Robust protein based organic electrocatalyst for hydrogen production through instant noodle wastewater electrolysis

Willy Satrio N, Winarto, Sugiono, ING. Wardana*
Department of Mechanical Engineering, Brawijaya University
MT Haryono 167, Lowokwaru, Malang 65145, Indonesia
*Email: wardana@ub.ac.id

Abstract. Natural resources carbonization doping using heavy atom is the common method to synthesize organic electrocatalyst for waste to hydrogen energy conversion. This study provides one-step solution to synthesize organic electrocatalyst using enzymatic protein of Lumbricus Rubellus. L.Rubellus extract and its combination with graphite flakes in powder form are tested as electrocatalyst for instant noodle wastewater electrolysis and pre-hydrolyzed instant noodle wastewater by direct mixing. Pre-hydrolysis has doubled the hydrogen production rate for each tested catalyst. Lumbricus Rubellus extract without graphite flake mix performs better in pre-hydrolyzed wastewater. Graphite flakes form polar substrates in pre-hydrolyzed wastewater. Positive substrates inhibit the hydrogen evolution reaction of pre-hydrolyzed wastewater by electron deposition. Negative substrate inactivates L.Rubellus protein due to competitive inhibition.

Keywords: hydrogen production, wastewater electrolysis, organic catalyst

1. Introduction
Waste to energy conversion is a solution attempted to minimize the global energy scarcity as well as minimize negative environmental impact of disposed waste. Biomass wastes which contain energetic sources such as nutritious macromolecules are able to be used as an energy source [1]. The biomass conversion to hydrogen as the cleanest energy source is also applicable [2]. Biomass electrolysis was shown can be more efficient than water electrolysis under the right strategy. The biomass used in this study is instant noodle wastewater.

The use right catalyst is considered as one of the key strategy to efficient biomass electrolysis. Metal catalysts always become the primary choice in any hydrogen production methods. For example, hydrogen production by kapok seed oil steam reforming use CuZn as cheap non-noble metal catalyst [3]. In electrolysis hydrogen production, the use of transition metal has successfully replace noble metal such as platinum. The use of zinc as an electron depositor in alkaline water electrolysis successfully increases hydrogen evolution reaction (HER) [4]. Nevertheless, the utilization of metal as the catalyst material can be replaced by some organic materials [5].

Some natural resources was utilized as organic electrocatalyst for hydrogen production. The natural sulfur doped onion was carbonized through pyrolysis to synthesize sulfur doped active carbon catalyst [6]. The nitrogen doped activated carbon catalyst was obtained using the same technique with the onion based activated carbon catalyst [7]. Coffee pulp also was able to be
converted to activated carbon catalyst doped with nitrogen and phosphor atom [8]. Activated carbon from carbonized algal also been utilized as electrocatalyst [9]. Heavy atom doped activated carbon catalyst have dual role as polar substrate binder and electron acceptor. The substrates bind to the catalyst structure are easily attack by the electrons [6–8].

Heavy atom doped activated carbon based organic catalyst requires two steps synthesis consists of carbonization and atom doping to obtain the final product. However, natural resources with active compounds such as antioxidant containing active protein consist of electron acceptor and electron donating chemical groups [10]. Methoxyphenyl content of phenolic antioxidant responsible for its electron donating capability [11]. One of the robust phenolic antioxidant active protein can be found in L,Rubellus earthworm [12]. DLBS-1033 protein of L,Rubellus extract is able to maintain fibrinogen synthesis after dissolved in gastric acid without any protection [13]. The robustness of L,Rubellus proteins was due to its disulfide bridges [14].

Despite the phenolic antioxidant content and the robustness of L,Rubellus, enzymatic properties of earthworm also playing an important role. Earthworm digestive system is all over its skin which contains glycolytic, proteolytic, lignolytic, cellulolytic, and lipolytic enzymes [15–17]. Earthworm enzyme widely use in flour industry to control the digestive quality of flours [17]. In relation to that, biomass content of wastewater is dominated by dissolved starch content. The dissolved starch is mostly consist of amylopectin which expected reach 59% of total organic compound in instant noodle wastewater [18]. Therefore, this study will test L,Rubellus ability as organic electrocatalyst for instant noodle wastewater electrolysis to hydrogen production.

