



Home (<https://jurnal.usk.ac.id/natural/index>) / About the Journal  
(<https://jurnal.usk.ac.id/natural/about>) / Editorial Team  
(<https://jurnal.usk.ac.id/natural/about/editorialTeam>)

## Editorial Team

### Chief Editor

---

1. Marwan Ramli, Department of Mathematics, Syiah Kuala University, Banda Aceh-Indonesia, Indonesia

### Managing Editor

---

1. Suhartono Suhartono, Department of Biology Faculty of Mathematics and Sciences Syiah Kuala University

### Editorial Board

---

1. Hadi Susanto, Department of Mathematical Sciences, University of Essex, United Kingdom
2. Rinaldi Idroes, Pharmacy Department, Universitas Syiah Kuala, Indonesia
3. Waltram Ravelombola, Department of Soil and Crop Sciences Texas A&M, United States
4. Peter Woodfield, Griffith School of Engineering and Built Environment, Gold Coast Campus Griffith University, QLD 4222 Australia, Australia
5. Anton Abdulbasah Kamil, İstanbul Gelişim Üniversitesi, Istanbul, Turkey
6. Syed Azhar Syed Sulaiman, Universiti Sains Malaysia, Malaysia
7. Rosli Saad, Universiti Sains Malaysia, Malaysia

### Associated Editors

---

1. Elin Yusibani, Physics Department, Faculty of Mathematics and Natural Science at Universitas Syiah Kuala, Indonesia
2. Rahmi Rahmi, Chemistry Department, Universitas Syiah Kuala
3. Muhammad Syukri, Physics Department, Faculty of Mathematics and Natural Science at Universitas Syiah Kuala, Indonesia
4. Febriani Febriani, Chemistry Department, Syiah Kuala University Banda Aceh-Indonesia, Indonesia

5. Mahdhivan Syafwan, Andalas University, Indonesia
6. Vera Halfiani, Department of Mathematics, Faculty of Mathematics and Natural Sciences,  
Universitas Syiah Kuala
7. Tedy Kurniawan Bakri, Pharmacy Department, Faculty of Mathematics and Natural Sciences,  
Universitas Syiah Kuala, Indonesia
8. Bodhi Dharma, Department of Biology FMIPA Universitas Mulawarman, Indonesia

# Accredited

# SINTA 2



(<https://sinta.kemdikbud.go.id/journals/profile/201>)

## SERTIFIKAT

Kementerian Riset dan Teknologi/  
Badan Riset dan Inovasi Nasional



Petikan dari Keputusan Menteri Riset dan Teknologi/  
Kepala Badan Riset dan Inovasi Nasional  
Nomor 148/M/KPT/2020  
Peringkat Akreditasi Jurnal Ilmiah Periode II Tahun 2020  
Nama Jurnal Ilmiah  
**Jurnal Natural**  
E-ISSN: 14118513

Penerbit: Unsyiah Press – UPT Percetakan dan Penerbit Unsyiah

Ditetapkan sebagai Jurnal Ilmiah

**TERAKREDITASI PERINGKAT 2**

Akreditasi Berlaku selama 5 (lima) Tahun, yaitu  
Volume 20 Nomor 1 Tahun 2020 sampai Volume 24 Nomor 3 Tahun 2024

Jakarta, 03 August 2020

Menteri Riset dan Teknologi/  
Kepala Badan Riset dan Inovasi Nasional  
Republik Indonesia,



*Bambang P. S. Brodjonegoro*  
Bambang P. S. Brodjonegoro

### ABOUT THE JOURNAL

Aims and Scope (<http://jurnal.unsyiah.ac.id/natural/about/editorialPolicies#focusAndScope>)

Publication Ethics (<http://jurnal.unsyiah.ac.id/natural/about/editorialPolicies#custom-0>)

## Immobilization of urease from *Phaseolus vulgaris* L. seeds using calcium alginate as a support matrix

ZUSFAHAIR<sup>1\*</sup>, DIAN RIANA NINGSIH<sup>1</sup>, AMIN FATONI<sup>1</sup>,  
ELY SETIAWAN<sup>1</sup>

<sup>1</sup>Department of Chemistry, Faculty of Mathematics and Natural Science, Jenderal Soedirman University, Purwokerto 53123, Indonesia

