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**Studies on the production of protease from agro residues *Convalia ensiformis* beans by solid state fermentation (SSF) using *Bacillus subtilis* 2724NCIM**

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**ABSTRACT**

The agro wastes are being dumped into the domestic environment causing unhygienic to the residents, and foul odor is becoming a common problem. Most of the agricultural wastes are contributing solids wastes, which are becoming hazardous to the public. By utilize the agro wastes for the bioconversion process we can reduce the environmental pollution problems. *Convalia ensiformis* beans have low nutritional quality and high content of crude fibre in the seed coat. Hence these beans are being neglected for consumption. In the present study an attempt was been made to utilize these beans for the production of protease enzyme by solid state fermentation (SSF) using *Bacillus subtilis* 2724NCIM. The study indicated, these beans were more suitable for the production of protease, which showed an activity of 680µg/ml. Various parameters like optimum  $p^H$ , temperature, substrate concentration, effect of carbon and nitrogen source were studied. The study indicated the best utilization of agro wastes for the production of protease enzyme which has wide applications in the field of food and pharmaceutical industries. These bioconversion studies of agro waste contributed the management of environmental safety and health by effective utilization of agro residues.

**Keywords:** *Convalia ensiformis*, Bioconversion, *Bacillus subtilis*, Agro residues, solid state fermentation, Plots.

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**INTRODUCTION**

Solid wastes from Indian cities contain high proportion of organic matter and also have high moisture content. The organic food content attracts flies and rodents that cause foul smell

(Krishnasureshbabu.*et.al*, 2005). The garbage has low combustible material and so its calorific value is usually low being 1500 kcal/kg (Kunamneni Adinarayana.*et.al*, 2003). Thus municipal solid waste is a resource for producing energy (Prakasham.*et.al*, 2006). Among the solid waste the agricultural wastes comprises 50-65 %. The agricultural waste from animal manure and crop residue (Subarea, *et.al*, 2005). The principle sources of solid waste are domestic commercial, industrial and agricultural activities. Agricultural wastes comprise both crop residues and animal wastes such as manure and urine. Animal and vegetable waste contains valuable minerals and nutrients. Humus from agricultural wastes contributes fertility of the soil and optimum plant growth (Subarea, *et.al*, 2005). Waste is a valuable raw material. It can be converted into useful product by making use of appropriate process technology (Patel, R.k *et.al*, 2006).

In the present study the agricultural residues like *Convalia ensiformis* beans are utilized for the production of protease enzyme by solid state fermentation. This reduces the agricultural waste by their effective utilization to produce useful enzymes.

### EXPERIMENTAL SECTION

The organism used in this *Bacillus subtilis* 2724NCIM, obtained from National Collection of Industrial Micro organisms, Pune.

The composition of the nutrient agar medium (g/l)

Yeast extract	1.5
Beef extract	1.5
NaCl	5.0
Peptone	5.0
Agar	15.0

In this research work, the fine powder of *Convalia ensiformis* beans used as substrate. Equipment and analytical instruments such as autoclave, incubator, orbital shaker, electronic digital weighing machine, hot air oven, laminar air flow station and visible spectrophotometer were used for synthesis and characterization of substrate.

#### Methods

The protease producing bacterial strain was grown and maintained on nutrient agar medium slants. After cultivating for 24 hours at room temperature 30<sup>0</sup>c. Cultures were subcultured for every 4 weeks. The agro waste material (agro residues) *Convalia ensiformis* beans were collected from the local vegetation and grind to fine powder using ball mill, sun dried and autoclaved before use. The bacterial culture on the other slant was scooped and washed from the slant with 5 ml of sterile water and 2 ml of inoculums was added to each conical flask (250 ml size) containing 10 grams of production medium.

#### Solid state fermentation

In solid state fermentation, 10 grams of fine powder of the *Convalia ensiformis* beans powder was taken into 250 ml Erlen Mayer flask and 4 ml of water was added, mixed thoroughly and autoclaved at 121<sup>0</sup>c for 15 lbs. after cooling these flasks were inoculated with 2 ml of 24 hours grown culture strain. The contents in the flask were mixed thoroughly and incubated at 30<sup>0</sup>c for 24 hours. After adjusting the p<sup>H</sup> of the medium to p<sup>H</sup> 7.0.

### Enzyme extraction

Enzyme was extracted from the nutrient broth (production media) , according to the methods described by (nagamani et. al.2003), fermented medium was mixed thoroughly with 50 mM glycine NaOH buffer(p<sup>H</sup> 11) and the extract was reported by filtering trough whatman No.1 filter paper. This process was repeated for 3 times and extracts were pooled together and then centrifuged. The supernatant was used as enzyme source.

### Enzyme assay

The protease p<sup>H</sup> 10.4 activities was assayed by the modified Anson method.1ml of enzyme was added to 6ml of casine (0.6 w/v 0.2 M glycine NaOH buffer p<sup>H</sup> 10.4) and the reaction mixture was incubated at 37<sup>0</sup>c for 10 minutes and then 6 ml of TCA was added.

