

Determination of Alkaline Protease Production In *Serratia Marcescens* Sp7 Using Agro Wastes As Substrate Medium, Optimization Of Production Parameters And Purification Of The Enzyme

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The enzyme alkaline protease production was determined under solid state fermentation using the soil bacteria *Serratia marcescens* sp7. The maximum production was obtained from wheat bran medium than ground nut shell and chemically defined medium. The physiological fermentation factors such as pH of the medium (pH 8), Temperature (40°C) and incubation time (48 hrs) played a vital role in alkaline protease production in all the above. 100Mm NaCl has given better resolution during elution of the enzymes. The enzyme production was found to be associated with growth of the bacterial culture.

KEY WORDS: Alkaline protease, Wheat bran, Ground nut shell, *Serratia marcescens*

I. INTRODUCTION

A huge amount of widespread microorganisms naturally existing in different environments are able to produce proteases. This is the most required enzyme in detergent industry which accounts more than 60% of sales than others in the international enzyme market [1], [2]. *Serratia marcescens* is a gram negative, rod shaped saprophytic organism can able to produce protease[3]. Protease enzyme has number of practical applications in industries such as detergent [4], [5], tanning [6], [7], dairy, baking, surgical cleaning [8], brewing [9] and medical applications [10], [11]. The most widely used of these proteases where function and mode of action is to remove a protein stains such as grease, egg and human sweat and blood by proteolytic degradation to polypeptides that are more soluble and amino acids.

Among the various vast pool of enzymes, proteolytic enzymes from microorganisms are the most wanted enzymes in the detergent industries worldwide. Look into the depth of microbial diversity, there is always a chance of finding microorganisms producing alkaline enzymes, which are suitable for the manufacture of "biocleaners" [8].

Moreover, the discovery of new bacteria or enzymes may open up new areas of basic research in the fields of nonaqueous enzymology and applications.

So far the in view of the above, the present study was aimed for the Proteases production using agro wastes through solid state fermentation. In this study we report on the screening of *S.marcescens* sp7 for its ability of producing proteases using agro wastes such as wheat bran, ground nut shell and their protease production efficacy was compared with chemical medium.

II. MATERIALS AND METHODS

Identification of Microorganism

The Strain *Serratia marcescens* sp7 used in this study was isolated from soil. The strain was identified by morphological and biochemical tests. The organism was maintained on nutrient agar slants at 4°C. Transfers were made every 4 to 6 weeks to maintain culture viability.

Determination of Protease Activity

The strain was inoculated on the Skim milk agar (skim milk – 100gms) and incubated for 24 – 48hours at 37°C.

Fermentation Studies

The production of proteases was carried out by solid state fermentation (SSF) method. Ground nut shell medium(sieved ground nut shell – 100gms, Sodium chloride – 0.5gms, Casein – 0.5 gms, Distilled water -250ml, wheat bran medium(sieved Wheat bran – 100 gms; sodium chloride, casein – 0.5gms; Distilled water – 250ml) and chemical medium (Glucose – 100mg; peptone – 0.5g; KH₂PO₄ - 0.5gms; MgSO₄.7H₂O – 0.2gms; FeSO₄.7H₂O – 0.01gms; Casein digest – 1.5gms; Lactose – 2.0 gms; Distilled water – 100gms) were used in this study. The contents of the flask were inoculated with 1mL of inoculums (1x10⁸cells/mL) after autoclaving. The contents were mixed thoroughly by gently beating the flasks on the palm of the hand and incubated in different incubation temperatures (12, 24, 36, 48 and 72 hrs).

Determination of pH and Temperature on Protease Production

The protocol adopted for the optimization of culture conditions influencing alkaline protease production was to evaluate the effect of individual parameters. The parameters optimized were: (a). various incubation temperatures range

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from 25, 35, 40, 45, 50°C (b). Various pH conditions range from 6, 6.5, 7, 7.5, 8, 8.5.

Enzyme Assay and Protein Estimation

After the fermentation, culture mass of solid medium was extracted with 1:10 volumes of distilled water (pH: 7) with shaking at 160 rpm for 1 h at 25-30°C, centrifuged and supernatant was used as the crude enzyme extract for the assay. Protease activity in culture supernatant was determined using spectrophotometric method [12]. Enzymes solution (1.0ml) was incubated with 1.0ml of 2.0% (w/v) casein in phosphate buffer(50mM, pH 7.0) at 50°C for 10 min and then the reaction was terminated by the addition of 5.0ml (5.0% w/v) aqueous solution of trichloroacetic acid. After 30 min of incubation at room temperature, the mixture was filtered and 2.0ml of filtrate was added to 4.0ml of 0.1N NaOH and 0.5ml of diluted folin-ciocalteu reagent. The tyrosine residues released by enzymatic hydrolysis of protein were determined [13]. A separate blank was set up to correct the non-enzymatic release of tyrosine. One unit of enzyme activity was defined as the amount of enzyme required to release one µg of tyrosine per min with bovine serum albumin (BSA) as standard under the assay condition described. The values were calculated at 660 nm for enzyme and 760nm for protein.