**Abstract.** Exploration of urease from various sources continues because of its many industrial uses. This research aimed to isolate urease from kidney bean (*Phaseolus vulgaris* L.) seed and immobilize it using a Ca-alginate support matrix and a trapping technique. Eight days were devoted to germinating kidney bean seeds to begin the investigation. Isolation of crude urease extract from kidney beans was carried out using phosphate buffer pH 7. It was then immobilized with Ca-alginate at different concentrations of Na-alginate and contact times. The crude free and immobilized urease extract was further characterized including pH, temperature and stability of repeated use. The urease activity was determined using the Nessler method using a spectrophotometer. The results demonstrated that urease immobilization from kidney bean seeds with a Ca-alginate matrix was most effective at a concentration of 5% Na-alginate and a contact period of 60 minutes, yielding a value of 5.92 U/mL. The optimal pH of free and immobilized urease was 7 and 8, respectively, and temperatures of 35 and 40 °C, respectively. The immobilization of urease from kidney bean seeds using a Ca-alginate support matrix increased the stability of recurrent use by fivefold, while the relative urease activity remained at 52%.

**Keywords:** alginate, immobilization, *Phaseolus vulgaris* L., urease

### INTRODUCTION

Urease is an enzyme that functions as a catalyst for the hydrolysis of urea into ammonia and carbon dioxide [1]. This enzyme is of the hydrolase class, a component of amidohydrolase [2]. Urease has been identified in bacteria, fungi, and plants that play a significant part in the natural nitrogen cycle [3]. Nuts contain high levels of protein so they can be used as a source of urease. Nut seeds that have been used as urease producers include peas (*Pisum sativum* L.) [2], Kayo beans (*Cajanus cajan*) [4], and long beans (*Vigna unguiculata* ssp *sesquipedalis* L.) [5]. Urease is an enzyme that plays a crucial function in nitrogen metabolism during plant germination [2].

Urease has been utilized as an antifungal [6], a method for detecting heavy metals in milk [7], and in industrial sewage treatment [2]. Due to the inclusion of an imported component, the

price of urease stays high. Therefore, it is vital to obtain urease from readily available basic materials. Kidney bean (*Phaseolus vulgaris* L.) seeds can be a source of urease due to their high protein content of 20 to 27% [8]. Furthermore, they belong to the same family as *P. sativum* L. and *Vigna unguiculata* ssp *sesquipedalis* L. This study used kidney bean seeds as a urease source to enhance their value.

The use of free enzymes as biocatalysts can usually only be used for one reaction. This issue can be remedied by immobilizing the enzyme [9]. Enzyme immobilization is the attachment of enzymes to an insoluble matrix in water. Immobilization can be done through several methods such as cross-linking, adsorption, and entrapping method [10]. Each technique has its benefits. This study used the entrapping method as its methodology. In the entrapping method, the enzyme does not bind to the matrix. Therefore, the enzyme's native structure and catalytic function are preserved [11].

Ca-alginate was used as the immobilization matrix in this investigation. Ca-alginate immobilization has the advantages of forming a solid gel, being non-toxic, and inexpensive [3]. The purpose of the investigation was to isolate urease from kidney bean seeds. The crude

\*Corresponding Author:  
zusfahair@gmail.com

Received: May 2022 | Revised: August 2022 |  
Accepted: September 2022

extract produced was immobilized using Ca-alginate and then analyzed.

## METHODOLOGY

### Instruments and Materials

The equipments and tools used in this research include centrifuge (Quantum), UV-Vis spectrophotometer (Shimadzu UV-1800 Spectrophotometer), pH meter (Hanna Instrument), incubator (Mettler), Scanning Electron Microscopy (JSM-6510LA) and analytical balance (Ohaus). This study utilized urea, acetic acid, commercial-grade Na-alginate,  $\text{CaCl}_2$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{NaH}_2\text{PO}_4$ ,  $\text{H}_2\text{SO}_4$ , Na-tungsten, and Nessler's reagent. All the chemicals used were of analytical grade and were acquired from Merck Chemical Company (Merck, Germany). The kidney bean seeds are used as the source of urease (Wage market, Purwokerto).