2. Materials and method

2.1 Substrate materials preparation

The substrate used in this study is instant noodle wastewater. The instant noodle used was regular size Indomie™ goreng. The noodle prepared by following the instructions behind its package. The water used to boil the noodle is Aqua™ from 19 L Polycarbonate gallon package. The water transported using electric water pump from the gallon to the stainless saucepan pot. The pre-hydrolyzed substrates were hydrolyzed by 11 gram of Saccharomyces Cerevisae bacteria obtained from yeast. The bacteria cultured for 1 hour in anaerobic environment.

2.2 Organic catalyst materials preparation

The organic catalyst materials used are Lumbricus Rubellus earthworm extract. The L,Rubellus extract used is Vermint™. The Vermint™ amount used in each test is 12 mg equivalent to 10 capsules. The graphite flakes material was HS-4402200 wooden charcoal powder obtained from CV. MAHKOTA CITRA MANDIRI, Malang, Jawa Timur, Indonesia. The charcoal used have 70% fixed carbon, 6800 calorific value, 1.7% ash content, and not been activated. For each mixed test, 5 gram of charcoal powder was mixed with 12mg of L,Rubellus extract.

2.3 Electrolysis test

The MQ-8 hydrogen sensor was used to detect the hydrogen gas. The sensor was connected to a Lenovo G-40 notebook computer with AMD-A8 CPU through the USB 3.0 port of Arduino Uno R3 microcontroller. A Styrofoam embedded under the jar cover to be used as a sensor placement. The electrodes were embedded between the Styrofoam. The depth of the electrodes is 12 cm inside the jar and leaving 3 cm on top of the jar as the connection point with the power source. The 5x12 cm PVC sheet was placed in the middle of the Styrofoam. The experiment arrangement was depicted in Figure 1. The gas collection space was 900 ml and the liquid substrate space was 600 ml. A 12 V dry cell battery was connected to electrodes by alligator clips as the power source.
Collected hydrogen gas concentration data were in parts per million (ppm) units measured relatively to the gas collection space volume. Data collection started after the sensor shows steady measurement pattern during electrolysis. Total resistance of all equipment’s during the electrolysis test was 10.01 Ω measured from the Arduino board without additional resistor. The electrodes were placed by following the battery convention. Therefore, the cathode is connected to negative side of battery and signed as negative electrode. The anode is positive electrode connected to battery positive side. The cathode material is stainless SS-201 shaft rod with 3 mm diameter obtained from material shop in Malang, Indonesia supplied from Surya Logam and the anode material is graphite obtained from Fabre Castle HB Pencil.

![Electrolysis test set-up](image)

**Figure 1.** Electrolysis test set-up

### 2.4 Material characterization
Elemental, Phase, and morphology characterization of *L.Rubellus* extract and graphite mixed *L.Rubellus* extract were performed by Fourier Transform Infrared Spectroscopy (FTIR) and (Energy Dispersive X-ray) EDX, (X-ray Diffraction Crystallography) XRD, and (Scanning Electron Microscopy) SEM respectively. The absorbance FTIR test was performed using Shimadzu IRPrestige21. The XRD phase analysis was conducted in Bragg-Brentano geometry using PanAnalytical type E’Xpert: Pro with Cu anode, Kα source (1.5405 Å) and Ni Kβ filter. The Bragg angle scan range was from 10° to 90° with 0.001° / 0.7 second angular speed for each scan step. The divergence slit length was 1.52 mm and the length of the fixed receiving slit is 0.10 mm. The sample morphology images and elemental compositions were obtained by carried out SEM imaging and EDX test using FEI Inspect-S50 electron microscopy instrument. The SEM mode was backscattered electron mode (BSE) using negatively charged beam (e-beam). The SEM images was taken in 100μm and 10μm magnification scale. EDX elemental data were collected in 100μm magnification scale to obtain general composition data of the samples.

### 2.5 Morphology surface charge mapping
Backscattered surface charges of the SEM images are mapped using digital imaging technique. The digital imaging technique used was brightness pattern mapping. The brightness patterns of the SEM images were plotted using imageJ software with the help of an extension package called 3D
surface plugin. ImageJ 3D surface plugin draw the surface brightness pattern of an image based on the 8-bits series arrangement of the image pixel brightness patterns obtained by converting the image type to 8–bit image. The SEM image file extension was in tagged image file format (TIFF) which has 32 bits consist of 24-bits of information and 8-bits of instructions for the image manager in operating system. The 8-bit image type has 8*32 bits information space which results in 256 bits from 0 to 255 represents the pixel brightness of an image [19]. The fire color scheme was assigned to the pixel brightness pattern to ease the surface charge region classification using a fire lookup table (Fire-LUT) matrix with specified value by imageJ. The Fire-LUT color scheme consists of blue for low brightness or dark pattern, violet for medium brightness or gray pattern, and yellow for high brightness or bright pattern.

