THE ISOLATION AND ANALYSIS OF PROTEIN FROM TOFU WASTE AS CORROSION INHIBITOR TOWARDS CARBON STEEL IN 1% NACl SOLUTION

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ABSTRACT

Corrosion could damage many aspects in life; therefore corrosion inhibitor was needed to overcome those problems. The aim of this research is to isolate protein from the foodstuffs waste that contain high protein, suchs as tofu, and at the same time is to increase the economical value of tofu waste. The isolation was carried out by two methods: precipitation by 10% v/v acetic acid solution and protein fractination by ammonium sulphate. The determination of corrosion inhibition activity utilized the Tafel method at the concentration of 80 ppm in 1% NaCl w/v solution. The corrosion inhibition activity of protein isolated by precipitation methods is 53.41%. Six protein fractions (fraction of 0%-60%) was obtained from fractination methods. The largest corrosion inhibition activity (70.89%) was shown on the fraction of 20-30%, which is larger than the corrosion inhibition activity of the tofu waste before being isolated (29.74%). Amino acids contained in isolated protein that have the influence to corrosion inhibition activity was analyzed by hydrolisis methods, which are: methionine, proline, tyrosine, asparagine, and thrytophane. The results of this research showed that protein isolated from the tofu waste have the potent as corrosion inhibitor towards carbon steel in 1%NaCl solution.

Keywords: *corrosion inhibitor, tofu waste, protein isolation, Tafel method*

INTRODUCTION

Problems caused by corrosion often gave negative effects to various companies in the world that attracted the attention of many researchers to overcome these problems. Both Indonesian and many researchers worldwide has been developing the substances that could be used to prevent the corrosion process. The cathodic protection, coating metals surface using paint, and the use of corrosion inhibitors are several ways to prevent the corrosion. Corrosion inhibitor is the substance that when being added to the environment in a small number, in a continuous or periodical manner, could reduce the rate of corrosion process of metals (Priandani, Manik, 2001). Corrosion inhibitor used recently is organic compounds that would prevent the corrosion quite effectively and more environmentally friendly than the inorganic corrosion inhibitors. "Back to nature" is the term used, so the communities tend to use chemicals from nature. "Back to nature" trend is based on various lacks found in the synthetic materials, such as the shortage of the safety, pollution of the environment, as well as the large cost from the use of the synthetic chemicals that used continuously (Hermawan, 2009). Indonesia as the country that has the second of the largest biological diversity in the world after Brazil has the potent to develop science and technology as well as their utilization for human life. Therefore, the extract of the naturally occurring materials that contained the nitrogen atom (N), oxygen (O), phosphorus (P), sulphur (S), and atoms that had lone pairs electrons could be used as alternative materials that can be used as corrosion inhibitor. In this research the protein was isolated from the tofu waste to be used as an alternative of corrosion inhibitor towards carbon steel, therefore it can eventually increase the economical value of the waste from the high protein containing food products in Indonesia. Moreover, it also can reduce the environmental pollution caused by the waste. Hopefully, the results of this research can increase the economic revenue of Indonesia and at the same time support the clean life for the Indonesian community. For those reasons mentioned this research was carried out to study the potent of isolated protein from tofu waste as well as its amino acid contents as corrosion inhibitor towards carbon steel in mild electrolyte condition.

MATERIALS AND METHODS

MATERIALS

All of reagents used in this research are GR grade. All of solvents were distilled prior to use. The material used in this research are: acetic acid, ethanol, diethylether, methanol, acetone, ammonium sulphate, Biuret reagent, ninhydrine reagent, universal pH indicator, KBr, TLC plate silica gel G-60 F254 alumunium (EM 5554), cotton, aquadest, and NaCl. Some amino acids were used as standard solution for thin layer chromatography (TLC) analysis: L-Glycine, L-Glutamine, L-Cysteine, L-Alanine, L-Tyrosine, L-Asparagine, L-Valine, L-Phenylalanine, L-Methionine, L-

Proline, L-Threonine, L-Histidine, and L-Tryptophane. The TLC used butanol-acetic acid-water (4:1:1) as developing solvents (eluents).

PROTEIN ISOLATION BY PRECIPITATION

Protein isolation by precipitation was prepared by heating the tofu waste at 55^{o}C, then 10% v/v of acetic acid added. The protein that has been coagulated was subsequently sentrifugated to separate it from the solution. The diethyl ether:ethanol (1:1) was added to the protein, then the mixtures were filtered, dried and evaporated to remove the solvents.