### Isolation of urease from kidney bean seeds

The isolation of urease from kidney bean seeds began with germination and continued through the extraction procedure. Germination was accomplished as follows: kidney bean seeds were steeped in distilled water for six hours, then drained, placed in a plastic container with wet cotton, and then covered with plastic. Germination was performed in the dark at room temperature [12]. The resulting sprouts were harvested as follows: 10 grams of kidney bean sprouts were crushed using a mortar and pestle and cooled in the freezer, followed by adding 40 mL of phosphate buffer pH 7 at 4 °C. The solution was agitated with a stirrer for three hours until two layers, the filtrate and the precipitate, developed. The filtrate and precipitate were separated by using a muslin cloth. Fifteen minutes were spent centrifuging the resulting filtrate at 4 °C and 12,000 rpm. The supernatant obtained was an extract of crude urease. The crude extract was subsequently evaluated for urease activity using Nessler's technique and immobilized using a Ca-alginate matrix.

### Free Urease Activity Examination [5]

A sample tube containing 1.90 mL of a pH 7 phosphate buffer solution was filled with 1 mL of 1000 ppm urea. The sample tube was filled with 0.1 mL of urease solution, incubated for 15 minutes at 35 °C, and cooled. To inhibit the urease enzyme activity, 1 mL of 2/3 N  $\text{H}_2\text{SO}_4$  was added to the sample solution in the test tube, followed by 1 mL of Na-Wolframate. The control test tube was filled with 2 mL of pH 7 phosphate buffer, followed by 1 mL of 2/3 N  $\text{H}_2\text{SO}_4$  and 1 mL of Na-Wolframate. After 15 minutes of incubation at 35 °C, the solution was cooled. Then, 1 mL of urea at 1000 ppm was

added to the control tube. The sample and control tubes were centrifuged for 15 minutes, after which the supernatant was collected. The 250  $\mu\text{L}$  of Nessler's reagent was added to approximately 1.5 mL of each solution. The absorbance of the solution was then measured with a UV-Vis spectrophotometer at a maximum  $\lambda$  of 500 nm. Ammonium sulfate standard solutions with 20, 30, 40, 50, and 60 ppm were applied to determine the standard curve. By using the standard curve of ammonium sulfate, urease concentration was determined. One unit of urease activity (U) was defined as the amount of ammonia produced per minute for every 1 ppm of urea hydrolyzed by the urease in the sample.

### Optimization of immobilized urease matrix Ca-alginate

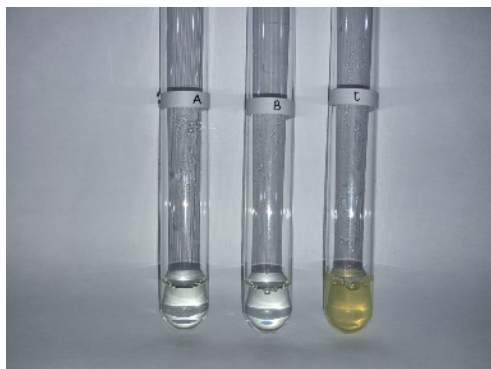
The concentration of sodium alginate and the contact period between immobilized urease beads and  $\text{CaCl}_2$  solution were varied to optimize immobilized urease. Alginate beads are used to immobilize urease by entrapping it. The 4 mL of Na-alginate solution with varying concentrations of 2; 3; 4; 5; and 6% (w/v) in phosphate buffer pH 7 was combined with 1 mL of crude kidney bean urease extract and stirred at 4 °C using a magnetic stirrer. The resulting solution was dropped into 30 mL of 0.2 M  $\text{CaCl}_2$  solution. Before filtering, the produced beads were soaked for one hour in a 0.2 M  $\text{CaCl}_2$  solution. Bead pores were analyzed using SEM. The activity of beads was assessed, and the concentration of Na-alginate with the highest activity was used to immobilize urease under varying contact times of 20, 40, 60, 80, and 100 minutes.

Measurement of immobilized urease activity was carried out by taking as much as 5 mL of 1000 ppm urea substrate in a pH 7 buffer and then adding as many as beads of immobilized urease beads formed in one formulation. A duration of 15 minutes was then spent to incubate the solution at 35°C. The Nessler method was used to determine the ammonia concentration in the supernatant after the filtered beads. The same procedure was performed as control by using beads devoid of urease.