3. Results

3.1 Hydrogen production performance test
Electrolysis performance test was done to observe the performance of the synthesized protein based catalyst in producing hydrogen. The hydrogen production performance test in pre-hydrolyzed and non-hydrolyzed instant noodle wastewater was performed in 7200 seconds. The hydrogen evolution pattern was recorded for each test as seen in Figure 2. There is no difference in hydrogen production performance between L,Rubellus extract (LE) and graphite flakes mixed L,Rubellus extract (GFLE) in instant noodle wastewater without pre-hydrolysis. LE catalyst performs better in pre-hydrolyzed instant noodle wastewater than GFLE catalyst. The use of LE catalyst results in 8452 ppm H₂ while the use of GFLE catalyst produces 7812 ppm H₂.

![Electrolysis Performance Test](image)

**Figure 2.** Hydrogen Evolution Pattern

3.2 Continual surface charge gradient
Morphology analysis of the GFLE catalyst is performed using SEM imaging to observe the role of the structure in order to build the catalyst kinetic mechanism GFLE sample consist of L,Rubellus extract and graphite flakes samples. The morphology of GFLE is presented in Figure 3(a) under 100 µm magnification scale. It is can be seen that, the GFLE sample is amorphous and consist of two
different structures. The structures are not mixed and ranged in different sizes. There is two visually differentiable structure, which is the rounded structure and the sharp edges structure. The rounded structure is shown in blue rectangle and the sharp edges structure is shown in the red rectangle. The sharp edges structure is the graphite flake and the rounded structure is the L, Rubellus extract.

The L, Rubellus extract structure image inside the blue rectangle is zoomed to 10 µm scale magnification to better understand its morphology. The zoomed image is presented in Figure 3(b). The image in figure 3(b) confirm that the L, Rubellus extract is a protein complex with large microstructure as stated by [12]. The L, Rubellus protein complex is shown as a combination of different microstructures. Therefore, to confirm the role of its morphology in the catalyst kinetics, the SEM images data are not sufficient. Thus, the brightness patterns of each SEM images are analyzed.

![Figure 3. SEM images and Surface Charge Patterns of graphite flakes mixed with L, Rubellus extract: (a)100um Scale, (b)10um scale, (c)Surface charge of 100um scale, (d)Surface charge of 10um scale.](image)

The GFLE SEM image in 100µm scale and its L, Rubellus extract on 10µm scale brightness patterns were analyzed to observe the role of GFLE and LE catalyst surface. Brightness pattern in high vacuum
mode in BSE SEM is depicting the surface charge of the material [20]. The SEM image of GFLE in Figure 3(a) showing some glowing regions which depicted as orange colored peak pattern in Figure 3(c) indicating positively charged region. Some dark regions also depicted as blue colored crater in Figure 3(c) indicating negatively charged region. Therefore GFLE surface is composed of continual positive and negative charge gradient. The LE surface composed of negatively charged regions and the strongest negative charge is located in the middle of the structure shown by the deeper blue crater in Figure 3(d).

The XRD phase analysis of the GFLE was also performed to confirm the surface reaction that occurs. The peak of the XRD was shown in between of 20° to 30° Bragg angle. The peak formed is broad and have two high hills as seen in Figure 4. The first hill was formed in 20° with x-ray intensity reflectance counts from 100 to 400. The second hill was formed near to 30° and reaches more than 400 reflectance counts. X-ray reflectance counts indicating the electron density of the surface on each Bragg angle [21]. Therefore, the peak regions is more electron dense than other regions. In the Bragg angle between 0° and 30° is more electron dense than the rest of the regions. The double hill peak with a single crater is indicating the peak is consists of a surface with different electron density. Therefore, the double hill XRD peak can be related to the continual surface charge gradient of GFLE as shown in Figure 3(c).

