RESEARCH PAPER | Environment | Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

Isolation of urease rich bacteria and determination of its optimal conditions

KEYWORDS: Urease, enzyme activity , optimal conditions.

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ABSTRACT *In the present study the activity of microbial urease and optimization of process parameters were carried out in the laboratory. The study was majorly focused on to isolate the urease rich bacteria from soil samples and also directed to determine the optimal conditions such as substrate concentration, enzyme concentration, time, pH and temperature that enhance the activity of the urease rich bacteria for bioreactor process.*

Introduction
Nitrogen is a crucial element for plant growth. It is an essential plant nutrient key to the sustainability and economical viability of agricultural systems. The highly dynamic nature of "N" makes it efficient use and Management a challenging task (Zaman, 2010) especially in intensive agriculture systems were huge N inputs are likely to result in significant N looses via surface runoff of NH_4^+,NO_3, leaching in to ground waters and gaseous emissions of NH_4^+And N_2O in to the atmosphere .Such N losses have economical implications and pose threat to environmental quality worldwide (Boyer et al, 2002) In addition to this in the living systems during catalytic destruction of waste materials nitrogen has to be rejected as innocuous material in to the environment while much of the wasteful nitrogen in the plant life is reused, the animals especially the vertebrates discard ammonical nitrogen in various forms of the amide urea. Human urine contains >2% urea, a non toxic compound.

Urea is a major nitrogenous product of biological actions. In nature, urea is short-lived and rapidly metabolized by microbial activities. It penetrates into bacterial cells by diffusion, where the enzyme urease catalyses the hydrolysis of urea, leading to production of ammonia and carbamate. In aquatic condition, carbamate spontaneously hydrolysis to ammonia and carbonic acid. The ammonia is not only used as nitrogen source, but also contributes to the ecological niche of bacteria. Urea constitutes the predominant source of industrial N fertilizer used in agriculture and represents 46% of world consumption of nitrogenous fertilizers (Watson, 2000). It contains major fraction (80%) of urine N (Zamam et al, 2007, 2009), while the rest is a mixture readily mineralizable amino acids, peptides and ammonium-N (NH^+_4) (Bolon et al, 2004). Considering these aspects investigations are carried out in recovery of nitrogen from urine which is a valuable fertilizer for urea production s and can result in a lower ecological burden in comparison to the use of chemically produced fertilizers (Maurer et al, 2003).

The present study was carried out in the laboratory to isolate and determine the activity of urease rich bacteria from urinated soil samples. The attention mainly directed towards the optimal conditions such as substrate Concentration, enzyme concentration, p^H and temperature that enhance the activity of microbial urease for bioreactor process.

Materials and methods
Sample Preparation
Soil sample enriched with urine (about 5 successive days) and garden soils were collected. A gram of each was weighed and dissolved in 10ml of sterile saline water and were serially diluted up to 10-7 dilutions. One ml from each of the dilution was poured and spread onto sterilized agar plates in duplicates to get isolated colonies.

Isolation and Sub culturing of Urease Rich Bacteria
Christenson's agar plates were prepared sterilized and inoculated with the isolated colonies of both garden soil and urinated soil sample and were incubated at 37^{0}C for about 24 hours later observed for the color change from orange to pink indicates the presence of urease rich bacteria. The isolated colonies were picked and were regularly sub cultured into fresh sterile agar medium for further use of immobilization studies

Determination of Enzyme activity
Urea solution of measured quantity was added into different test tubes and microbial enzyme extract was added into each of the test tubes and were incubated over a time period of 6 hrs and 24hrs. After incubation of the respective time interval the enzyme activity was estimated by quantifying the ammonia produced by the activity of immobilized microbial urease on urea. The quantification of ammonia was done at 640nm by following the process of phenol hypochlorite

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
Isolation and enrichment of Urease Rich Bacteria
Christenson's agar plates inoculated with garden soil and urine enriched soil samples were examined for the presence of urease rich bacteria by observing the color change of plates from orange to pink. After incubation period of 24 hours at 37^{o}c it was found that the plates inoculated with urine enriched sample had the color change from orange to pink compared to control indicating the presence of urine rich bacteria. This is due to breakdown of urea in the medium by urease rich bacteria present in the sample, while no Color change was observed in plates of garden soil indicating the absence of urease rich bacteria.

The colonies formed in the Christenson's agar medium were picked and isolated into agar slants enriched with urea. Colonies formed were regularly sub cultured for immobilization studies.

Fig-1 Comparison of sample plates with control

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

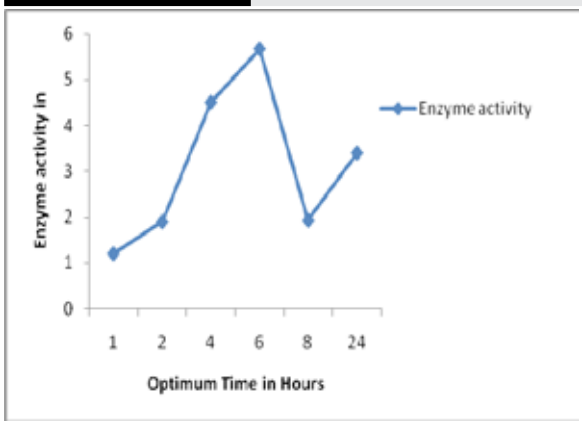

Fig- 2 Optimum time interval for microbial urease

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, 8 and 24hours. The results of the experiment are represented in fig- 2 which shows that the enhanced activity of urease was found to be at a time period of six hours while decreases with an increase in the time interval, this is due to the saturation of 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 microbial urease 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 six and twenty four hours. Experimental results showing the enhanced enzyme activity at varying enzyme concentration are presented in fig-3(a) which states the activity found to be optimum at 0.2%, 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 enzyme 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-3(b). It shows the maximum urease activity was obtained at a substrate concentration of 3%. Initially there is an increase in 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.