### Characterization of free urease and immobilized urease

The characterization of urease was conducted at various pH levels and incubation temperatures to identify the optimal conditions. The activity test was utilized to determine the optimal pH for free urease and immobilized urease activity. However, it was conducted at different pH levels for the urea substrate solution, including





**Figure 1.** Test activity using the Nessler method. a) blank (aquades) b) control (urea without the addition of enzymes) c) reaction of urea with urease from kidney beans.

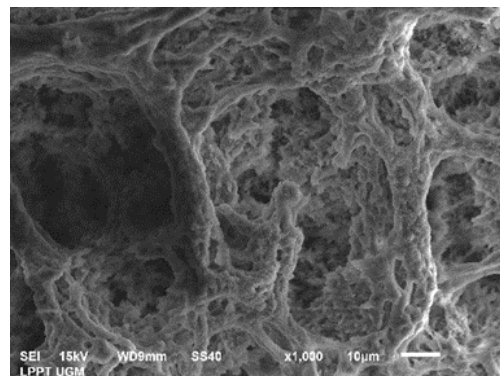
pH 5, 6, 7, 8, and 9. The urease activity was measured at the optimal pH at several incubation temperatures ranging from 30, 35, 40, 45, and 50 °C. The characterization of immobilized urease also included repeated usage experiments. This experiment was repeated until it was observed that the relative urease activity dropped by approximately 50%. Under optimal conditions, the reaction was conducted. The reaction was terminated by filtering the beads via filter paper, and the beads were then reused for urea hydrolysis. On the initial use of the beads, the activity was set to 100%.

## RESULTS AND DISCUSSION

### Enzyme isolation from kidney bean seeds

Germination is the initial stage in separating urease from kidney bean seeds. Germination is accomplished by soaking kidney bean seeds in water to absorb water into the plant tissue's cavities so that the plant tissue's cells become actively growing [13]. The soaked kidney bean seeds are then placed on moist cotton and kept in the dark to prevent sunshine from interfering with the auxin hormone's effectiveness. Auxin contributes to the growth and elongation of plant cells. The auxin concentration is more significant in the portion of the plant that is not exposed to light than the lit portion [14].

The activity test was carried out by measuring the amount of ammonia produced from the reaction of urea with urease from kidney bean seeds. The activity test was carried out using the Nessler method. The principle of measuring ammonia using the Nessler method is that Nessler's reagent ( $K_2HgI_4$ ) when reacted with ammonia in an alkaline environment will form a colloidal dispersion with a yellowish brown color  $[HgOHg(NH_2)I]$ . The intensity of the color that occurs is directly proportional to the concentration of ammonia present in the



**Figure 2.** Alginate beads pores analysis results using SEM at magnification 1000 x.

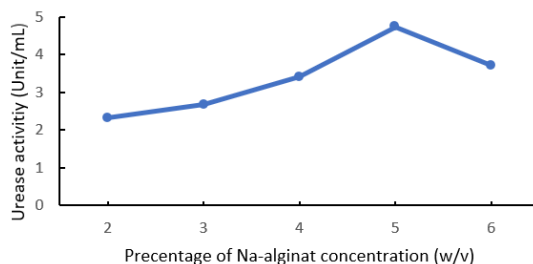
sample. The absorbance of the solution was measured using a UV-Vis spectrophotometer at 500 nm. The results of the urease activity test obtained a value of 11.84 U/mL. Figure 1 shows the activity test using Nessler method: a) blank (aquades) b) control (urea without the addition of enzymes) c) reaction of urea with urease from kidney beans.

### Ca-alginate matrix immobilization of urease *Effect of alginate concentration on urease immobilization*

Urease from kidney bean was immobilized by utilizing a trapping approach with an alginate matrix. Using a micropipette, urease was immobilized by dropping a mixture of Na-alginate solution and urease enzyme into a 0.2 M  $CaCl_2$  solution. Ca-alginate beads subsequently formed, trapping the enzyme inside. The pores in the beads are formed as a result of cross-linking between the carboxyl anion ( $COO^-$ ) of guluronic acid in alginate and the bivalent cation  $Ca^{2+}$  produced from  $CaCl_2$  (Figure 2) [15].