PROTEIN FRACTINATION WITH AMMONIUM SULFATE

Ammonium sulphate, $(NH_4)_2SO_4$, was added to the tofu waste by stirring it until the concentration of ammonium sulphate was 10% (Berg, 1984). If the precipitation was occurred, the centrifugation was carried out to separate the solvent from protein. Fractionation was continued until the concentration of ammonium sulphate was 70%. Isolated protein was dissolved in aquadest and ready to be analyzed.

THE DETERMINATION OF CORROSION INHIBITION ACTIVITY

The isolated protein was dissolved in NaCl 1% w/v solution to make a solution with concentration of 8 ppm and other concentrations. The blank solution used is 1% NaCl solution without sample. Into 110 mL specialized chamber equipped with magnetic stirrer was introduced 100 mL of blank solution or sample solution. The working electrode (carbon steel), the reference electrode (SCE), and auxiliary electrode (platinum electrode) were immersed into the electrolyte solution. Carbon dioxide gas was introduced into the electrolyte solution until saturation reached, approximately 20 minutes. The carbon steel type used is API 5L X65 with compositions (in percentage, %): Fe (97,9327); C (0,0737); Si (0,2882); S (0,0068); P (0,0153); Mn (1,5353); Ni (0,0129); Cr (0,0224); V (0,0276); Cu (0,0051); W (0,0029); Ti (0,0169); Sn (0,0005); Al (0,0282); Nb (0,0396); Zr (0,0009); Zn (0,0014). The measurement utilizing Potentiostate/Galvanostate PGZ 301 VoltaLab® 30 model and VoltaMaster® software program until the curve of potential measurement towards time was completely formed well. The measurements of each sample solution should be initiated by the measurement of blank solution. The inhibition activity can be calculated using following equation:

$$\%\ \text{inhibition efficiency} = \frac{\text{Corrotion rate of blank solution (mm/Y)} - \text{corrosion rate of sample (mm/Y)}}{\text{Corrotion rate of blank solution (mm/Y)}} \text{x} 100\% \quad (2\text{-}1)$$

or

$$\% \text{ inhibition efficiency} = \frac{I_{Blank}\ (mA/cm^2) - I_{Sample}\ (mA/cm^2)}{I_{Blank}\ (mA/cm^2)} \times 100\% \qquad (2\text{-}2)$$

with I_{blank} is corrosion current density of blank solution (uninhibited system), in mA/cm^2, and I_{sample} is corrosion current density of sample solution (inhibited system).

AMINO ACIDS ANALYSIS

Amino acids analysis of the protein fraction that have the highest corrosion inhibition activity (fraction of 20-30%) was carried out by the hydrolysis of protein in 6N Hydrochloric acid (HCl) solution. The reaction mixture was refluxed for 24 hours. The viscous solution was obtained from the removal of solvent by rotary evaporator in reduced pressure. Amino acids content of the isolated protein was determined by thin layer chromatography (TLC) with buthanol:acetic acid:water (4:1:1) as eluents. Ninhydrine reagent was used to reveal the Rf (retention factor) values of TLC chromatograms. Rf values of amino acids that detected was measured and compared to the standards.

RESULTS AND DISCUSSION

The determination of corrosion inhibition activity of isolated protein at various concentrations utilized the electrochemical method, which is Tafel polarization method. According to the standard of the use inhibitor that was known, the initial concentration standard to be used is at concentration of 8 ppm because at this state the corrosion process tends to be decreased. The 1% NaCl solution was used as electrolyte condition that would initiate the corrosion process in the system. Table 1 presents the measurements results. The conclusion that could be taken from the data on Table 1 was that the protein isolation by the fractination method with ammonium sulphate in the concentration of 80 ppm showed the respectable corrosion inhibition activity. The highest corrosion inhibition activity was achieved by the protein fraction of 20-30% that is of 70.89%. This result showed that this protein fraction contain the largest amount of protein extracted from water phase. Moreover, if these results compared to the sample of the tofu waste, the isolated proteins by fractination with ammonium sulphate have bigger value of its corrosion inhibition activity. This result showed that the process of the protein isolation was the crucial stage in the process of the production of corrosion inhibitor derived from the tofu waste. The good results were also shown from the corrosion inhibition activity of proteins isolated by the precipitation process in 10% v/v acetic acid solution, which are also better than the crude tofu waste, although it is lower than the value of protein isolated by the fractionation process in ammonium sulphate

solution. Therefore it can be concluded that the fractionation method is the better technique to isolate protein from the tofu waste compared to the precipitation method. The results are also presented in Figure 1. From Figure 1 it can be concluded that the optimum concentration of the isolated protein is 8 ppm. This result is in accordance with the standard data that mentioned that the concentration of 8 ppm was the best that was used to determine the corrosion inhibition activity towards metal.

