Analysis of bio-oil effectiveness from coconut shells pyrolysis as biopesticide by potentiometric biosensor

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Abstract. Coconut shell (CS) is one waste that can be processed to produce bio-oil by pyrolysis method to be used as an active ingredient of biopesticides. The purpose of this study is to analyze the effectiveness of bio-oil from coconut shells as biopesticides by optimizing the performance of potentiometric biosensors based on acetylcholinesterase enzymes including: % inhibition test, range of work concentration, Limit of detection (LoD) and accuracy. The results of the measurement of percent inhibition of the concentration of natural pesticides from the lowest concentration of 10⁻⁶ to 10⁻² % (v/v) obtained the percent inhibition value respectively were 62.16; 58.08; 54.75; 49.15; 38.99; 26.98 and 17.27 %. The results of the analysis of the concentration range of work showed a linearity with a correlation coefficient of 0.951 in the measurement range of 10⁻⁸ % - 10⁻² % (v/v). LoD in the range of 10⁻⁷ % (v/v) and accuracy obtained an average value of percent recovery of 98.17 %, therefore testing the effectiveness of biopesticides can be done using potentiometric biosensors based on the acetylcholinesterase enzyme.

1. Introduction

Improving the quality and quantity of agricultural products generally farmers use pesticides as away the most effective and economical [1]. Pesticides are chemicals (natural or synthetic) that are used in agriculture to control pests, weeds and diseases in plants. Therefore, applications of various types of pesticides on food crops are needed to confront pests and increase crop production [2]. However, the use of pesticides has caused significant consequences not only for public health but also for the quality of food. Improper pesticide application affects the whole ecosystem by entering residue in the food chain and polluting the soil, air and water surfaces [3].

Other alternatives were available for controlling crop production due to pest, weed and disease, namely the application of various biopesticides from natural materials or biomass. Coconut Shell (CS) is biomass that can be utilized as an active ingredient of biopesticides. Coconut shell processing technique as biopesticide using the pyrolysis method [4]. Biomass pyrolysis has gained increased attention. Pyrolysis is a thermochemical technique in which there is a breakdown of the organic elements of biomass resulting in three items: bio-oil, gas, and bio-char [5]. Bio-Oil CS used as raw materials for biopesticides and preservatives [4–6].

Pesticide use at plants always leaves residues due to high toxicity and impact for human health, animals and the environment [7]. Inhibition of acetylcholinesterases activity (AChE) by pesticides may
cause impaired normal neuron function and death [8]. Therefore, it is necessary to measure pesticides in water and reliable and fast food is very important. Recently, various technologies and methods of analysis have been used to determine pesticide residues, such as liquid chromatography-mass spectrometry (LC-MS) [9], high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) [10].

Although analytic methods have high sensitivity but are limited to laboratory analysis, due to the high cost of experimental instrumentation and the length of examination time [11]. Therefore, electrochemical biosensors have attracted the attention of many people due to its low cost, simple operation, in-place inspections, rapid response and excellent selectivity [2, 12]. A biosensor is an analytical tool that is a combination of a biological component (enzyme) and a physical transducer to detect a target compound [13]. Biosensors have extensive use in food safety measurements, environmental issues monitoring, as biological and chemical warfare agents, agricultural product safety, clinical diagnostics and biomedical research [14]. Electrochemical biosensors are based on potentiometric [15], amperometric or conductometric mode [16] for measurement.

Electrochemical biosensors influenced by catalytic activity in the enzyme immobilization of the acetylcholinesterase (AChE) [17]. Enzyme-based biosensor provide inhibition of enzyme activity directing the presence of biopesticide. Pesticide poisoning is detected by the inhibitory level of the enzyme that causes the decreased concentration of enzyme reaction products than normal values [18]. Analysis of the effectiveness of bio-oil from CS pyrolysis as a biopesticide can be seen from the ability of the inhibition of AChE enzyme activity on the potentiometric biosensor electrode by observing the potential value of the potentiometer. In this study, the effectiveness of bio-oil was observed to be used as a biopesticide with a potentiometric biosensor through the parameters: % inhibition, work concentration range, limit of detection (LoD) and accuracy.