Meanwhile, the test results of immobilized urease activity with variations in the Na-alginate solution can be seen in Figure 3. Figure 3 demonstrates that the optimal concentration of Na-alginate was achieved at a concentration of 5% with an activity value of 4.74 U/mL. When the concentration of the Na-alginate solution was less than 5%, the pores of the produced beads were prominent, making it more straightforward for the trapped enzymes to escape the matrix, which resulted in low activity. If the concentration of the Na-alginate solution is above 5%, the pores of the beads formed will be more tightly packed, this is because the cross-links formed are also increasing so that the substrate diffusion process becomes inhibited and the product will

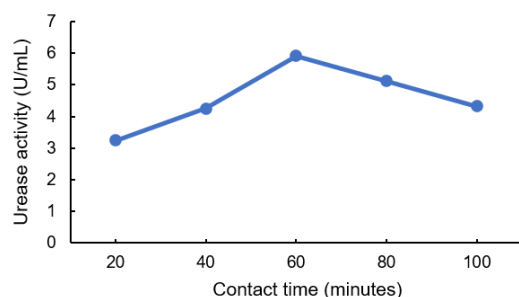
be difficult to form so that its activity decreases [3].



**Figure 3.** Effect of Na-alginate concentration on bean urease activity of kidney bean seeds

#### Effect of contact times on urease immobilization

The effect of contact time on urease activity can be seen in Figure 4.



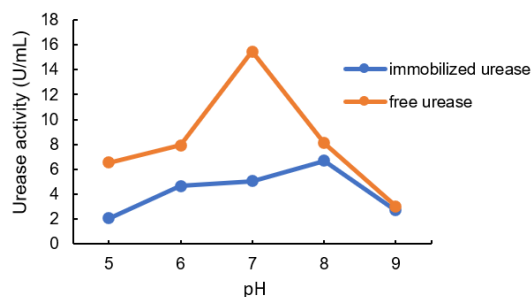
**Figure 4.** Effect of contact time on the activity of urease beads from kidney bean seeds

As presented in Figure 4, the optimal contact duration was 60 minutes, with an activity level of 5.92 U/mL. A lack of urease activity resulted from beads coming into contact with  $\text{CaCl}_2$  before the optimal time. Ca-alginate beads can only form on the surface. Bead formation would continue in the deep section until the optimal contact time, at which point the beads would be homogeneous and solid. After exceeding the optimum contact time, the activity of the urease beads decreased because the enzyme was exposed to Ca metal for a more extended period, thereby denatured [11].

#### Characterization of free urease and immobilized urease

##### Effect of pH on enzyme activity

The study results on the effect of pH on urease activity can be seen in Figure 5. The optimal pH for free urease is pH 7, with an activity value of 15.45 U/mL. In contrast, the optimal pH for immobilized urease is pH 8, with an activity value of 6.68 U/mL, as shown in Figure 5.

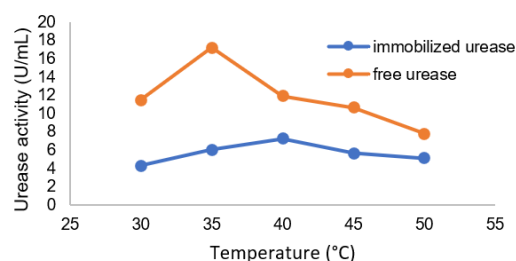


**Figure 5.** Effect of pH on urease activity of kidney bean seeds

Under low pH conditions, the enzyme would undergo protonation and lose its negative charge. Under high pH conditions, the substrate would undergo ionization and lose its positive charge, making the formation of the enzyme-substrate complex more challenging. The enzyme can be denatured if the pH conditions are excessively high or low. At the optimal pH, the conformation of the enzyme's active site corresponds to the shape of its substrate [16]. The difference between the optimal pH for free urease and immobilized urease is that, after immobilization, the supporting matrix would influence changes in environmental conditions [17].

##### Effect of temperature on the activity of urease

The research results on the effect of temperature on urease activity can be seen in Figure 6. Figure 6 shows that the optimal temperature difference between free urease and immobilized urease is 35 °C and 40 °C, with 17,18 U/mL and 7.25 U/mL. The interaction of the enzyme structure with the immobilization matrix led to the formation of secondary and tertiary hydrophobic interactions, resulting in a change in the enzyme's conformation. It necessitates higher temperatures to attain the proper conformation for optimal activity [17].