Table 1 The corrosion inhibition activities of the tofu waste and the isolated protein at various concentration in 1% NaCl solution according to Tafel method

Sample	Concentration	% Inhibition Efficiency
Tofu waste	5% (v/v)	29,74%
Protein isolated by the precipitation in 10% v/v acetic acid solution	8 ppm	52,30%
	80 ppm	53,41%
Protein isolated by fractination process in ammonium sulphate solution	80 ppm (fraction of 0-10%)	43,03%
	80 ppm (fraction of 10-20%)	46,49%
	80 ppm (fraction of 20-30%)	70,89%
	80 ppm (fraction of 30-40%)	47,44%
	80 ppm (fraction of 40-50%)	56,12%
	80 ppm (fraction of 50-60%)	67,14%

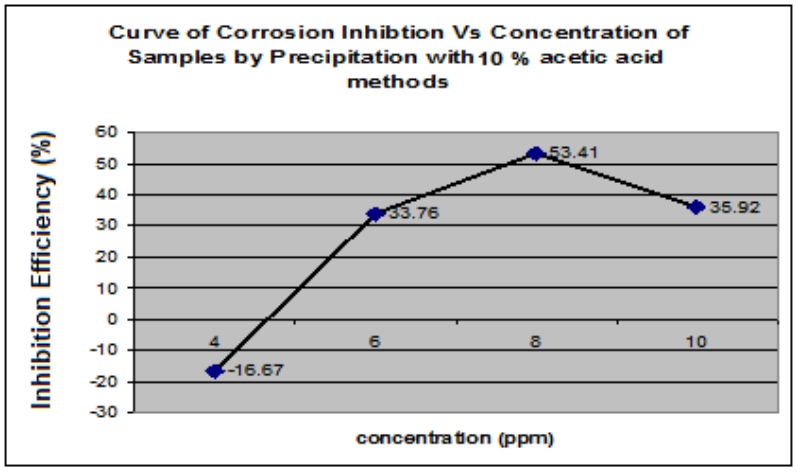

Figure 1 The relationship between the corrosion inhibition efficiency (%) and the concentration of isolated protein by precipitation in 10% acetic acid

The determination of corrosion inhibition activity was also carried out for samples of isolated protein from the tofu waste that has been prolonged kept for several weeks, which is presented in Table 2. The sample composition that was measured was 5% v/v of protein solution.

Table 2 The corrosion inhibition activity of isolated proteins from the tofu waste that has been prolonged kept for several weeks

Concentration of sample	**Time of the tofu waste being prolonged kept (week)**	**Inhibition Efficiency (%)**
4 ppm	2	52,11
	4	81,06
	6	49,13
	8	34,56
	10	47,53
8 ppm	8	52,30
	24	33,76
	40	35,92

Table 3 showed that the more the tofu waste being prolonged kept has caused the decrease in corrosion inhibition activity of the isolated protein. It can be seen that in the first two weeks the maximum corrosion inhibition efficiency was found to be 81.06 % at the concentration of 4 ppm, however after the last two months the highest corrosion inhibition efficiency was only found to be 52.30 % at the concentration of 8 ppm. These facts are the result of the degradation process of proteins contained in the tofu waste that was increased along the time, therefore both of the amount and the corrosion inhibition potency of some potential proteins contained in the tofu waste was also decreased.

In order to analyze which amino acids that would be responsible to the corrosion inhibition activity of the isolated protein therefore the most potent protein fraction isolated was then hydrolyzed in 6M HCl for 24 hours. The amino acid contents were qualitatively analyzed by thin layer chromatography (TLC) using butanol:acetic acid:water (4:1:1) as the developing solvents or eluents and thirteen amino acids as standard. The spots of TLC chromatogram was developed using ninhydrine reagent. The results are presented on Table 3. The results of TLC analysis showed that the isolated protein from the tofu waste at the fraction of 20-30 %, which has the highest corrosion inhibition activity, contains methionine, tyrosine, proline, asparagine, and

tryptophane, as the Rf values are the same as the amino acids standard. Moreover, it also could be seen from the color of the spots that were produced which is also the same as the standards, such as follows: the purple color with Rf that is the same as methionine, the purplish orange color with Rf that is the same as tryptophane, and the purple color with Rf that is almost the same as tyrosine.

