Research Paper

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Performance Evaluation and Optimization of Process Parameters of Urease Enzyme Extracted from Bio-Waste Materials

Environment

KEYWORDS : Urease, Bio-waste materials, Citrullus vulgaris, enzyme activity, process parameters

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ABSTRACT

Studies on enzyme activity and optimization of process parameters were carried out in laboratory to determine the enhanced activity of Urease enzyme extracted from different biomaterials which include unwanted inedible seeds. The present study attention was mainly directed to investigate the maximum potency of the Urease activity extracted from unusable inedible seeds disposed as a waste i.e. seeds from different fruits such as Pouteria sapota, Tamarindus indica, Citrullus vulgaris, Citrus limonium and Citrus aurantium. A detailed study of these seeds along with its inner and outer layers were studied for enzyme activity, interestingly it was observed that the enzyme activity of Citrullus vulgaris was found to increase with subsequent time interval. The various process parameters like pH, Temperature, Substrate concentration, Enzyme concentration enhancing the urease activity of Citrullus vulgaris were also studied. The study reveals that the activity of the enzyme was found to be more at a pH 7 and has increased with an increase in substrate concentration. The optimum enzyme concentration was at 0.5% with an optimum temperature of 37 0c. Citrullus urease is for this purpose, distinctly superior than other fruit preparation because it determines urea quantitatively which gives abnormally high values when the sources of urease are used.

Introduction

Agriculture needs inputs of many subsidies like soil nutrients, pest control and energy inputs. Among soil nutrients NPK are bulk essentials in demand. In these three, nitrogen is an essential plant nutrient key to the sustainability and economical viability of agricultural systems, that most influences crop production and it is generally applied to soil with fertilization representing the largest amount (Camilla giovannini, etal 2009). The highly dynamic nature of "N" makes it efficient use and management a challenging task (Zaman, M 2010). This increase in the use of nitrogen fertilizer has led to massive increase in agricultural yield and has, infact allowed humans to largely avoid shortages historically predicted to accompany our recent population boom. In this sense nitrogen fertilizer has been an enormous boon to humans. However the recent in nitrogen use may have serious potential drawbacks as well, such as aquatic pollution and the increased production of green house gases leading to global climate change. Human urine is rich in nitrogen and the growing requirements of nitrogen for our food security can be easily met with urine harvesting as in the living systems during catalytic destruction of waste materials nitrogen has to be rejected as innocuous material into the environment, the animals especially the vertebrates discard ammonical nitrogen in various forms of the amide urea (Vinneras 2002). Human urine contains >2% of urea, a non toxic compound. The major fraction of Urine N is Urea (Zaman et al., 2007, 2009), while the rest is a mixture of readily mineralizable amino acids, peptides and ammonium-N (NH_4^+) (Bolon et al., 2004). Urea constitutes the predominant source of industrial N fertilizer used in agriculture and represents 46%of world consumption of nitrogenous fertilizers (Watson C.J.2000).

Urea can be an inefficient N source due to its rapid hydrolysis caused by soil urease (Watson. C. J 2000). Ammonia accumulation can cause NH_3 losses by volatilization that, especially in sand alkaline soils and urea surface application, can exceed 50% of the N applied (Terman,G.L. 1979) . Considering these aspects investigations are carried out in recovery of nitrogen from urine which is a valuable fertilizer and can result in a lower ecological burden in comparison to the use of chemically produced fertilizers (Maurer, et al 2003). The nutrients in urine reflect the components necessary for plant growth, which make it suitable as a fertilizer in agriculture applications. The rate of hydrolysis of urea is related to Urease activity, pH, Temperature and the form in which urea is applied (vlek, P.L.G et al, 1983).

