Rice Husk: A Potent Source for Life Sustainability

P.Seshabala[1], A.V.S.Prabhakar Rao[2] and K. Mukkanti[3]

[1-3]Centre for Environment, IST, Jawaharlal Nehru Technological University,

Kukatpally, Hyderabad – 500 085 (India)

Abstract

Nitrogen fertilizer has been an enormous boon to humans as it is an essential plant nutrient and key to the sustainability and economical viability of agricultural systems. The same nitrogen which is rich in urine, rejected as innocuous material in various forms of amide urea into the environment during catalytic destruction of waste material in the living systems. Considering these aspects the growing requirements of nitrogen for food security can be met easily with urine harvesting in a bioreactor. Studies were carried out in laboratory for recovery of nitrogen from urine using bio-waste materials (seeds from different fruits such as Pouteria sapota, Tamarindus indica, Citrullus vulgaris, Citrus limonium, Citrus aurantium and Rice husk) as a source for preparation of enzyme and adsorbent beds in bioreactor. The present study was mainly directed to focus on the best suitable material as an adsorbent from the available resource materials in preparation of adsorbent beds for enzyme immobilization studies in a bioreactor. Various process parameters such as pH, temperature, substrate concentration, enzyme concentration and time interval that influence the activity of the immobilized enzyme were also studied.

IJERT

1. Introduction

India is an agriculture oriented country 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 [1].The highly dynamic nature of "N" makes it efficient use and management a challenging task [2]. 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. Human urine contains >2% of urea, a non toxic compound. The major fraction of Urine N is Urea [3][4], while the rest is a mixture of readily mineralizable amino acids, peptides and ammonium-N (NH_4^+) [5]. Urea constitutes the predominant source of industrial N fertilizer used in agriculture and represents 46%of world consumption of nitrogenous fertilizers [6].

Urea can be an inefficient N source due to its rapid hydrolysis caused by soil urease [6]. 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. 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 [7]. 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 [8].

Enzymes have been utilized in a large number of practical applications, particularly in biomedical and biotechnological fields, through immobilization on a variety of supports. These immobilized products were intended for use in the construction of artificial organ systems, biosensors or bioreactors. Immobilization is advantageous because it extends the stability of bioactive species by protecting the active material from deactivation, enables repeated use, it provides significant reduction in the operation costs; facilitates easy separation, speeds up recovery of the bioactive agent. Many methods exists for the immobilization of enzymes but usually one of four methods is used: physical adsorption, entrapment, copolymerization, and covalent attachment,[9][10] the methods and supports employed for enzyme Immobilization are chosen to ensure the highest retention of enzyme activity and its stability and durability [11][12].

Urease (urea amidohydralase, E C 3.5.1.5) 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. This was the first enzyme ever crystallized [13] and found to be in large quantities in plant seeds, in some animal tissues and intestinal micro-organisms[14] and is found both as intra and extra cellular enzyme [15][16]. Immobilized urease has been widely used in biosensors for diagnostic purposes, in the determination of urea in biological fluids, in artificial kidney devices for the removal of urea from blood for extracorporeal detoxification, in enzyme reactor for the conversion of urea present in fertilizer waste water effluents or in the foods, [17][18] industry for removal of urea from beverages and foods. The most effective way removing urea from aqueous solutions is the utilization of immobilized urease as no efficient adsorbent is available for urea.

Studies were carried out in laboratory for recovery of nitrogen from urine using bio-waste materials (seeds from different fruits such as *Pouteria sapota, Tamarindus indica, Citrullus vulgaris, Citrus limoni*um, *Citrus aurantium and Rice husk)* as a source for preparation of enzyme and adsorbent beds in bioreactor. The present study was mainly directed to focus on the best suitable material as an adsorbent from the available resource materials in preparation of adsorbent beds for enzyme immobilization studies in a bioreactor. Various process parameters such as pH, temperature, substrate concentration, enzyme concentration and time interval that influence the activity of the immobilized enzyme were also studied.

IJERT

2. Materials and Methods

2.1 Sample Preparation

Seeds of *Citrullus vulgaris* were collected from surrounding area of Kukatpally, Hyderabad. Approximately 100 seeds of this variety were taken, washed and dried in sunlight for two successive days. The dried seeds were grinded into find powder and are sieved to < 2mm in order to get uniform particle size.

2.2 Enzyme extraction

Enzyme was extracted from *Citrullus vulgaris* by dissolving small amount 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 activity of immobilized enzyme and the conditions favoring its activity under controlled conditions.