2. Experimental

2.1. Materials

The materials used in this research are acetylthiocholine chloride (ATCC) substrate (Type A5626), AChE enzyme from Electrophorus electricus (electric eel) (Type C3389) 1.17 mg with activity 425.94 U/mg (EC. 3.1.1.7)), glutaraldehyde (GA) 25% from Aldrich-sigma, cellulose acetate (CA), parafilm, NaOH 1 M, KCl 0.1 M, NaH2PO4·H2O, Na2HPO4·12H2O, acetone p.a and ethanol p.a from Merck. Biopesticide sample of bio-oil from CS pyrolysis at temperature 600 °C. Biopesticide as a solution of inhibitors made in the concentration variation of 10^-9 to 10^-2% (v/v) using ethanol:water solution (1:1).

2.2. Apparatus

The equipment used are: pH meter Orion Model 710A/potentiometer, digital multimeter model: UX-837TR, magnetic stirrer, battery, analytical scale, stopwatch, solder, plastic tip, aurum (Au) wire, bronze (Cu) wire, platinum (Pt) wire, silver (Ag) wire, tin (Sn) wire and glassware.

2.3. The manufacture of potentiometric biosensor

Design of a potentiometric biosensor apparatus based on the modified method of Mashuni et al [13, 19]. The potentiometric biosensor electrode body is made of copper wire (Cu) measuring 7 cm connected with gold wire (Au) with a size of 2.5 cm by soldering the Sn wire as a connector. Then the electrode wire is inserted into the plastic tip with the gold wire protruding 1.5 cm at the end of the plastic tip, then the two ends of the plastic tip are wrapped with parafilm. The electrode bodies that have been made, at the edges, are immersed in the membrane material. The edge of the electrode body, namely gold wire, was immersed in a homogeneous solution of CA 15%, then the electrode was rinsed with distilled water and then immersed in a glutaraldehyde solution 25% for 4 hours. After coating cellulose acetate containing glutaraldehyde was formed, the electrodes were rinsed with distilled water and phosphate buffer then the electrode membrane was formed immersed in AChE enzyme for 48 hours. Before use, the electrodes were stored immersed in a phosphate buffer pH 8.0 at 4 °C. Preparation of standard
Ag/AgCl electrodes was carried out by electrolyzing silver wire (Ag) with KCl 0.1 M solution for ± 25 minutes. The Pt and Ag wires, each 5 cm long, are connected to the battery. The silver wire is electrolyzed until the wire is coated with AgCl, which is indicated by the change in color of the wire to grayish black. The Ag/AgCl wire that has been formed is dipped with 0.1 M KCl solution in a plastic tip that has been closed with silica gel, then the top of the electrode body is coated with parafim.

2.4. Enzyme inhibition measurements

Enzyme inhibition for the determination of biopesticide using AChE enzymatic biosensor with potential measurement by potentiometer. The initial potential is measured by immersing the working electrode and the standard electrode into a ATCC 10⁻³ M substrate solution (I₀). The potential measurement was carried out by immersing the enzymatic biosensor electrode with a biopesticide inhibitor of bio-oil from the coconut shell pyrolysis, then the electrodes were rinsed with a phosphate buffer solution pH 8.0 then immersed in a 10⁻³ M ATCC substrate solution connected to a potentiometer transducer and an Ag/AgCl standard electrode. Then, a variation of the known biopesticide concentration is added to inhibit enzyme activity (I). The value of percent inhibition (% I) of AChE enzyme activity can be calculated through equation 1.

\[
\text{%I} = \frac{I_0 - I}{I_0} \times 100\%
\]

Potentiometric biosensor equipment design can be seen in Figure 1.

![Figure 1. The equipment of potentiometric biosensor.](image)

3. Results and discussion

Biopesticide was the CS bio-oil from pyrolysis method at heating temperature 600°C modification of the research Mashuni et al. [6]. The one method of biopesticide analysis is to use an enzymatic biosensor with a potentiometer transducer. This research is a solution and an alternative in designing a potentiometric biosensor based on the performance of bio-oil from CS pyrolysis as a biopesticide in inhibiting AChE enzyme activity. This research was conducted by making a biosensor electrode from gold wire (Au) coated with CA membrane, glutaraldehyde and then immobilized with the AChE enzyme. The role of the AChE enzyme on the biosensor electrode as a catalyst to hydrolyze the ATCC substrate to thicholine chloride (TCC) and acetic acid. The presence of biopesticides can inhibit the activity of the AChE enzyme in hydrolyzing the ATCC substrate so that the resulting concentration of acetic acid is reduced, this is indicated by a decrease in the potential value generated by the potentiometer. The equation of the substrate hydrolysis reaction by the AChE enzyme catalyst can be seen in equation 2.
Experimental conditions of biopesticide power as inhibitors against the performance of the AChE enzyme observed from percent inhibition measurement, as well as the measurement of the working concentration range, limit of detection (LoD) and performance % recovery discussed for the purpose of biosensor accuracy.

