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Uric Acid Biosensor Using Immobilised *Lactobacillus plantarum* Mar8 on Zeolite/κ-Carrageenan Membrane

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Abstract. Determination of uric acid is crucial in the uric acid related diseases management. Uric acid biosensor has been developed as an alternative for conventional spectrophotometric methods. A bacteria of *Lactobacillus plantarum* Mar8 producing uricase was immobilised on zeolite/κ-carrageenan membrane. Then, the system was optimised and both kinetics and stability of the uricase were determined using cyclic voltammetry method. The optimum condition was revealed at 3 mM of uric acid, 50 mg of zeolite, and 2% (w/v) of κ-carrageenan. The kinetics of Michaelis-Menten constant and maximum velocity (K_M and V_{max}) obtained from Lineweaver-Burk equation were 2.86 mM and 0.0018 mA, respectively. A linear measurements was at 2-2.6 mM with $R^2 = 0.9959$. The sensitivity of biosensor was 8×10^{-4} mA mM- while the limit of detection and limit of quantity were 0.478 mM and 1.598 mM, respectively. Finaly, the stability of the biosensor could extend up to 23 days.

Keywords: uric acid, biosensor, zeolite, κ-carrageenan, *Lactobacillus plantarum*

Introduction

Uric acid (2,6,8-trihydroxypurine) is the final product of purine metabolism and excreted by kidney and intestinal tract. The normal level of uric acid in human serum is between 240-520 μM. An excessive level of uric acid leads to some diseases such as hyperuricemia, gout, Lesch-Nyhan syndrome and kidney disorders (Luo *et al*., 2006). Consequently, rapid and reliable determination of uric acid would be urgently required in diagnostic and treatment. The current conventional detection method of UV-Vis sprectroscopy is sensitive to light, low specificity and relatively expensive due to the spectrophotometer cost (Hamzah *et al*., 2013). Other alternatives such as chemiluminescence, flouresence, HPLC mass spectrometry, ion chromatography, capillary electrophoresis are laborious, expensive, time consuming and complex to perform (Erden and Kilic, 2013). The alternative of high sensitivity, rapid and portable enzymatic biosensor kit is also expensive due to the cost of pure uricase (Attala *et al*., 2009). Uricase is an enzyme that catalyses the reaction of uric acid to allantoin, where its redox reaction is used to generate sensing in biosensor system.

Lactobacillus plantarum Mar8 is a lactic acid bacterial isolate producing uricase that has good catalytic activity on uric acid (Iswantini *et al*., 2014). Therefore immobilisation of this isolate on a membrane that facilitates the bacteria to extend its life time and produce catalytic uricase, would be an advantage in an uric acid biosensor development.

There are various methods of good immobilisation, such as physical adsorption using zeolite and entrapment using κ-carrageenan. The zeolite interacts with the immobilised cells through a simple adsorption process (Krekeler *et al*., 1991, Monsan *et al*., 1987). Yet this simple adsorption produces a relatively weak interaction and hence low stability (Gorecka and Jastrzebska, 2011; Hsu *et al*., 2004). Entrapment of adsorpted cells with a porous matrix suc as κ-carrageenan had been reported to increase the stability of superoxidase biosensor until 30 days (Campanella *et al.,* 1999) and oxidative stress biosensor of *Escherichia coli* (Ooi *et al.,* 2015).

This research applied both adsorption on zeolite and entrapment by κ-carrageenan on the immobilisation of *L.plantarum* Mar8 producing uricase. Optimisation experiment and characterisation of both kinetics and stability were carried out to develop a more rapid and simpler uric acid biosensor.

Materials and Methods

Zeolite activation. Natural zeolite obtained from Bayah, Soutwest of West Java, Indonesia was crush-mashed

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until 400 mesh and washed with aquadest to its neutral pH. Zeolite was acidly activated by HCl of 0.5; 1; and 3 M by stirring for 1 h. Activated zeolite was washed again with aquadest. The activated zeolite was then dried at 300 °C for 3 h prior to its cation exchange capacity measurement.