III. RESULTS AND DISCUSSION

The Solid State Fermentation process was observed to be less sensitive to contamination than submerged fermentation. In contrast general belief that the Solid State Fermentation technique is not suitable for bacterial and other cultivation because of their requirement for higher water activity, the enzymes titres produced SSF were higher than those in submerged fermentation.

In SSF, the selection of a suitable solid substrate for fermentation process is a critical factor and thus involves the screening of a number of agro-industrial materials for microbial growth and product formation. All the substrates used in the study supported the growth and enzyme production, while proved higher to other substrates. A high titre of protease activity was obtained in a medium containing wheat bran as substrate followed by ground nut shell and chemical substrates.

Effect of pH and Temperatures

The effect of physical factors, such as, pH and temperature, on the production of crude enzyme was comparatively investigated using wheat bran, ground nut shell and chemical mediums.

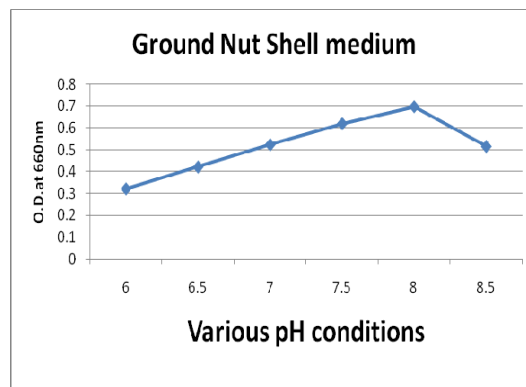


Fig. 1(a) Determination of pH conditions on the production of Protease enzyme from Ground nut shell as substrates

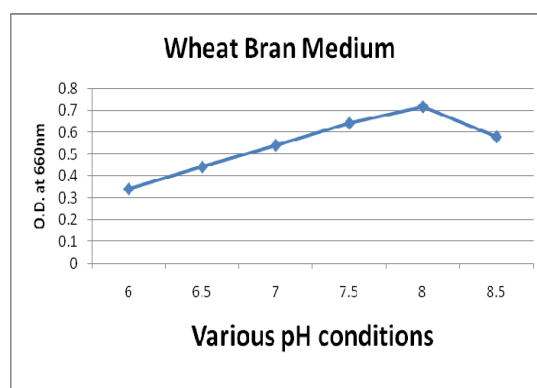


Fig. 1(b) Determination of on the production of Protease enzyme from Wheat bran medium

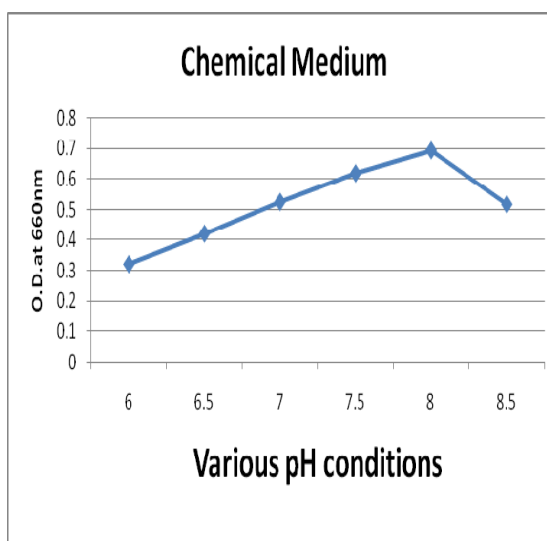


Fig. 1(c) Determination of on the production of Protease enzyme from Wheat bran medium

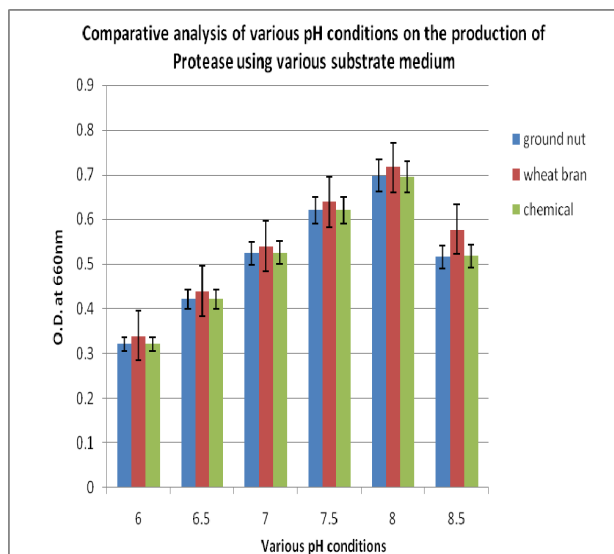


Fig. 1(d) Comparative analysis of pH condition on the production of Protease enzyme with various substrates