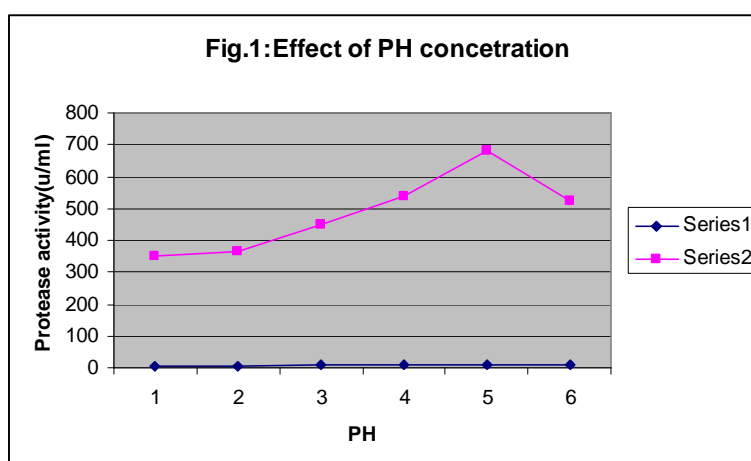
The TCA terminated for 30 minutes at room temperature the precipitates were removed by filtration through whatman no.1 filter paper and to 1 ml of filtrate 2 ml of FC reagent (folin ciocalteu's) was added and observe the optical density at 660 nm.after study for 30 minutes. One unit of alkaline protease activity was defined as the amount of enzyme liberated in 1 gram of tyrosine for minute under assay conditions. Enzyme units were measured using tyrosine (10-100 mg).

## RESULTS AND DISCUSSION

Protease enzyme was produced using *Convalia ensiformis* beans by *Bacillus subtilis* 2724 NCIM. The enzyme Production was optimized under the parameters like incubation time, substrate concentration, pH level, temperature. And also the nutritional supplementation was carried out using various carbon and nitrogen sources to enrich the production medium to enhance the enzyme yield. The results of the present study discussed here.

### Effect of time

To determine the effect of time on the production of protease under the various time intervals like 12, 24, 48, 72 hours, the maximum has obtained at 24 hours



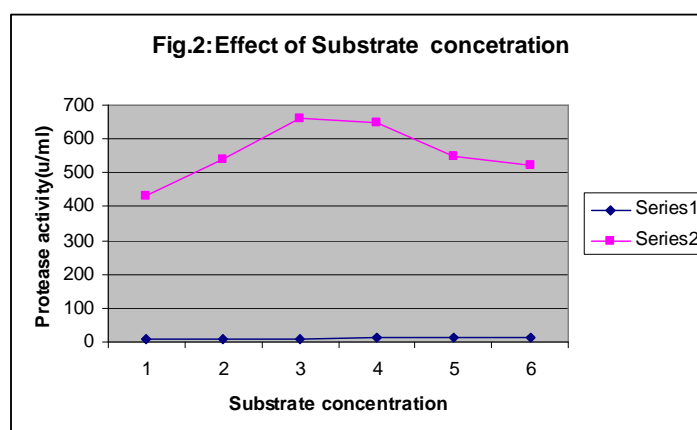
### Effect of pH

p<sup>H</sup> variation brought drastic change in the production of protease.The production medium was adjusted to different p<sup>H</sup> ranges such as 6,7,8,9,10,11.flasks were incubated for 24 hours.

The enzyme activity was maximum at  $p^H$  10. The maximum enzyme activity was obtained 680.1 U/ml.

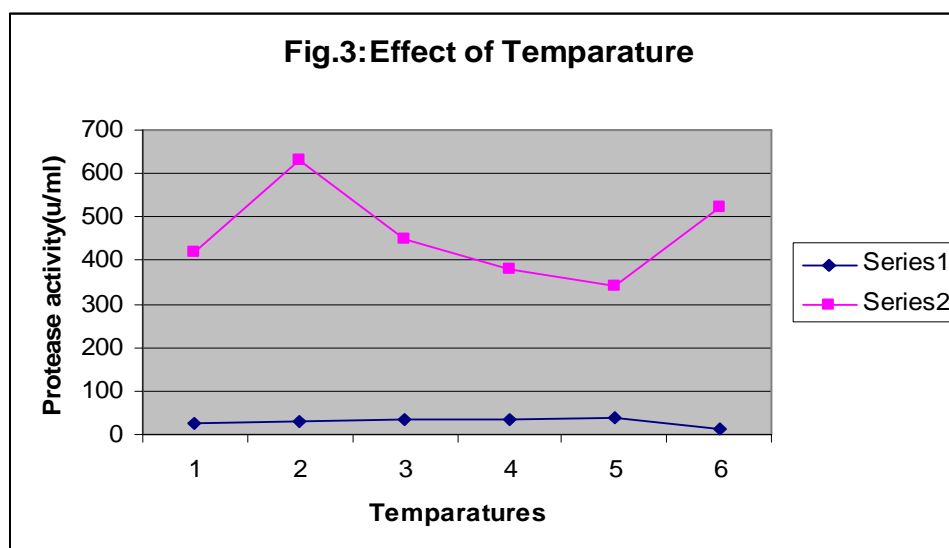
### Effect of substrate

The substrate concentration varies from 8, 9, 10, 11, 12.grams were prepared at  $p^H$  10 into the 250ml flasks were inoculated for 24 hours and the maximum enzyme activity was obtained 660.4 U/ml at 10 grams.