Preparations of *L. plantarum* Mar 8 cells. The *L. plantarum* Mar8 was grown on Glucose Yeast Peptone medium for 24 h at 37°C. A reculture was carried out to reach cell density of absorbance OD_{600}=0.5. The cells were then harvested by centrifugation at 8000 g for 15 min, washed with aquadest 3 times, resuspended in borate buffer pH 8, and were kept at 4 °C.

Preparation of Zeolite/κ-Carrageenan membrane. Natural zeolite was resuspended in aquadest using ultrasonication at 50°C for 10 min. Then, κ-carrageenan at test concentration were added. The suspension was poured into a 10 cm diameter glass and allowed to gel at 4 °C for 5 h. The membrane was cut at the size of 3 mm^2 and stored at 4 °C overnight.

The carbon paste electrode fabrication and immobilisation of *L. plantarum* Mar8 cells on Zeolite/κ-Carrageenan membrane. A carbon paste electrode was fabricated by packing a mixture of graphite powder, paraffin liquid, and 2,3-dimethoxy-5-methyl-1, 4 benzoquinone (Q_0) as a mediator into the end of glass tube. The zeolite/κ-carrageenan membrane was immersed in 20 µL the *L.plantarum* suspension (~10^6 CFU/mL) and kept at 4 °C for 3 h. Then the membrane was applied on the surface of carbon paste electrode. Finally the electrode membrane surface was covered with a dialysis membrane and fixed with O-ring rubber.

Electrochemical measurements and optimisation. Electrochemical measurements were carried out with eDAQ potensiostat (Ecorder 410) and completed using eChem v2.1.0 software. An Ag|AgCl, Pt and carbon paste electrode was used as reference, counter, and working electrode, respectively. The measurements were conducted at 30 °C in a 2 mL borrate buffer pH 8 with each of 2mL uric acid at test concentration. The optimisation experiment was carried out based on Respon Surface Methods in the Minitab v.16.2.4 software.

Kinetics properties. Kinetics properties of *L.plantarum* uricase were determined using Michaelis-Menten equation and Lineweaver-Burk plot, where:

$$V = \frac{V_m\,[\text{uric acid}]}{K_M + [\text{uric acid}]}$$

where:

V_m is maximum rate and K_M is the constant of Michaelis-Menten.

Analytical parameters. The determined analytical parameters were linearity, sensitivity, limit of detection (LOD) and limit of quantity (LOQ). Linearity was determined by finding the correlation coefficient (R^2). Limit of detection and limit of quantity were determined by the formula:

$$Q = \frac{k.sb}{b}$$

where:

Q is the Limit of Detection (LOD) or Limit of Quantity (LOQ), sb is standard of deviation, k =3 for LOD, k=10 for LOQ, and b is the slope (Harmita, 2004).

Biosensor stability. Biosensor stability of *L.plantarum* uricase activity was determined relatively from initial time to some definite times (Iswantini *et al*., 2014).

Results and Discussion

Zeolite activation. The cation-exchange capacity of the activated zeolite was affected by the hydrochloric acid concentration and the relatively good response of current was obtained at 0.5 M. as presented in Table 1.

Characterisation of carbon-paste electrodes. The electrodes showed a constants current cyclic response of both anodic and cathodic current peaks each at 0.500V and 0.300V, respectively when elecrodes were characterised using a solution of $K_3[Fe(CN)_6]$ 0.01 M in a solution of KCl 0.1 M. In contrast, there was no peaks when the electrode was characterised in a solution of KCl 0.1 M only (Fig. 1).

Uricase activity of the immobilized *L. plantarum* on Zeolite/κ-Carrageenan membrane. The electrode

Table 1. The cation exchange capacity values

HCl (M)	CEC (me/100 g)
0	82,05
0,5	81,45
1	76,20
3	68,14

containing immobilised cells showed a cyclic pattern voltammogram with an anodic of oxidation reaction at 0.400V to 0.007V. In contrast, the blank electrode in the buffer showed no activity peak (Fig. 2).

