation. Oil mill waste and molasses however also produced maximum protease 19.7 µg /ml 13.9 µg/ml respectively) at 40 °C incubation temperature after 7 days of incubation. However fruit waste comparatively produced least amount of protease (4.5 µg /ml) at 50°C after 5th days of incubation.

KEYWORDS

Agro industrial waste, *Fusarium oxysporum*, protease

INTRODUCTION

Microbial proteases are gaining attention due to its prominent commercial importance. Protease enzyme constitutes two thirds of total enzymes used in various industries and this dominance in the industrial market need to increase production (Gupta et al. 2002). This enzyme occurs widely in plants and animals, but commercially proteases are produced exclusively from microorganisms. Globally, 140 billion metric tons of biomass wastes are generated every year from agriculture. If managed carefully, however, solid state fermentation offer the best possible use of these agro industrial waste as substrate for protease production through different manipulations. High protein and moisture content of these underutilized wastes facilitate their use as substrate in solid state fermentation with the supplementation of nitrogen sources (Saxena and Singh 2010). Advantages of solid state fermentation include lower manufacturing costs with large volumes of production, less preprocessing energy and effluent generation along with easy process management and better product recovery. (Prakashan et al. 2006; Oliveira et al. 2006).

MATERIALS & METHOD

Five agro-industrial wastes such as Dal mill waste, Oil mill waste, Molasses, Fruit waste and Vegetable garbage were employed as substrates for the production of protease by *Fusarium oxysporum*. All the five agroindustrial waste were collected from local area.

Substrates preparation

Agro industrial waste such as dal mill waste, oil mill waste, molasses, fruit waste and vegetable garbage were powdered to size about 2 mm in homogenizer and 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-Ciocalteu's reagent and incubated at 37°C for 30 min. The Absorbance was measured at 660 nm. in Spectrophotometer .

Effect of Incubation Temperature

Five agro industrial wastes such as dal mill waste, oil mill waste, molasses, & fruit waste & vegetable garbage were tested for the protease production at different incubation temperatures such as 25°C, 37°C, 40°C, 50°C and 60°C. Protease production in SSF was estimated over 7 days.

RESULTS & DISCUSSION

All the agro industrial substrates were incubated at different incubation temperatures such as 25°C, 37 °C, 40 °C, 50 °C, 60 °C for 7 days of incubation and protease production was estimated and depicted in Table 1 and Fig. 1, 2, 3, 4 and 5 As far as dal mill waste and vegetable garbage are concerned maximum protease production was obtained at 40 °C after 6 days of incubation (12.7 µg /ml and 19.9 µg /ml respectively). Oil mill waste and molasses also produced maximum protease (19.7 µg /ml 13.9 µg/ml respectively) at 40 °C incubation temperature after 7 days of incubation. However fruit waste comparatively produced least amount of protease (4.5 µg /ml) at 50°C after 5th days of incubation. It can also be noted that 40% moisture content exerted maximum production of protease enzyme when vegetable garbage, molasses and dal mill waste were the substrates even after 120 hrs of incubation. Exceptionally fruit waste substrate showed maximum protease production at 50°C incubation after 120 hrs of incubation.

Marcy et al. (1984) recorded that maximum production of protease from *Thermoasus aurantiacus var cevisporus* occurred at 50°C. In the study carried out by Oyeleke et al. (2012) the optimum temperature for protease enzyme by *B. Subtilis* was 60°C and the optimum temperature for *A. niger* was 50°C. Wang et al. (2006) reported that the protease production was high in 45°C by marine yeast. Megalla et al. (1990) reported that 45°C was the optimum temperature for protease production by *Thermoas thermophilum*. The optimum temperature for maximum protease production was found at 25°C in the study carried out by S. Sajeed Ali and N.N Vidhale (2013).

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

Type of waste	Temperature 0C	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	3.3	3.8	4.5	4.6	5.4	5.3	4.48 ±0.75
	37	4.7	3.9	4.9	5	8.1	8.8	5.90 ±1.85
	40	7.3	8.5	8.6	10.7	12.7	12.6	10.07±2.08
	50	8.5	9.7	9.8	9.9	9.3	9.2	9.40±0.48
	60	2.2	2.3	2.4	2.5	2.4	2.4	2.37±0.09