The present study results showed maximum protease production was obtained in all the production medium at the pH of 8 in Figure 1(a), (b), (c), (d). Wheat bran medium showed highest activity than groundnut shell medium and chemical mediums. It was observed that the pH of the fermentation medium reached a high value 8 on all the production mediums. The enzyme activity dropped gradually at pH after 8.5. This enzyme is stable at 8 pH [6 ramakrishna].

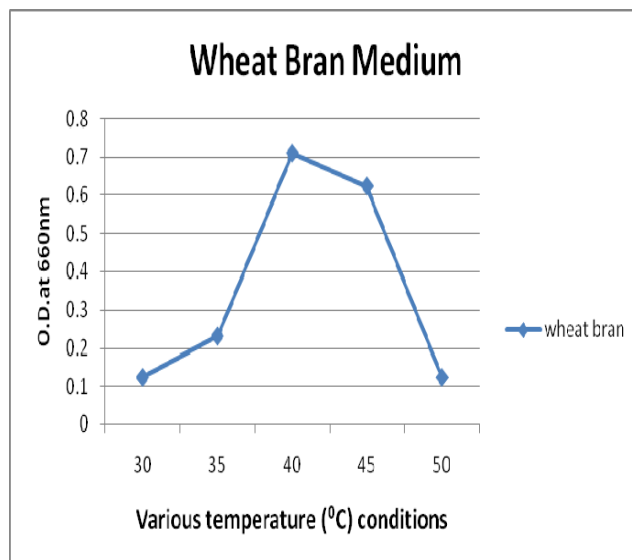


Fig. 2(b) Determination of on the production of Protease enzyme from Ground nut shell as substrates

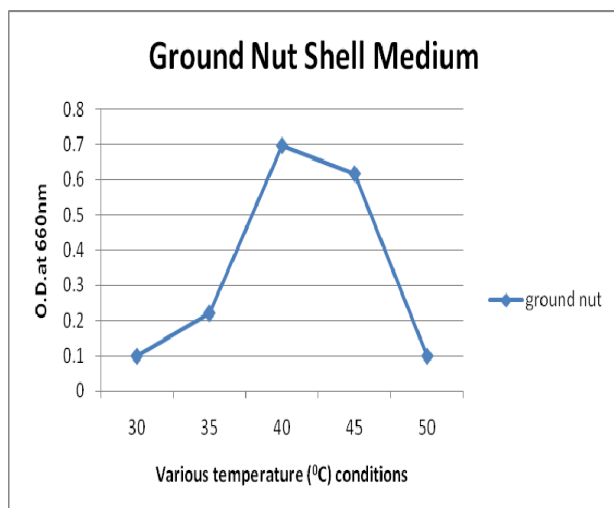


Fig. 2(a) Determination of Temperature on the production of Protease enzyme from Ground nut shell as substrates

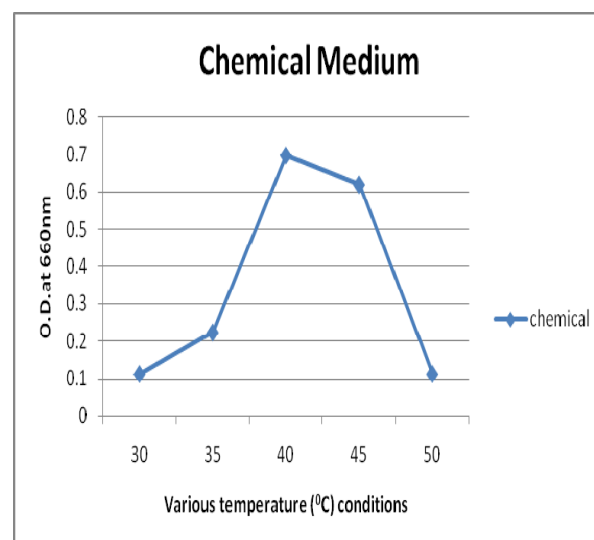


Fig. 2(c) Determination of on the production of Protease enzyme from Ground nut shell as substrates