Enzyme plays an essential role in the metabolism of all organisms. They catalyze and control most biochemical reactions in our body from the replication of genetic information (DNA polymerase) to digestion. Enzyme activity however, is not always easy to visualize. (WWW.scienceinschool.org/print/607). Urease is a nickel metallo enzyme that catalyses the degradation of urea to ammonia and carbamine acid. The latter compound decomposes to generate a second molecule of ammonia and carbon dioxide (Mulvancey and Bremner, 1981). This plant protein was the first enzyme ever crystallized (Sumner, 1926) and the first enzyme to contain nickel ion, the crystal structure of the active centre of Urease contain probably two simple coordinated water molecules and a bridging –OH group. The substrate binding site of Urease is perfect constructed and the specificity of enzyme is closely related to the shape of its active centre (WWW.chemic.uniregensburg.de). The enzyme found to be in large quantities in plant seeds, it also occur in large quantities in plant seeds, it also occur in some animal tissues and intestinal microorganisms (Ainsworth, 2008).

The present study is carried out in laboratory to determine the maximum activity of Urease enzyme extracted from bio-waste materials and mainly focused on various process parameters enhancing the activity of Urease enzyme.

Materials and methods

Sample preparation

Seeds of different varieties i.e., Pouteria sapota, Tamarindus indica, muskmelon, Citrullus vulgaris, Citrus limonium and Citrus aurantium were separated from unwanted waste material collected from juice shops located in the surrounding area of Kukatpally, Hyderabad. Approximately hundred seeds of each variety were taken, washed and were dried in sunlight for two successive days. The dried seeds were grinded into fine powder and are sieved to <2mm in order to get uniform particle size.

Enzyme extraction

Enzyme was extracted from each variety by dissolving 0.5gm of fine powder into 100ml of phosphate buffer and stirred continuously for about half-an-hour with the help of magnetic stirrer. The supernatant obtained containing enzyme was collected for estimating the respective enzyme activity by following phenol hypochlorite method.

Determination of Enzyme activity

Urea solution of 25ml was added into each test tube followed by 1ml of the different enzyme extract were added and incubated over a time period of 1,2,&24 hrs. After incubation of the respective time intervals enzyme activity was estimated by quantifying the ammonia produced by the activity of urease enzyme on urea.

Quantification

Ammonium chlorides of different standards were prepared whose concentration varies from (1-10µg/ml) while the test solution (urea solution+enzyme extract) of 1ml each were added into the test tubes and made to final volume of 5ml by adding distilled water. Phenol and sodium nitroprusside of 0.2ml each were added followed by 0.5ml of oxidizing solution was added, mixed and incubated at room temperature for about 1 hour for colour development. The intensity of the colour developed was measured spectrophotometrically at 640nm.The concentration of ammonia produced by the Urease enzyme was determined by plotting a graph taking concentration on x-axis and absorbance on Y-axis.

Determination of optimal conditions

Optimal conditions for the enhanced activity of Urease enzyme were determined by varying factors like time, ph, temperature, substrate concentration and enzyme concentration.

Results and discussions

For the present study the maximum activity of urease enzyme was determined by extracting it from different seeds of fruits that were disposed as waste. A detailed study of the seeds along with its inner and outer layers were studied for enzyme activity, interestingly it was found that the enzyme activity of water melon was found to increase with subsequent time interval. The decreasing order of the enzyme activity among the seeds chosen were the Citrullus vulgaris followed by musk melon seed, Tamarindus indica, Pouteria sapota, Citrus aurantium and Citrus limonium seeds respectively which is shown in figure-1 that the maximum activity of enzyme was found to be in Citrullus vulgaris. This is due to the presence of urease in considerable quantity in the cotyledons of water melon and decomposes more than their own weight of urea every hour. Urease activity in the cotyledons changes during growth, showing an initial rise followed by an abrupt drop almost to zero. These changes in cotyledonary urease constitute merely one aspect of the "protoplasmic differentiation" that takes place as a cell matures (Williams, W.T. 1950). The maximum activity of the urease in the seeds of watermelon is shown to be a potent source of urease suitable for use in urea determination.

