Fabrication of novel electrode developed by modified graphene oxide with L-cysteine deposited on iron nanoparticle for enhancing the electricity generation from MFCs

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Table of contents

Abstract

Nowadays, electricity shortage due to insufficient resources and a huge population growth rate and wastewater pollution are the main problems which severely affect the environment. In this research, Microbial fuel cell (MFC) - novel alternative energy generators and wastewater treatment utilizing organic wastes in wastewater as substrates – was introduced. However, microbial fuel cell still inconstantly generates only low amounts of electricity. Consequently, novel electrode synthesized from graphene oxide synthesized from bamboo with L-cysteine deposited on iron nanoparticle (L-Cys grafted Fe NPs on GO) was synthesized in order to enhance electricity production efficiency due to the higher bacteria adsorption ability. L-Cys grafted Fe NPs on GO was characterized under Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive X-ray (EDX), X-ray Diffraction (XRD) and Raman Spectroscopy. As a result, Novel electrodes were tested under different conditions, wastewater, and bacteria solution. The results suggested that a novel electrode under condition can show the highest performance which can generate electricity up to 15.13 watts per cubic meter which is 28.5 times compared to the ordinary electrode. Furthermore, results suggested that the wastewater treatment efficiency and bacteria adsorption ability are up to 53.89% and 24.24% respectively. Additionally, generated electricity voltage is enough for charging a lead acid battery when MFC were connected in series (8 cells).

Introduction

Electric energy is the main source of human life in the modern era. It can be produced from many sources. Whether fossil fuels, crude oil, natural gas, water, solar cell, wind power, electric power has many benefits in daily life, such as used in electronics, electrical equipment, vehicles, and communication tools. From the Ministry of Energy Thailand, crude oil was consumed approximately 75.2 megatons within one year. And Thailand has a power generating capacity of 164,000 kilowatts per hour. To use electricity in Thailand, people still have to bring energy from neighboring countries, such as Laos and Myanmar when the resources are limited. However, the population and the need for energy have increased causing a lack of energy problems, which is considered one of the important social and environmental issues.

In addition, the acute shortage of electricity in Africa is literally serious problems that result in many side effects to society. Africa is home to almost a fifth of the world's population, but accounts for less than 4% of global electricity use. North Africa enjoys near-universal access to electricity, yet more than half of the sub-Saharan population—600m people—live in the dark. This can hinder the provision of basic services. Half of the secondary schools in sub-Saharan Africa do not have power; many clinics and hospitals in the region also lack access to reliable electricity.

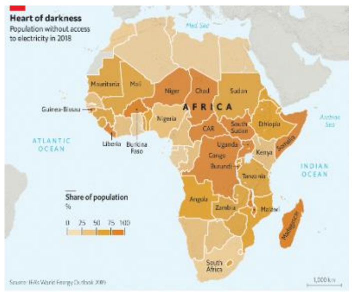


Figure 1: shows the electricity shortage areas in Africa

Besides, Forty percent of the global population—or 2.5 billion urban residents—practice open defecation or otherwise lack adequate sanitation, and an additional 2.1 billion urban residents use facilities that do not safely dispose of human waste. About 1.5 million children die every year from contaminated food and water, and in developing countries, half of all patients in hospitals are there because of problems with water and sanitation. These problems contribute to negative impacts on health issues.

Therefore, wastewater is one of the crucial problems in Thailand that has not been fully solved. Wastewater affects both humans and the environment: It also affects the life cycle of aquatic animals. Wastewater acted like a source of spreading germs, including the surrounding scenery as well wastewater from the household is contaminated with organic waste and many bacteria, especially *E. coli* bacteria, since the waste was generated from various activities in the house is mostly organic matter. This kind of bacteria is a bacterium that is found in the digestive tract of animals and humans. Therefore, the ability to trap bacteria in wastewater for enhancing electricity generation and wastewater treatment is one option for increasing the efficiency of microbial fuel cells.