### Effect of temperature

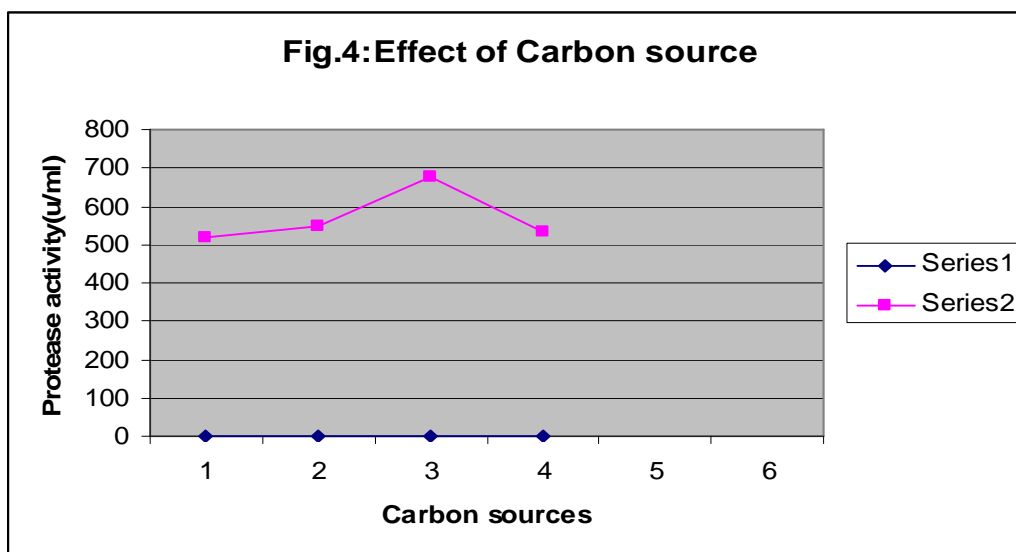
The production medium was applied for various temperature levels at  $27^0, 30^0, 33^0, 36^0, 38^0$  centigrades.the maximum enzyme yield obtained at  $30^0$  c and the maximum enzyme activity was founded as 629.7U/ml.



### Effect of Carbon supplementation

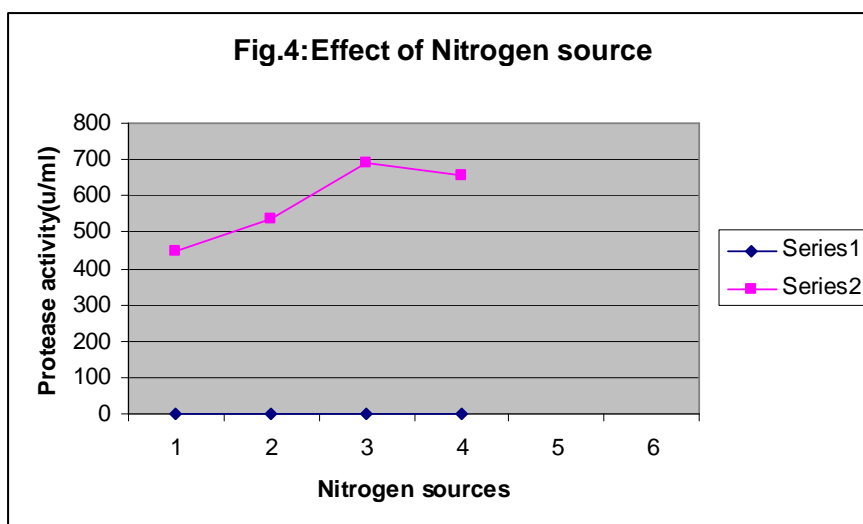
To determine the effect of carbon supplements were added into the production of protease.The supplements which included in that are glucose, galactose, Dextrose and maltose. Each of these at a concentration of 1% w/w was added to the production medium. The flask were inoculated and incubated. After inoculation period over the enzyme was

extracted and assayed. The result indicated that the maximum enzyme activity was obtained as 675.9 U/ml.



#### Effect of Nitrogen supplementation

To determine the effect of different Nitrogen sources on the production of protease enzyme. The Nitrogen sources Ammonium Chloride, Ammonium Sulphate, Peptone, beef extract were added to the production medium and flask were inoculated and incubated for 24 hours and the samples of enzyme were extracted and assayed finally observed the Optical density (O.D). The results indicated that the maximum enzyme production enhanced by the enriched nitrogen supplements. Among these supplements beef extracts consisted flask obtained 690.2 U/ml of the enzyme activity was founded.



#### CONCLUSION

These results indicated that the agro residue *Convalia ensiformis* beans powder was more useful for the production of protease enzyme by solid state fermentation. Thus several agro

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residues can be converted as useful feeds for the microorganisms, hereby production of enzymes like proteases which have significant role in the food, textile and pharmaceutical industries. The solid waste removal and management through SSF is recommended.

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