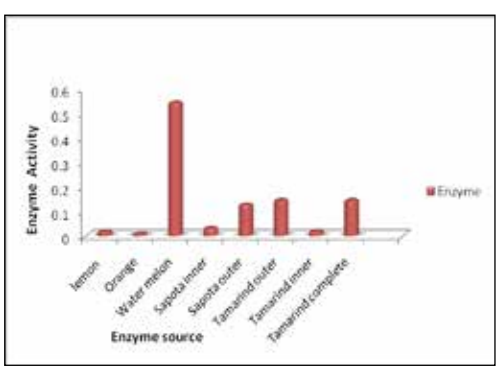

Fig-1 Activty of urease enzyme extracted fron different seeds

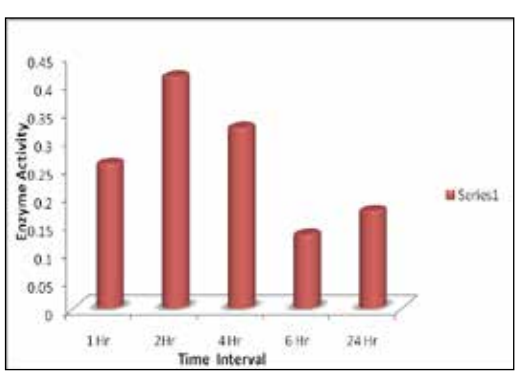

Fig- 2 Optimum time interval for urease enzyme

Effect of time on activity

This experiment was conducted to enhance the activity of urease enzyme by incubating at different time intervals of 2, 4, 6, and 24hours. The results of the experiment are represented in fig- 2 which shows that the enhanced activity of the urease was found to be at a time period of two hours while decreases with an increase in the time interval, this is due to the saturation of the active sites of the enzymes by the substrate molecules and no longer involved in breakdown of it (Lehinger, 2002).

Effect of enzyme and substrate concentration

The enhanced activity of urease enzyme of varying concentration was carried out by preparing different enzyme concentration of enzyme extract and incubating in different test tubes containing urea solution over a time period of thirty, sixty, ninety, minutes and two hours. Experimental results showing the enhanced enzyme activity at varying enzyme concentration are presented in fig-3(a) and 3(b) which states that the activity found to be optimum at 0.5%, initially at low enzyme concentration there is a great competition for the active sites as the concentration increases the reaction proceeds at a faster rate due to more active sites. Eventually increase of concentration beyond the optimum level has no effect as the enzymes active sites are no longer saturated because substrate concentration becomes rate limiting. While the optimum substrate concentration for the enhanced activity of urease enzyme is shown in fig- 4. It shows the maximum urease activity was obtained at a substrate concentration of 5%. . Initially there is an increase in the activity of enzyme as there are many active sites that are not occupied by the substrate (Pozniak G, et al 1995) in addition an abrupt decreased was observed in the activity with increased substrate concentration because of formation of enzyme substrate complexes due to the saturation of active sites of the enzyme.

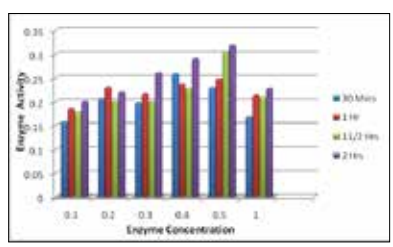

Fig- 3(a) Optimum Enzyme concentration for urease enzyme activty

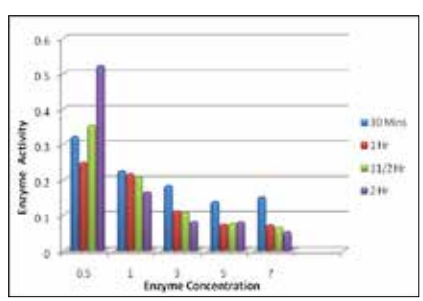

Fig- 3(b) Optimum Enzyme concentration for urease enzyme activty

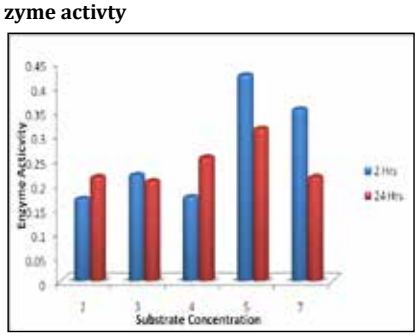

Fig- 4 Optimum Substrate concentration for urease enzyme activty

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Effect of pH and Temperature