3.1. Inhibition measurements
Inhibition (%) was a biopesticide inhibition against the performance of the AChE enzyme. This analysis aims to see inhibition of enzyme activity in hydrolyzing ATCC substrate to thiocholine and acetic acid due to the presence of biopesticide inhibitors. Biopesticides can damage the active site of the amino acid serin on the enzyme molecules. The enzyme activity and concentration of the resulting product are influenced by the administration of inhibitors so that potential values are acquired small. The administration of inhibitors resulted in enzyme damage caused by hydrolysis or oxidation reactions. Interactions between the AChE enzyme and inhibitors will result in covalent bond formation between the active site of the enzyme and inhibitors [20]. The presence of additional inhibitors through a phosphorylation process against enzymes can reduce the value of the resulting product, the greater the concentration of inhibitors, the weaker the strength of acetic acid (H+ ion) so that the potential value is produced smaller. Inhibition measurement presented in Table 1 and Figure 1A.

3.2. Working concentration range
The working concentration range of a method is a concentration range where the accuracy and precision obtained is still acceptable, and usually also include linearity. The measurement result of a good working concentration range is that the value of the correlation coefficient ($r^2$) approaches 1 [21, 22]. The working concentration range can be obtained through the link graph of the potential value of the working electrode and $-\log$ [inhibitor]. Fig. 2B, shows the value of $r^2 = 0.951$ so that the measurement range with that concentration can be used.

| [Inhibitor] (%) | -log [Inhibitor] | Potential value | Inhibition (%) |
|----------------|-----------------|----------------|----------------|
| $10^{-2}$      | 2               | 199.80         | 75.60          | 62.16          |
| $10^{-3}$      | 3               | 199.80         | 82.70          | 58.61          |
| $10^{-4}$      | 4               | 199.80         | 90.40          | 54.75          |
| $10^{-5}$      | 5               | 199.80         | 101.60         | 49.15          |
| $10^{-6}$      | 6               | 199.80         | 121.90         | 38.99          |
| $10^{-7}$      | 7               | 199.80         | 145.90         | 26.98          |
| $10^{-8}$      | 8               | 199.80         | 165.30         | 17.27          |
Table 1. Measurements of inhibition, working concentration range and LoD.

| Mean of inhibition (%) | 43.98 |
|------------------------|-------|
| Linear regression equation | $y = 15.25x + 35.664$ |
| $r^2$  | 0.9511 |
| SB     | 745.33 mV |
| LoD    | 146.62 mV |

Figure 2. Curve of inhibition measurements (A) dan working concentration range (B).

3.3. Limit of detection
LoD is the smallest concentration of an analyte that can be determined or detected by biosensors after inhibition by pesticides. The detection limit is the lowest analyte concentration that still signals large enough and can be distinguished by the signal obtained from Blanko with a confidence level of 99%. The smaller the concentration that can be detected the better the characteristics of the biosensors and can be calculated based LoD = 3 SD / S, where SD is the standard deviation and S is slope [23]. Table 1, LoD value of 146.62 mV which ranges at a concentration of $10^{-7}\%$. Thus, LoD Biosensor to detect biopesticides at a concentration of $10^{-7}\%$. The research of Azizah et al. (2014) [24], LoD for electrodes conductometry biosensors each organophosphate concentration is $10^{-5}\%$ in diazinon and $10^{-4}\%$ in malathion. The research of Zheng et al. 2011 [25], to meet current detection requirements with high precision (according to EU pesticide residue standard (EC 149-2008)), the pesticide concentration that can be detected in fruits and vegetables should be lower than 20 g/L for paraoxon, 50 g/L for parathion, 100 g/L for dichlorvos and 200 g/L for omethoate.

3.4. Accuracy
Accuracy can be interpreted as a measure that shows the degree of proximity of analysis to the actual value that has been determined by the standard method. Accuracy testing using sample concentrations ($C_A$) That is $10^{-8}\%$ and the analyte concentrations added to the sample ($C_{*A}$) are $10^{-6}$, $10^{-7}$ and $10^{-8}\%$. Based on Table 2, the average recovery obtained is 98.17%. The value of recovery obtained is acceptable according to the level of analyte concentrations, according to Gonzalez et al. (2007) [26], the recovery value of 60-115% for analyte concentration $10^{-8}$ to $10^{-6}\%$.