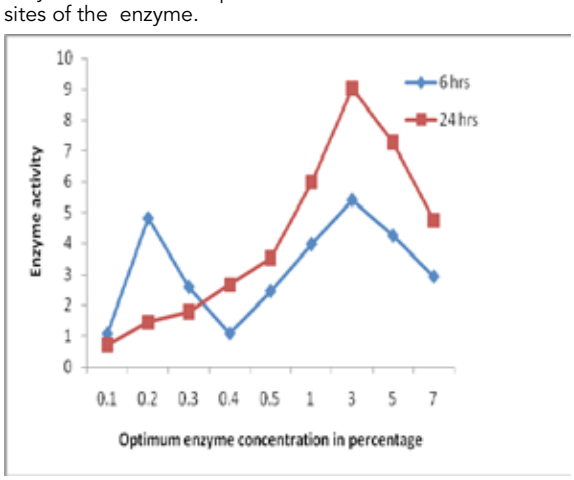

Fig- 3(a) Optimum Enzyme concentration for microbial urease

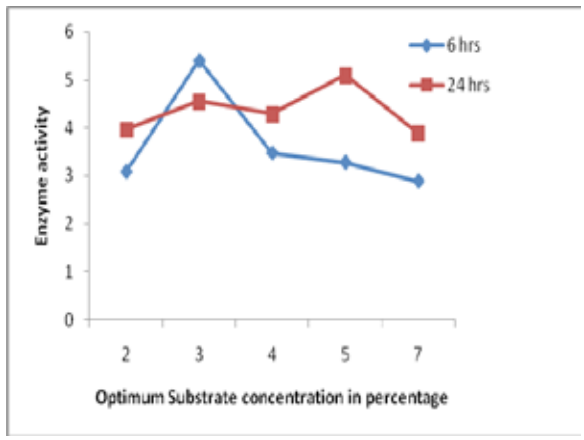

Fig- 3(b) Optimum Substrate concentration for microbial urease

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 urease is presented in fig-4(a) 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 loss of hydrogen bonding leads to the loss of binding efficiency and eventually enzyme activity (Marzadori C. et al, 1988). The activity of microbial urease is strongly dependent on the temperature and a sharp optimum was obtained at 35 ^{0}c represented in the figure- 4(b). 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.

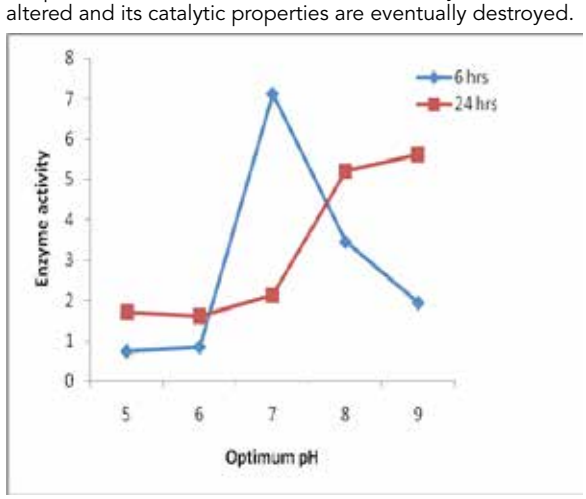

Fig-4(a) Optimum pH for microbial urease

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

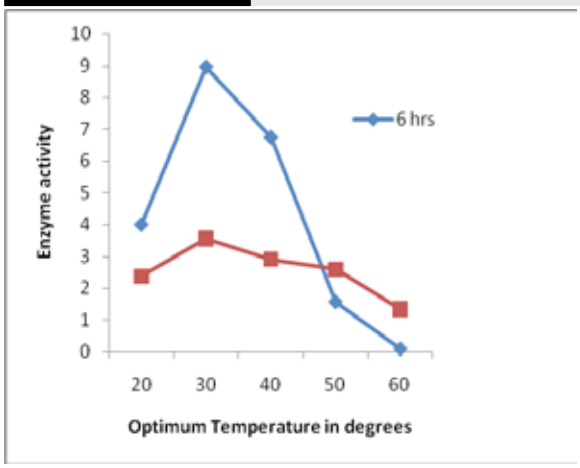

Fig-4(b) Optimum Temperature for microbial urease

Conclusion

The study reveals that the urease enzyme isolated from urinated soil sample observed to be a source of urease suitable for use in urea determination. The enhance activity of microbial urease was 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 0.2% with an optimum temperature of 30±5^{0}c. Microbial urease is for this purpose, distinctly superior than other 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.

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