Oil mill waste	25	2.1	2.2	2.2	2.3	2.4	2.5	2.28±0.13
	37	2.7	2.5	2.4	2.4	2.5	2.4	2.48±0.11
	40	2.8	5.1	5.8	8.2	12.5	19.7	9.02±5.65
	50	3.6	3.8	4	4.4	4.6	4.5	4.15±0.37
	60	3.2	3.2	3.3	3.3	3.4	3.2	3.27±0.07
Molasses	25	2.1	2.2	2.3	2.4	2.6	2.5	2.35±0.17
	37	1.6	1.3	1.9	2	2.2	2.1	1.85±0.31
	40	3.1	4.2	6	8.8	13.9	13.9	8.32±4.32
	50	3.1	4.3	5	6.5	7.2	7.3	5.57±1.56
	60	2.7	2.8	2.8	3	2.8	2.8	2.82±0.09
Fruit waste	25	3	3.1	3.2	3.3	3.6	3.6	3.30±0.23
	37	3.1	3.2	3.3	3.4	3.5	3.7	3.37±0.20
	40	2.9	2.9	3	3.1	3.2	3.4	3.08±0.18
	50	3.9	4	4.2	4.5	4.5	4.5	4.27±0.25
	60	3.6	3.7	3.7	3.8	4	4.2	3.83 ±0.21
Vegetable garbage	25	3.4	3.2	3.6	4	4.7	5.5	4.07±0.80
	37	4.3	5.6	4.8	6.4	6.9	8.7	6.12±1.45
	40	2.2	3.4	11.9	19.8	19.9	17.6	12.47±7.34
	50	3.3	3.4	4.4	8.9	9.9	10.8	6.78±3.15
	60	3	3.1	3.5	4	3.8	3.7	3.52±0.36

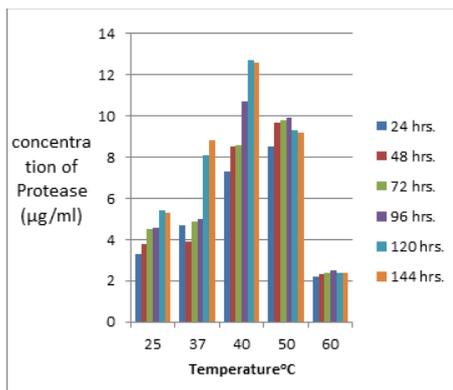


Fig 1: Effect of temperature on production of protease by *Fusarium oxysporum* in a SSF using dal mill waste.

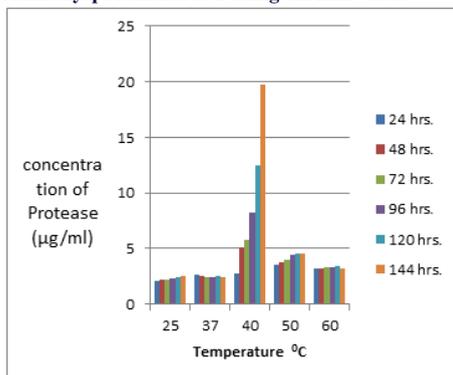


Fig2: Effect of temperature on production of protease by *Fusarium oxysporum* in a SSF using oil mill waste.

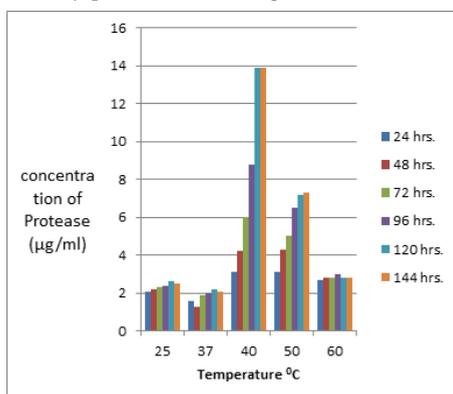


Fig 3: Effect of temperature on production of protease by *Fusarium oxysporum* in a SSF using molasses.

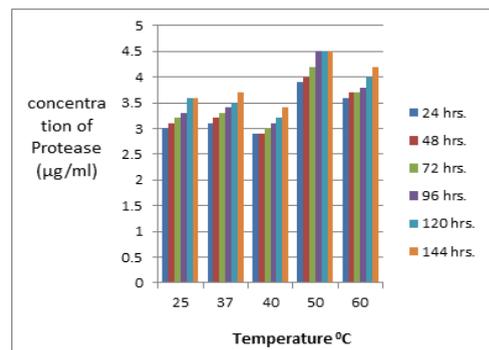


Fig 4: Effect of temperature on production of protease by *Fusarium oxysporum* in a SSF using fruit waste.

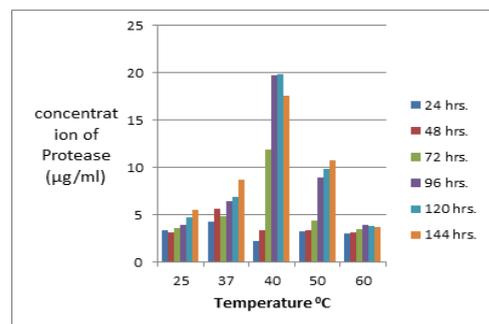


Fig 5: Effect of temperature on production of protease by *Fusarium oxysporum* in a solid state fermentation using vegetable garbage.

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