Table 2. Percentage recovery of potentiometric biosensors.

| $I$ (mV) | % Recovery |
|----------|------------|
| $C_{*A}$ | $C_A$ | $C_F$ | % Recovery |
| 121.90  | 165.50 | 118.40 | 97.12 |
| 145.90  | 143.30 | 98.21 |
| 165.90  | 164.13 | 99.17 |
| % Recovery mean | 98.17 |
C* = the analyte concentrations added to the sample, C = sample concentration, C = the total concentration of samples obtained from measurement, I = potential value.

4. Conclusion

Potentiometric biosensors is an enzyme-based biosensors design that can be used by technicians as well as the public in analyzing the ability of natural pesticides or biopesticides in inhibiting the enzyme activity of AChE. Results of performance measurement of biosensors for the biopesticide analysis of bio-oil from CS pyrolisis effectively inhibit enzyme AChE, obtained percentage inhibition value were 62.16, 58.08, 54.75, 49.15, 38.99, 26.98 and 17.27 %, respectively. The analysis results of the working concentration range of 10^{-6} to 10^{-2} (v/v) showed linearity with a correlation coefficient of 0.951. Limit of detection (LoD) in the range of 10^{-2} (v/v) and accuracy value is 98.17 %. This method is good for the analysis of biopesticides residue at low concentrations with accurate.

References

[1] Sharma A 2019 et al. *SN Appl. Sci.* 1 1446
[2] Abhilash P C and Singh N 2009 *J. Hazard. Mater.* 165 1–3
[3] Sharma A, Kumar V, Bhardwaj R and Thukral A K 2017 *Toxicol. Environ. Chem.* 99 1
[4] Mashuni, Jahiding M, Kurniasih I and Zulkaidah 2017 *AIP Conf. Proc.* 1823
[5] Sarkar J K and Wang Q 2020 *Energies* 13 1970
[6] Mashuni, Yanti N A, Jahiding M, Kadidae L O, Djaila R and Hamid F H 2020 *Asian J. Chem.* 32 7
[7] Wei M and Feng S 2017 *Microchim. Acta* 184 9
[8] Yang M, Kainuma S and Jeong Y S 2018 *J. Constr. Steel Res.* 141
[9] Shin Y et al. 2018 *J. Agric. Food Chem.* 66 13
[10] Santos C, Oppolzer D, Gonçalves A, Barroso M and Gallardo E 2018 *J. Anal. Toxicol.* 42 5
[11] Gumpu M B et al 2017 *Bull. Environ. Contam. Toxicol.* 98 5
[12] Pundir C S and Chauhan N 2012 *Anal. Biochem.* 429 1
[13] Mashuni, Ramadhan L O A N, Jahiding M and Herniati 2016 *Mater. Sci. Eng.* 107
[14] Pundir C S, Malik A and Preety 2019 *Biosens. Bioelectron.* 140 p
[15] Du D, Huang X, Cai J and Zhang A 2007 *Sensors Actuators, B Chem.* 127 2
[16] Simonian A L, Good T A, Wang S S and Wild J R 2005 *Anal. Chim. Acta* 534 1
[17] Patel H, Rawtani D and Agrawal Y K 2019 *Trends Food Sci. Technol.* 85
[18] Hassani S et al. 2017 *Arch. Toxicol.* 91 1
[19] Mashuni, Ramadhan L O A N, Jahiding M and Syarfiah 2016 *IJCEBS.* 4 2
[20] Kostelnik A and Pohanka M 2018 *Biomed Res. Int.* 2018
[21] Prichard L and Barwick V 2003 *Preparation of calibraton curves.*
[22] Skoog D A, West D M and Holler F J 2004 *Fundamentals of analytical chemistry,* 9th ed. (Philadelphia: Saunders College)
[23] Guider R et al. 2015 *Sens. Bio-Sensing Res.* 6
[24] Azizah A, Mulyasuryani A and Sutrisno 2014 *Kim. Student J.* 1 1
[25] Zheng Z, Zhou Y, Li X, Liu S and Tang Z 2011 *Biosens. Bioeletront.* 26 6
[26] González A G and Herrador M A 2007 *TrAC - Trends Anal. Chem.* 26 3