Optimisation of uricase activity of the immobilised *L. plantarum.* The optimisation results showed that the affecting variable on the measurement current was the concentration of uric acid (P<0.05) (Table 2). The analytical contour indicated an optimum condition could be reached at a 3 mM of uric acid, 50 mg of zeolite, and 2% (w/v) of κ-carrageenan (Fig. 3).

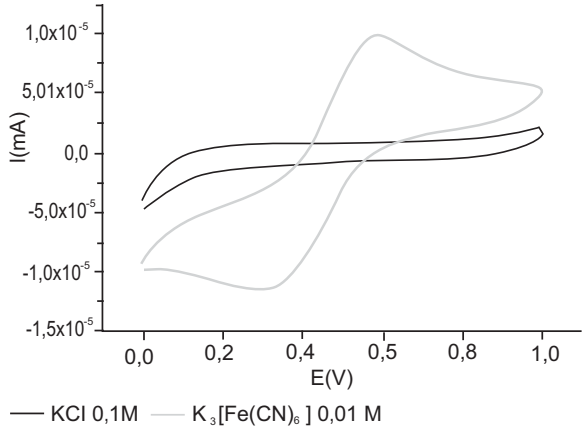

Fig. 1. Voltammogram of caracterisation carbon paste electrode using $K_3[Fe(CN)_6]$ 0,01 M solution in KCl 0,1 M solution. KCl 0,1 M solution (—) and $K_3[Fe(CN)_6]$ 0,01 M solution (—).

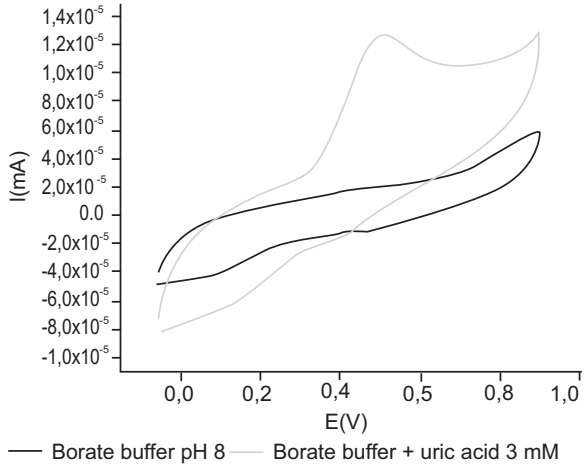

Fig. 2. Voltammogram of cells immobilisation analysis on zeolite/κ-carrageenan membrane. Borate buffer pH 8 (—) and borate buffer + uric acid 3 mM (—).

Table 2. The effect of parameters to current responses

Parameter	Coefficient	P
Constanta	$5{,}45 \times 10^{-3}$	0,000
Mass zeolite	$1{,}39 \times 10^{-3}$	0,064
Concentration κ-carrageenan	$-0{,}36 \times 10^{-3}$	0,582
Concentration uric acid	$2{,}19 \times 10^{-3}$	0,008
R-sq = 92,81%	R-sq (adj) = 82,02%	

Kinetic properties of uricase from *L. plantarum.* The uricase activity of immobilized cells had a value of KM and V_{max}, respectively of 2.86 mM and 0.0018 mA. These values were determined based on the relationship between 1/[S] with $1/I_{pa}$ of the Michaelis-Menten equation and Lineweaver-Burk plot (Fig. 4).

Analytical parameters. The uricase activity of immobilised cells on the electrode showed a linear plot in the range of uric acid concentration between 2.0 to 2.6 mM with $R^2 = 0.9959$. The linearity graph also revealed that this electrode had sensitivity of 0.0008 mA/mM and both limit of detection (LOD) and limit of quantity (LOQ) of 0.478 mM and 1.598 mM, respectively (Fig. 5).

The stability of uric acid biosensor. The uric acid sensing of the immobilised cells on the electrodes could relatively withstand up to 23 days from the initial activity (Fig. 6).