2.3 Adsorbent Preparation

Seeds of different varieties (*Pouteria sapotae, Tamarindus indica, Citrus limonium* and *Citrus aurantium*) were collected from the surrounding area of Kukatpally Hyderabad. Approximately 100 seeds of each variety were taken, washed and 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, and other adsorbents like plastic, husk, mud piece were also collected for immobilization studies.

2.4 Enzyme Immobilization

Small quantity of adsorbent powder of each variety (*Pouteria sapota*, Tamarindus *indica, Citrus limonium and Citrus aurantium, Rice husk*) and pieces of plastic, mud were taken and are incubated overnight in test tubes containing the urease enzyme extract which provides a medium for the immobilization of enzyme on to adsorbent. The adsorbent was washed thoroughly with distilled water and the immobilized enzyme was collected and checked for its activity by quantifying ammonia.

2.5 Determination of Enzyme activity

Urea solution of measured quantity was added into different test tubes and immobilized enzyme extract was added into each of the test tubes and were incubated over a time period of 2hrs and 24hrs. After incubation of the respective time interval the enzyme

activity was estimated by quantifying the ammonia produced by the activity of immobilized urease enzyme on urea

2.6 Quantification

Ammonium chloride solution of different standards were prepared whose concentration varies from (1 to 10 mg/ml) while the test solution (Urea solution + immobilized enzyme extract) of 1ml each were added into the test tubes and the final volume was made up to 5ml by adding distilled water. Phenol of 0.2 ml followed by 0.2 ml of sodium nitroprusside was added into the test tubes and mixed well. Oxidizing solution of 0.5ml was added, mixed and incubated at room temperature for about 1 hr for color development. The intensity of the color developed was measured spectrophotometrically at 640nm. The concentration of the ammonia produced by the immobilized urease enzyme was determined by plotting a graph, taking concentration on x- axis and absorbance on y- axis.

2.7 Determination of Adsorptional conditions

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

3. Result and Discussion

3.1 Immobilization of urease via adsorption

For the present study the activity of immobilized urease enzyme and the optimization of process parameters were carried out in the laboratory to determine the enhanced activity of urease enzyme adsorbed on to different adsorbents such as seed powders of *Pouteria sapota, Tamarindus indica, Citrus limonium, Citrus aurantium* and *Rice husk*. The other resource materials like pieces of mud and plastic were also used as adsorbent for immobilization. Urease enzyme extracted from *Citrullus vulgaris* immobilized via adsorption on different adsorbents was carried in the pH range of 7, at room temperature for a time interval of two hours and twenty four hours, and initial urea concentration was 2% in each adsorption medium. Fig 1 shows the amount of enzyme adsorbed onto different adsorbents states that the maximum activity was found to be majorly in *Rice Husk*, followed by *Tamarindus indica, Citrullus aurantium, Citrullus Limmonium, Pouteria sapota* by respectively. The materials like plastic and mud have least enzyme activity because the amount of enzymes immobilized on these materials were less while the maximum activity for *Rice husk* was due to its maximum surface area with both rough and smooth surface and has maximum pore size. Moreover to immobilize any enzyme on a solid support, the support must have some characteristics like large surface area, large pore size, distribution and hydrophilicity [19] as Rice husk has the characteristics of large surface area and pore size used as a solid support for immobilization and is considered as best suitable adsorbents than those chosen.