![Figure 4. XRD characterization result of graphite flakes mixed L, Rubellus extract](image)

3.3 Molecular insights of the synthesized catalyst
The deeper insight in the molecular scale is needed to unravel the GFLE and LE catalyst mechanism of action. Hence, the functional elemental analysis using FTIR was performed on GFLE. The FTIR absorbance of GFLE was analyzed to determine some functional groups role in the catalytic reaction. By matching the formed patterns on some FTIR database, some molecular bonds are able to be decoded as seen in Figure 5. The polar functional groups founded are hydroxyl group (O-H), single carboxyl group (C-O), and double carbonyl group (C=O). The magnetic group founded is CH aromatic group. The existence of hydroxyl and CH aromatic is consistent with the classification of LE as phenolic compound.

The elemental mapping of the GFLE was done using EDX. Based on the atomic percentage on energy dispersion in 100µm scale magnification, three main atomic compositions was founded as seen in figure 6. There is 71.93% Carbon, 27.82% Oxygen, and 00.24% Calcium. The dispersed X-ray for each atom is K shell as the K letter depicted beside the name of each detected element.
4. Discussion
Neither GFLE nor LE catalyst are showing hydrogen evolution differences in instant noodle wastewater electrolysis without pre-hydrolysis. However, in terms of pre-hydrolyzed instant noodle wastewater substrate electrolysis the LE catalysts outperformed GFLE catalyst. LE is an enzymatic protein and has a role as hydrolytic agent. Therefore, during the electrolysis of instant noodle wastewater it is also hydrolyzed by LE. The hydrolysis of saccharides breaks down bigger polysaccharide to smaller polysaccharide such as pentasaccharide, disaccharide, or disaccharide [22]. Therefore the pre-hydrolyzed instant noodle wastewater has smaller saccharides content
than the wastewater without pre-hydrolysis.

The size differences between two or more molecules define its reactivity [23]. The large polysaccharides length ranged from 75µm to 110µm while the medium polysaccharides length ranged from 45µm to 65µm. Monosaccharide, disaccharide, and trisaccharide length ranged from 3 to 7 carbon atom length which is 0.0231µm to 0.0539µm [24]. Correspond to that, the size of each continual crater and continual peak in continual surface charge gradient of GFLE as shown in figure 3(c) is ranged from 10-14µm. Therefore, GFLE surface charge gradient only effective to break down polar molecule longer than 20µm. In contrast, LE with bigger surface area reaction has strong negative surface charge. Each crater length shown in figure 3(d) is ranged from 2-4µm with uniform charge gradient. Therefore, the uniform negatively charged surface is a substrate binder for The LE and GFLE catalyst are both effective in instant noodle wastewater without pre-hydrolysis. The continual surface charge gradient on GFLE surface is able to bind and deform large polar molecules such as large polysaccharides. Both LE and GFLE are reduction capable. The correlation between XRD double hill peak and GFLE surface indicating the negatively charged crater of the surface charge pattern on GFLE and LE are both electron dense. There is also a Calcium element detected on EDX result. The electron dense molecule or atom have a small band gap makes it capable to donating electron [25]. Therefore, the presence of calcium atom makes the GFLE and LE catalyst able to donate electrons which trigger the hydrogen evolution reaction during electrolysis.

The functional elements reconstructed from the FTIR results have a combinatorial chance to form a methoxyphenyl. Methoxyphenyl is an electron donating phenolic group which contain in most phenolic antioxidant [11]. Methoxyphenyl as an electron dense molecule with aromatic magnet can have dual role. The aromatic ring magnet activated by the incidence electron or negatively charged ions during electrolysis can attract positively charged ions. The methoxyphenyl then transfer its electron to the positively charged ion. If the positively charged ion is hydronium then the hydrogen evolution reaction will occur. Therefore, the aromatic ring of LE in both GFLE and LE catalyst is a proton binding. The GFLE and LE are both effective to be used as biomass electrolysis catalyst with medium to large sized polysaccharide content. The LE catalyst is more effective than GFLE for biomass electrolysis with smaller polysaccharide content due to its uniform negatively charged surface charge gradient in smaller size. The hydrogen evolution reaction occurs due to methoxyphenyl content and Calcium content of LE. The large molecule binding with the GFLE and LE surface based on surface charge polarity. The small proton binding with GFLE and LE based on methoxyphenyl aromatic magnet.

5. Conclusions
The GFLE and LE are effective to be used as hydrogen evolution catalyst for instant noodle wastewater electrolysis. The LE catalyst is more effective than GFLE catalyst in smaller biomass substrate electrolysis.

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