The immobilisation of *L.plantarum* Mar 8 to zeolite/κ-carrageenan membrane improved both activity and stability of the constructed uric acid biosensor. The zeolite should be activated by acid treatment using HCl to facilitate effective cation exchange capacity (Sekulic *et al*., 2014). The acid activation cleared the zeolite and increased hydrophobic properties for better adsorption of the bacterial cells (Ozkan and Ulku, 2005). The κ-carrageenan net entraped the bacterial cells and provided moisture required for bacterial life and uricase activity during the storage and use. Indeed, the uricase activity of immobilised *L. plantarum* Mar 8 could be then effectively optimised where its response was greatly affected directly by the measured uric acid concentration only. This indicate an effective biosensor.

The zeolite/κ-carrageenan membrane also had relatively increased the affinity of the uricase of immobilised *L.*

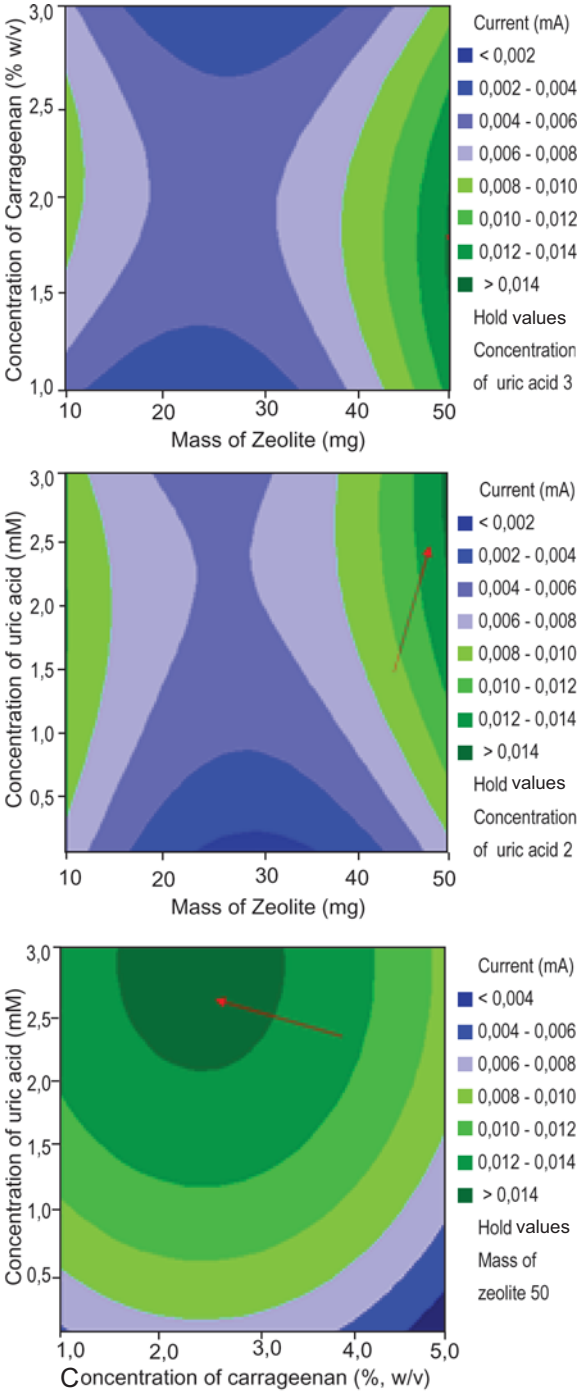

Fig. 3. Contour of the relathionship between parameters and current responses. Dark green showed the highest current and optimum condition.

plantarum Mar 8 as compared with the one immobilised on the natural zeolite only. This indicated by the value of K_M and V_{max} of 2.86 mM and 0.0018 mA versus 3.1397 mM and 7.4936 µA (Iswantini *et al*., 2014). The

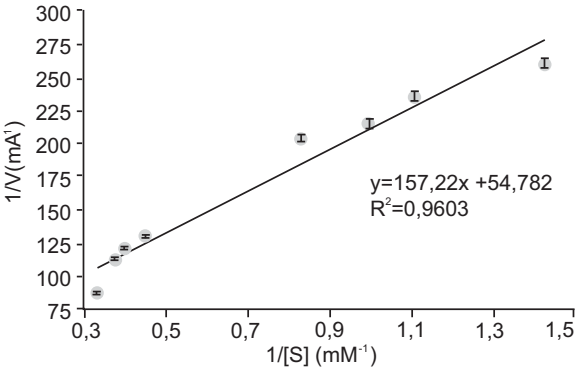

Fig. 4. Lineweaver-Burk plot of uricase from *Lactobacillus plantarum* immobilised on zeolite/κ-carrageenan membrane.