Table 3 The TLC results of the isolated protein (fraction of 20-30%) compared with the standard amino acids

No.	**Sample**	**Results**	
		Rf	**Color of spots**
1.	L-Glycine (standard)	0,27	Brownish Orange
2.	L-Glutamine (standard)	0,21	Reddish Purple
3.	L-Cysteine (standard)	0,10	Brown
4.	L-Alanine (standard)	0,53	Red
5.	L-Tyrosine (standard)	0,29	Purple
6.	L-Asparagine (standard)	0,37	Yellowish Purple
7.	L-Valine (standard)	0,45	Brownish Purple
8.	L-Phenylalanine (standard)	0,67	Brown
9.	L-Methionine (standard)	0,63	Brownish Purple
10.	L-Proline (standard)	0,37	Yellow
11.	L-Threonine (standard)	0,38	Red
12.	L-Tryptophane (standard)	0,39	Yellowish Purple
13.	L-Histidine (standard)	0,10	Brown
14.	Protein Fraction of 20-30 % (largest % efficiency inhibition)	0,63	Purple
		0,39	Purplish Orange
		0,29	Purple
		0,37	Yellowish Purple
		0,37	Yellow

Based on the results it can be concluded that the five main amino acids contained in the isolated protein play the important role in the corrosion inhibition activity. The role of these amino acids mainly caused by the ability of functional groups within the structure of amino acids that would

interact with the metal surface. The interaction between metal and amino acids are presented in Figure 2.

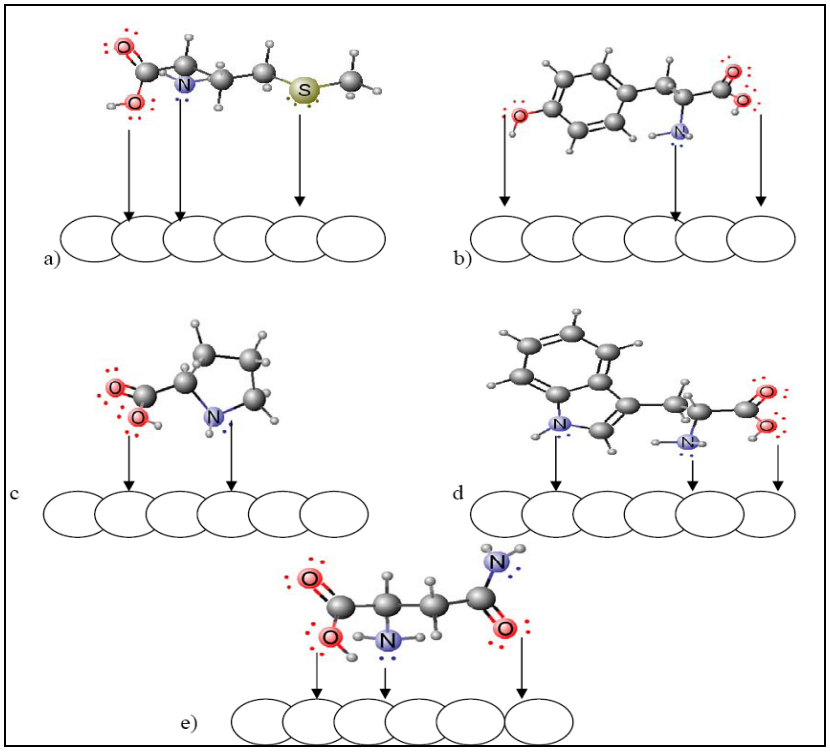

Figure 2 The illustration of the interaction between functional groups of amino acids and metal surface: (a) methionine; (b) tyrosine; (c) proline; (d) tryptophane; (e) asparagine

Figure 4 showed the interaction between the lone pair's electrons of N, S and O atoms as well as the cloud of phi electrons of aromatic rings within the amino acids structure and the metal surface. The protection mechanism of the amino acids towards the iron or carbon steel was assumed to be the adsorption process, chemically or physically. The adsorption process of these functional groups within protein structure would make a protective layer that would prevent the metal from its corrosive environment. Therefore the carbon steel can be prevented from corrosion process by means of this adsorption mechanism of protein molecules, which has been shown from the relatively high value of corrosion inhibition activity of the isolated protein. These results can be used as the initiator to develop the utilization of waste as potent corrosion inhibitor.

ACKNOWLEDGEMENTS

1. The Ministry of Research and Technology of Republik Indonesia for granted the research.
2. To all parties associated to this research that can not be mentioned one by one

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