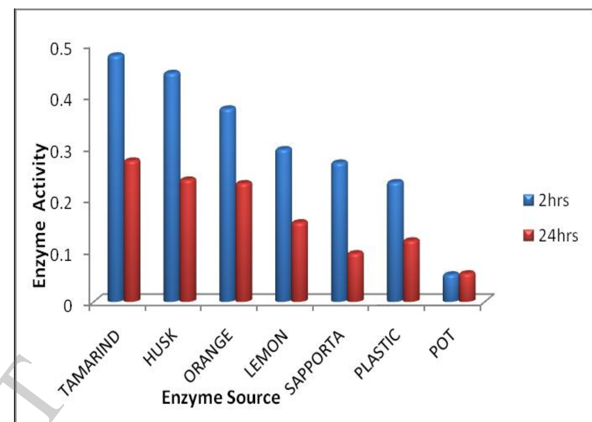

Fig- 1 Enhanced activity of Immobilized urease on to different adsorbents

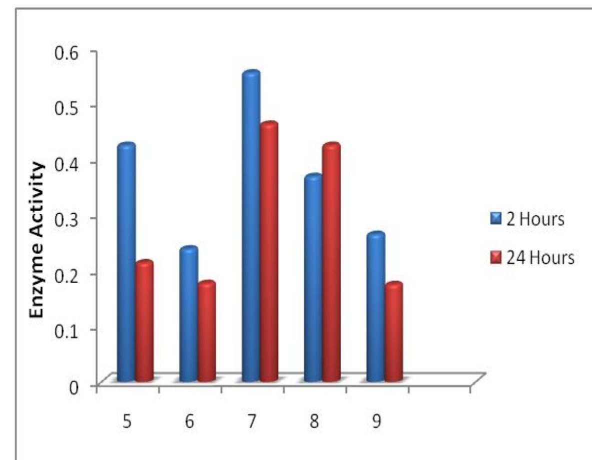

Fig- 2 Optimum pH for immobilized urease enzyme activty

IJERT

ISSN: 2278-0181
Vol. 1 Issue 8, October - 2012

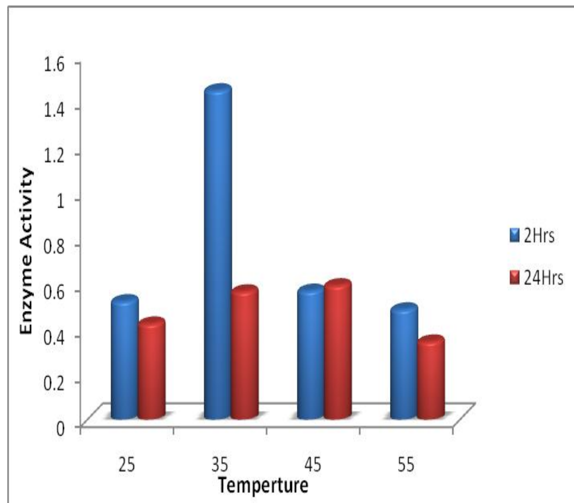

Fig- 3 Optimum temperature for immobilized urease enzyme activty

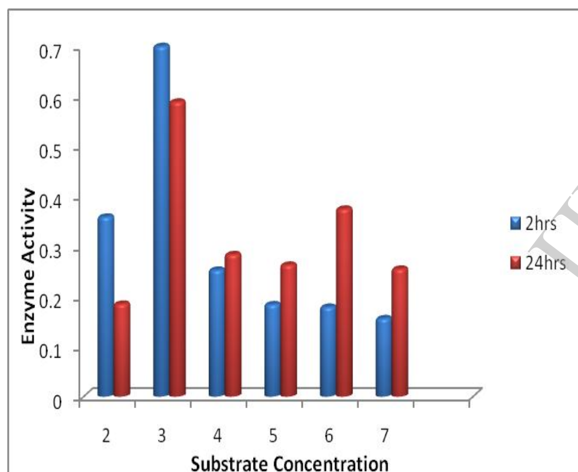

Fig- 4 Optimum Substrate concentration for immobilized urease enzyme activity

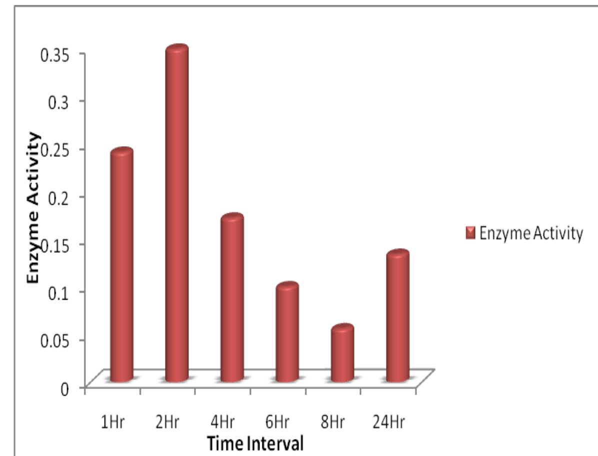

Fig- 4 Optimum Time interval for immobilized urease enzyme activity

3.2 Effect of pH & temperature on activity

The important parameters pH and temperature should be controlled during immobilization of enzyme via adsorption to obtain the reproducible results. Since these parameters influence the stability and conformational structures of proteins [20] urease immobilization via adsorption on *Rice husk* was carried out in the pH range 5 to 9, at room temperature for two hrs and twenty four hrs with an initial urease concentration of 2% in each adsorption medium. The pH dependence of the immobilized enzyme activity is characteristic of the nature of enzyme, immobilization method used and of the carrier used. The reaction rate of the urea hydrolysis by the free adsorbed urease as a function of pH and temperature is represented in Fig 2 & 3. As Proteins have no net charge at their isoelectronic points, and therefore, the maximum adsorption from aqueous solution is usually observed at this pH. Urease has an isoelectronic point of 4.9 [21]. In this work the maximum urease adsorption was obtained at pH 7.0, for *Rice husk*. While there is an abrupt decrease in the enzyme activity with an increase in the pH level due to loss of H^+ ions from the side chain groups of –CooH and $-NH_2$ altering the chemical nature of amino acids resulting in the disruption of the active site of the enzyme. Microenvironment of enzyme molecule is possibly modified depending on the surface and residual charges on the solid matrix and the nature of the bound enzyme due to immobilization resulting in retaining activity over an extended range of pH and also causing a shift in the pH optimum of the enzyme [22].

Optimum temperature for immobilized urease was investigated by changing the temperature from 20°c

IJERT

ISSN: 2278-0181
Vol. 1 Issue 8, October - 2012

to 50°c. The effect of temperature on the activity of urease enzyme immobilized on *Rice husk* is shown in fig 4. The activity of immobilized urease enzyme is strongly dependent on the temperature and a sharp optimum was obtained at 35°c. The denaturation of the immobilized enzyme could take place on the tertiary structure of peptidic chains of the urease which would occur above 40°c after two hours [20]. In other words as the temperature increases the structure of the enzyme becomes altered and its catalytic properties are reduced and eventually destroyed. The activity of the immobilized preparation is more resistant than that of the soluble form against heat and denaturing agent [23].