Parameters of pH and temperature are very much important to be controlled to obtain reproducible results as they influence the stability and conformational structure of proteins (Akogal s, et al, 2002). The optimum pH enhancing the activity of urease is carried out by incubating the enzyme with substrate by varying pH of 5, 6, 7, 8, and 9 for a time interval of two hours and twenty four hours respectively. The reaction rate of urea hydrolysis by the urease is presented in fig -5 shows that the activity increases upto an optimum pH 7 and then decreases due to gradual ionizing of side chains (R- groups) of tertiary protein structure of enzyme. As the precise shape of an enzyme (and hence its active site) depends on the tertiary structure of the protein held together by weak bonds (including 'H' bonds) between R- groups (Side chains) the changing can cause these side chains to ionize resulting in the loss of hydrogen bonding leads to the loss of binding efficiency and eventually enzyme activity (Marzadori C. et al, 1988). The optimum temperature for enhanced urease activity was observed by varying the temperature from 20±5^{0}c to 50±5^{0}c. The activity of urease enzyme is strongly dependent on the temperature and a sharp optimum was obtained at 35 ^{0}c represented in the figure- 6. Initially the rate of hydrolysis observed to increase with increase in temperature due to more kinetic energy inducing collisions between enzyme and substrate enhancing the activity beyond which denaturation of the enzyme could take place on the tertiary structure of peptidic chains of the urease which would occur above 40 ^{0}c after two hours (Akogal s, et al, 2002). In other words as the temperature increases the structure of the enzyme becomes altered and its catalytic properties are eventually destroyed.

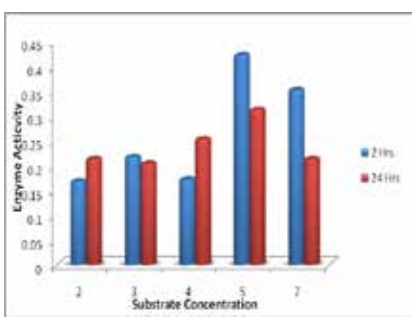

Fig- 5 Optimum pH for urease enzyme activty

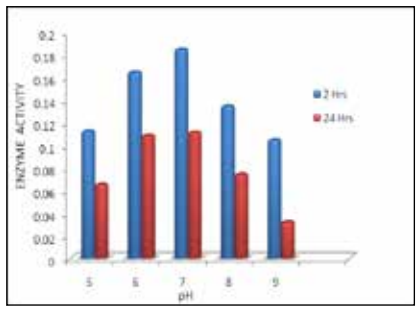

Fig- 6 Optimum Temperature for urease enzyme activty

Conclusion

The study reveals that the seed of Citrullus vulgaris shown to be a potent source of urease suitable for use in urea determination and the use of Citrullus vulgaris seeds as a source of urease is free from the error that pertain to the now widely used horse gram, jack beans and soya beans. The enzyme activity found to be more at a pH 7 and has increased with an increase in substrate concentration. The optimum enzyme concentration was found to be 0.5% with an optimum temperature of 37^{0}c. Citrullus urease is for this purpose, distinctly superior than other fruit preparation because it determines urea quantitatively which gives abnormally high values when the later sources of urease are used. Thus this highly stabled enzyme with optimized parameters can successfully be used for recovery of fertilizer by continuous decomposition of urea from biological fluid in a bioreactor.

The scope of further studies is to establish the state of knowledge on the immobilization of the enzyme and responses of immobilized enzyme activity under processed conditions for recovery of fertilizer from bio waste materials.

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