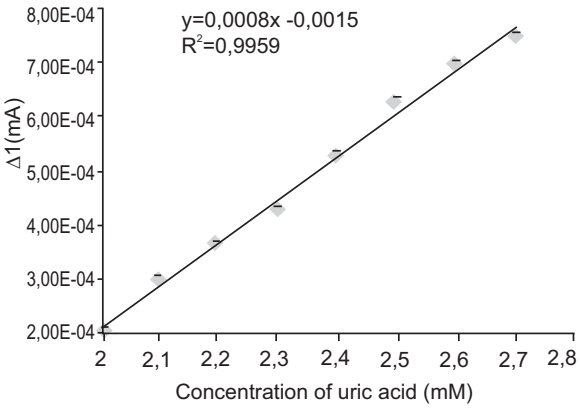

Fig. 5. Relationships between uric acid concentration and current responses.

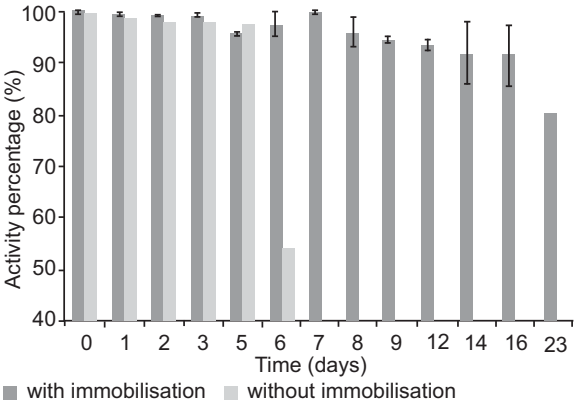

Fig. 6. Stability of uric acid biosensor. With immobilisation (■) and without immobilisation (■).

smaller K_M value shows the higher affinity to the substrate, so that lower substrate concentration can saturate the enzyme active site immediately.

The analytical parameter of this constructed biosensor also showed a slight improvement. The linearity range measurement was 2 to 2.6 mM and the calculated correlation coefficient (R^2) was 0.9959 which was lower than that of the previous report of 0,1 to 800 µM (Devi and Pundir, 2014). Limit of detection (LOD) was calculated to be 0.478 mM which was better than that of the previous report of 0.58 mM (Hamzah *et al*., 2013). Limit of quantity (LOQ) and sensitivity of biosensor were 1.598 mM and 0.0008 mA/mM. The sensitivity of this biosensor was better than that of the previous report of 1,278 mA/mM (Arora *et al*., 2011).

The *L. plantarum* Mar8 immobilised on the membrane of zeolite/κ-carrageenan might have a much-longer stability compared to that of without immobilisation process. The cells immobilised could survive up to 23 days with remaining activities of 80.43%, while the cells without any immobilisation could survive just for 7 days with remaining activities of 54.17%. Ali *et al.* (2011) reported that uric acid biosensor using pure uricase enzyme had three weeks stability with 80% remaining activity. Mulyasuryani and Srihardiastutie (2011) reported the stability of uric acid biosensor using nata de coco membrane had three days stability.

Conclusion

The membrane of zeolite/ κ-carrageenan immobilises uricase producing *L. plantarum* Mar8 and in turn facilitates enzymatic reaction of uricase and uric acid. The reaction redoks is then effectively measured in the constructed biosensor. This uric acid biosensor has better performance in terms of activity, sensitivity and stability and can be developed in to a rapid kit.

Acknowledgement

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