3.3 Effect of substrate concentration & time on activity

Optimum substrate concentration for immobilized urease enzyme was carried out by varying substrate concentration in a range of 2% to 7% respectively. The effect of substrate concentration on the activity of immobilized urease enzyme is shown in fig 5. It shows the maximum urease adsorption was obtained at a substrate concentration of 3% for *Rice husk*. Initially there is an increase in the activity of enzyme as there are many active sites that are not occupied by the substrate [24] while 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.

IJERT

The stability of the immobilized enzyme urease on matrices of *Rice husk* was determined by incubation at different time intervals of 1, 2, 4, 6, 8, and 24hrs.The activity was then assayed under standard conditions. Immobilized urease showed improved stability retaining a considerable amount of activity at a time interval of two hours shown in fig-6 while decreases with an increase in the time interval due to the saturation of the active sites of the enzymes by the substrate molecules and no longer involved in the breakdown of it [25].

Conclusion

In the present study the bio-waste material such as *Rice husk* shown to be a potent source for immobilization of urease suitable for use in urea determination. These are considered to be as best suitable adsorbents for immobilization studies which are free from the error pertain to the now widely used synthetic adsorbents. Immobilized urease showed improved stability retaining a considerable amount of activity at a pH of 7 with an optimum temperature of 35°c and substrate concentration of 3% for a time interval of 2hrs.Thus the high stability obtained with urease immobilized via adsorption on to matrices of *Rice Husk* indicates that this immobilized enzyme can successfully be used for recovery of fertilizer by continuous decomposition of urea from biological fluid in a bioreactor.

References

[1]. Camilla Giovannini., Jose, M., Ciavatta, c. and Marzadori, C. 2009. Ureic nitrogen transformation in multilayer soil columns treated with urease and nitrification inhibitors. *Journal of Agricultural and food chemistry*. 57: 4883-4887.

[2]. Zaman. M. and Blennerhassett, J.D. 2010. Effects of the different rates of Urease and nitrification inhibitors on gaseous emissions of ammonia and nitrous oxide, nitrate leaching and pasture production from urine patches in an intensive grazed pasture system. *Agriculture, Ecosystems and Environment*.136: 236-246.

[3]. Zaman, M., Nguyen, M.L., Matheson, F., Blennerhassett, J.D. and Quin, B.F.2007. Can soil amendments (Zeolite or lime) shift the balance between nitrous oxide and dinitrogen emissions from pasture and wetland soils receiving urine or urea N. Aus. *J. Soil Res*. 45: 543-553.

[4]. Zaman, M., Saggar, S., Blennerhassett, J.D. and Singh, J.2009. Effect of urease and nitrification inhibitors on N transformation gaseous emissions of ammonia and nitrous oxide, posture yield and N uptake in grazed pasture system. *Soil. Boil Biochem*. 41: 1270-1280.