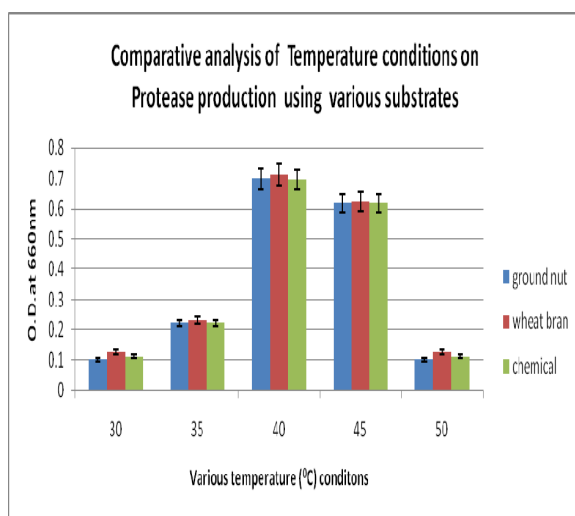


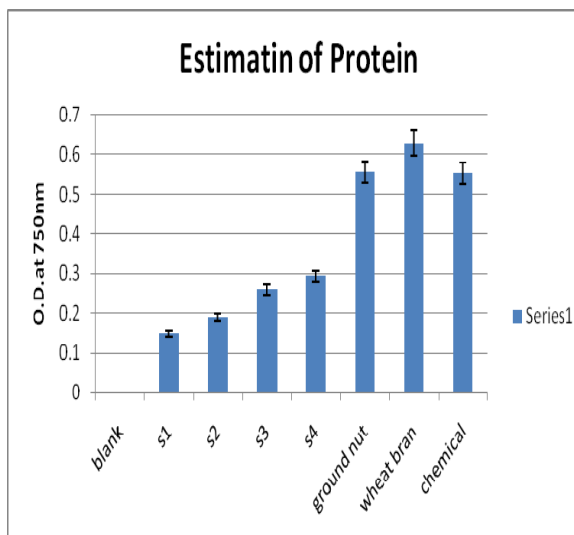
Fig. 2(d) Determination of on the production of Protease enzyme from Ground nut shell as substrates

Determination of the temperature on enzyme production was presented in Figure 2a-2d (2a,2b,2c,2d). The temperature chart showed that the enzyme production was gradually increased from 30 to 40°C, followed by a gradual decline thereafter. The maximum activity attained at 40°C [14] by all the production mediums. Especially wheat bran has more production range while compare with other two mediums. Even they are also showed its maximum production activity at the same temperature. However while compare with Chemical medium the ground nut shell medium also has no huge production differences. Previous report estimated the maximum enzyme production was obtained from Wheat bran medium pH of 9 and temperature 45°C [8].

ESTIMATION OF PROTEIN:

At the pH 8 and 40°C temperature conditions yielded enzyme proteins were measured and its results were plotted on Figure 3.

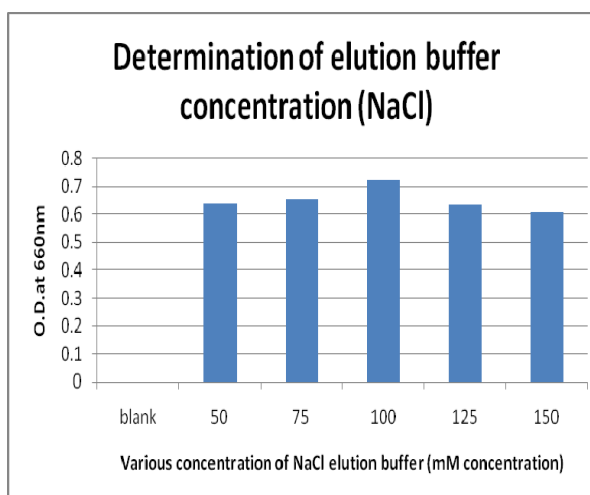
Figure (3): Estimation of Protein



The Wheat bran medium yields the maximum protein yields than ground nut shell medium and chemical medium. This result interprets that the agro waste products like Wheat bran and ground nut shell would be the best source for the protease enzyme production.

ELUTION BUFFER CONCENTRATIONS:

Figure 4: Determination of NaCl elution buffer concentration:



As in Figure 4, the efficiency of elution buffer concentration which is more important during purification of the enzyme. 100mM of NaCl along with 25mM HCL as constant has showed best elution concentration. But, at higher NaCl concentrations (125, 150, 175) the purification of protease enzyme level is decreased. 100mM NaCl elution buffer concentration gives the better resolution in this study.

CONCLUSION:

Finally, from the above results, the role of agro waste [14], [15] role in Protease enzyme production was identified and production parameters were determined. The wheat bran and ground nut shell can be the less expensive alternative active substrates in the production of alkaline proteases enzyme [2gupta]. These significant results gives new hope in the enzyme based detergent industries. The soil isolated *Serratia marcescens* sp7 has been potentially act on the wheat bran [16] and ground nut shell medium and yields maximal enzyme products. This new area of research would be the challenging topic in the future bioprospecting world. Further studies of protein sequence and strain development are in progress.

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