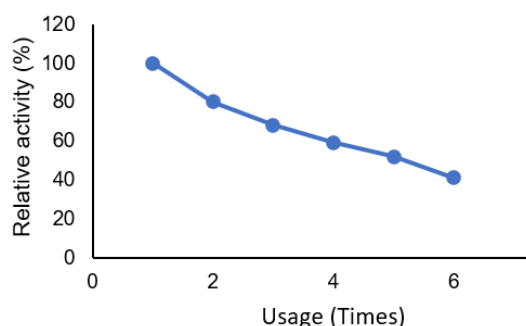
**Figure 6.** Effect of temperature on urease activity of kidney bean seeds

The support matrix can absorb a certain amount of heat and prevent the denaturation of the enzymes. It may increase the optimal temperature of immobilized urease. The majority of industrial applications of enzymes are carried out at room temperature or higher

temperatures. Typically, immobilization increases the enzyme's resistance to higher temperatures, making the immobilized enzyme more valuable and cost-effective in the industry [18].

#### Effect of repeated use of immobilized enzymes

With optimal bead conditions, repeated use of immobilized urease was conducted. At the end of the activity test reaction, the immobilized enzyme was collected by filtration using filter paper, washed with buffer, and added back into the new substrate to start a new cycle. The results can be seen in Figure 7.



**Figure 7.** Stability of repeated use of urease beads from kidney bean seeds

Figure 7 shows that urease beads can be reused up to five times with a 52% relative residual activity. Reduced activity after repeated use because the beads become very brittle and the surface of the beads is harmed, causing the enzymes to become inactive and consequently reducing their activity. Immobilized urease from chickpea with alginate maintained 60% of its initial activity at the end of five cycles [19]. Immobilized urease from jack bean using alginate can also be used up to 5 times with an activity of about 40% [20]

## CONCLUSION

Kidney bean seeds' urease activity was significantly affected by the concentration of Na-alginate and the contact time between immobilized beads and  $\text{CaCl}_2$ . The obtained beads can be reused up to five times with a remaining activity of 52%.

## ACKNOWLEDGMENT

The writers would like to thank LPPM UNSOED which has provided BLU Funds with the "Basic Research" Research Scheme in 2022, so that this research can be completed

## REFERENCE

[1] Singh, A.K.; Singh, M.; Verma, N. 2017, Extraction, Purification, Kinetic

Characterization and Immobilization of Urease from *Bacillus sphaericus* MTCC 5100. *Biocatal. Agric. Biotechnol.* **12**, 341–347, doi:10.1016/j.bcab.2017.10.020.

- [2] El-hefnawy, M.E.; Sakran, M.; Ismail, A.I.; Aboelfetoh, E.F. 2014, Extraction, Purification, Kinetic and Thermodynamic Properties of Urease from Germinating *Pisum sativum* L. Seeds. *BMC Biochem.* **15**, 1–8.
- [3] Maharani, L.D.; Prasetyawan, S.; Mahdi, C. 2013, Optimasi Amobilisasi Urease dari *Schizosaccharomyces pombe* Menggunakan Matrik Ca-Alginat (Optimization of Urease Immobilization from *Schizosaccharomyces pombe* Using Ca-Alginate Matrix). *Kim. Student J.* **2**, 421–427.
- [4] Sujoy, B.; Aparna, A. 2012, Isolation, Partial Purification, Characterization and Inhibition of Urease (EC 3.5. 1.5) Enzyme from the *Cajanus cajan* Seeds. *Asian J. Bio Sci.* **7(2)**, 203–209.
- [5] Zusfahair, Z., Ningsih, D. R., Fatoni, A., & Pertiwi, D.S. Pemurnian Parsial dan Karakterisasi Urease dari Biji Kacang Panjang (*Vigna unguiculata subsp sesquipedalis* L.). *Alchemy J. Penelit. Kim.* **14 (1)**, 72–83.
- [6] Postal, M.; Martinelli, A.H.S.; Becker-Ritt, A.B.; Ligabue-Braun, R.; Demartini, D.R.; Ribeiro, S.F.F.; Pasquali, G.; Gomes, V.M.; Carlini, C.R. 2012, Antifungal Properties of *Canavalia ensiformis* Urease and Derived Peptides. *Peptides* **38**, 22–32, doi:10.1016/j.peptides.2012.08.010.
- [7] Kaur, H.; Kumar, S.; Verma, N. 2014, Enzyme-Based Colorimetric and Potentiometric Biosensor for Detecting Pb (II) Ions in Milk. *Brazilian Arch. Biol. Technol.* **57**, 613–619, doi:10.1590/S1516-8913201402160.
- [8] Pitura, K.; Arntfield, S.D. 2018, Characteristics of Flavonol Glycosides in Bean (*Phaseolus Vulgaris* L.) Seed Coats. *Food Chem.*, doi:10.1016/j.foodchem.2018.07.220.
- [9] Tantowidjojo, R.V.; Roosdiana, A.; Prasetyawan, S. 2013, Optimasi Amobilisasi Pektinase dari *Bacillus subtilis* Menggunakan Kitosan-Natrium Tripolifosfat. *Kim. Student J.* **1**, 91–97.
- [10] Mawaddatul, W. 2017, Amobilisasi Enzim Bromelin dari Buah Nanas (*Ananas comosus* L.Merr) Menggunakan Matriks Kitosan Untuk Pengurangan Kandungan Protein Pada Air Limbah Pabrik Tahu. *J. Fak. Mat. Inst. Teknol.*