[5]. Bolan, N.S., Saggar, S., Luo, J., Bhandral, R. and Singh, J.2004. Gaseous emissions of nitrogen from grazed pastures: Processes, measurements and modeling, environmental implications and mitigation. *Adv. Agron*.84: 37-120.

[6]. Watson. C.J. 2000. In Urease Activity and inhibition-Principles and Practice, Proceedings No.454, *The International Fertilizer Society*: York, UK.

[7]. Maurer, M., Schwegler, P., and Larsen, T.A. 2003. Nutrients in urine energetic aspects of removal and recovery. *Water Sci Technol.* 48(1): 37-46.

[8]. Boyer, E.W., Goodale, C.L., Jaworsk, N.A.and Howarth, R.W., 2002. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the Northeastern USA. Biogeochemistry 57, 137–169.

[9]. Arica, M.Y., Hasirci, V. and Alaeddingolu, NG.1997. Covalent immobilization of x-amylase onto pHEMA microspheres: preparation and application to fixed bed reactor. *Biomaterials*.16:761-8.

[10]. Gacesa, P. and Hubble, J. 1987. Immobilization studies of urease enzyme. *Enzyme technology*. Oxford: Alden Press: 45-54.

[11]. Arica, M.Y., Denizli, A., Salih, B., Piskin, E. and Hasirci V. 1995. Urease adsorption on to Cibacron Blue F3GA and Fe (III) derivatized pHEMA membranes and application to a continuous system. *J Memner Sci*.129:65-76.

[12]. Busto, MD., Ortega, N. and Perez-Mateos M. 1987. Effect of immobilization on the stability of bacterial and fungal b-D-glucosidase. *Process Biochem*.32:441-9.

[13]. Sumner, J. B. 1926. The isolation and crystallization of the enzyme urease. *Journal of Biol. Chem*. 69: 435- 441.

[14]. Mulvancey, R.L., Bremner, J.M.1981. Control of urea transformations in soils. *Soil Biol. Biochem*.5: 153-196.

[15]. El-Sheriff, H., Martelli, PL., Casadio, R., Potataccio, M., Bencivenga, U. and Mita, DG. 2001. Urease immobilization on chemically grafted nylon membranes part 1: isothermal characterization. *J. Mol Catal B: Enzymatic.25*:14-19.

[16]. Ciurli, S., Benini, S., Deiana, S., Marzadori, C. and Gessa, C. 1996. Ureases form the soil bacterium bacillus pasterII: immobilization on Ca- Polygalacturonate. *Soil Biol Biochem*. 28:811-822.

[17]. Laska, J., Wlodarczyk, J. and Zabirsja, W. 1999. Polyaniline as a support for urease immobilization. J Mol Catal B: Enzymatic.6:549-556.

[18]. Hearn, E. and Neufeld, RJ. 2000. Poly (methylene-co-guanidine) coated alginate as an encapsulation matrix for urease. *Process Biochem.* 35: 1235-53.

[19]. Senel, S., Say, R., Arica, MY. and Denizli, A.2001. Cibacron Blue F3GA and Cu (ll) derived Poly (2-hydroxyethylmetacrylate) membranes for lysozyme adsorption.Colloides Surf A: *Physicochem Eng Aspects.*182:161-178.

[20]. Akogal, s., Yagmur, Y., Gulay, B. and Adil Denizli, M. 2002. Reversible immobilization of urease on to Procion Brown MX-5BR-Ni (II) attached Polyamide hollow fiber membranes.38:675-683.

[21]. Marzadori, C., Miletti, S., Gessa, C. and Ciurli, S.1998. Immobilization of jack bean urease on hydroxyapatite: urease immobilization in alkaline soils. *Soil Biol Biochem.* 30:1485.

[22]. Mosbach, K. 1971. Enzymes bound to artificial matrixes. *Nuclear Agriculture and Biotechnology* 224: 26-33

[23]. Ulbrich, HR. and Selisko, B. 1993. Soluble and immobilized enzymes in water-miscible organic solvents: gluco-amylase and invertase. *Enzyme Microb Technol*.15:33_ 41.

[24]. Pozniak., Krojewska, B. and Trochisczuk, W.1995. Urease immobilized on modified polysulphone membrane, Preparation and properties. *Biomaterials.* 16:129-134.

[25]. Lehninger, David L. Nelson., Michael M. Cox.2002.Factors affecting the activity of enzyme. *Principles of Biochemistry*. 45: 247-263.

IJERT