- Sepuluh Nop. 1–104.
- [11] Zusfahair, Ningsih, D. R., Kartika, D., Fatoni, A. & P. 2017, Immobilization of Lipase from *Azospirillum* sp. PRDI Using Chitosan Alginate as Supporting Agent. *Malaysian J. Fundam. Appl. Sci.* **10** (4), 195–199.
- [12] Zusfahair, Z.; Ningsih, D.R.; Fatoni, A.; Ika, D.; Age, L. 2022, Immobilization and Characterization of Urease from *Phaseolus vulgaris* L Using Bentonite Chitosan Matrix. *AIP Accepted*.
- [13] Annisa; Mardhiansyah; Arlita, T. 2016, Response of Saga (*Adenanthera pavonina* L.) Seeds Germination Capacity Effect of Water Submerged Time. *Jom Faperta* **3**.
- [14] Wiraatmaja, I.W. 2017, Bahan Ajar Zat Pengatur Tumbuh Auksin dan Cara Penggunaannya dalam Bidang Pertanian. *Bahan Ajar* 182–191.
- [15] Mazumder, M.A.J. 2013, Bio-Encapsulation for the Immune-Protection of Therapeutic Cells. *Adv. Mater. Res.* **810**, 1–39, doi:10.4028/www.scientific.net/AMR.810.1.
- [16] Murray, R.K.; Granner, D.K.; Mayes, P.A.; Rodwell, V.W. 2003, *Harper's Illustrated Biochemistry*; ISBN 0071389016.
- [17] Shaheen, R.; Asgher, M.; Hussain, F.; Bhatti, H.N. 2017, Immobilized Lignin Peroxidase from *Ganoderma lucidum* IBL-05 with Improved Dye Decolorization and Cytotoxicity Reduction Properties. *Int. J. Biol. Macromol.* **103**, 57–64, doi:10.1016/j.ijbiomac.2017.04.040.
- [18] Kumar, S.; Dwevedi, A.; Kayastha, A.M. 2009, Immobilization of Soybean (*Glycine Max*) Urease on Alginate and Chitosan Beads Showing Improved Stability: Analytical Applications. *J. Mol. Catal. B Enzym.* **58**, 138–145, doi:10.1016/j.molcatb.2008.12.006.
- [19] Tetiker, A.T.; Ertan, F. 2017, Investigation of Some Properties of Immobilized Urease from *Cicer arietinum* and Its Using in Determination of Urea Level in Some Animal Feed. *J. Innov. Pharm. Biol. Sci.* **4**, 2–7.
- [20] Pithawala, K.; Mishra, N.; Bahadur, A. 2010, Immobilization of Urease in Alginate, Paraffin and Lac. *J. Serb. Chem. Soc.* **75**, 175–183, doi:10.2298/JSC1002175P.