Consequently, these problems have brought many innovations that were developed to be the alternative options, such as clean energy from various renewable sources, or, even from bacteria. MFC, is gaining a lot of attention recently because of its ability to create electricity from the respiration reaction of bacteria from the decomposition of organic substances. Moreover, this cell was considered as a clean energy resource. Nevertheless, the low power output is still the main problem of practical MFC. This research is therefore aimed to create a bacterial fuel cell that can generate higher electricity by trapping more bacteria, as well as being able to treat wastewater much efficiently.

Figure 2: shows different types of wastewater pollution which are household wastewater (left) and industrial wastewater (right)

Background Research

Green alternative energy has been demanded a sustainable society in the future generation. MFC is an eco-friendly innovation that can generate electricity and treat wastewater simultaneously. MFC is self-sufficient innovation for producing electricity using microbes. MFC uses bacteria to generate electricity from the oxidation of organic matter [1–5].

Bacteria capable of electricity generation have been enriched from domestic wastewater [6], ocean sediments [6], animal wastes [7], and anaerobic sewage sludge [8,9]. Electricity generation is supported by a range of biodegradable substrates, including glucose, acetate, lactate, butyrate, ethanol and organic matter in wastewater [10], produced from the electron transportation.

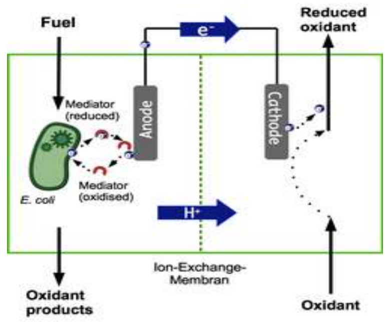


Figure 3: shows the component and electricity generation process of microbial fuel cell

Low generated electricity is the limitation of MFC. Therefore, the electrode will be the significant part that should be developed in order to improve the electricity generating efficiency of MFC. Consequently, this research aims to improve the efficiency of MFC by newly modified electrode. Additionally, this novel electrode probably reduces the toxicity of wastewater by increasing the organic waste consumption of bacteria. The higher bacteria were trapped by novel electrode, the electricity will be generated [9,10]. The above idea is the principle of how the electrode will be developed in order to improve efficiency. Graphene Oxide [12,13,16] is one of the materials which has its own high electricity conductivity properties – one of the significant properties of electrode. In addition, GO can be eco-friendly synthesized by using agricultural wastes [14,15], for example, bamboo.

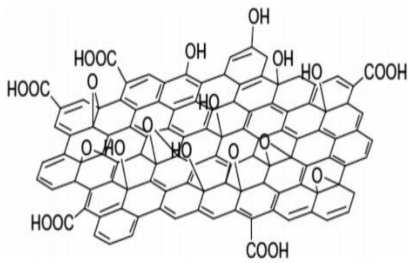


Figure 4: shows the chemical structure of Graphene Oxide (GO)

However, GO is unable to capture bacteria in the wastewater for producing the electricity. Modified graphene oxide with L-cysteine deposited on iron nanoparticle [18-21] (L-Cys grafted Fe NPs on GO) is novel material which was developed for being used in MFC for generating higher electricity and diminish wastewater pollution [22,23]. L-cysteine is one of the amino acids that contained sulfhydryl functional group (-SH) which can pass through the bacterial cell wall because of its strong nucleophilic properties. Nevertheless, the positivity of the amine functional group (-NH) can greatly attach with the negativity of the bacteria cell wall [24-25]. Iron nanoparticles are used for increasing the surface area because of its low-toxicity, availability, appropriate size and shape. Therefore, L-Cys grafted Fe NPs on GO was synthesized for enhancing the bacterial trapping efficiency of MFCs. In addition, this novel electrode can trap both positive and negative-gram bacteria represented the real condition of wastewater because both positive- and negative-gram bacteria has their same negatively surface charge.

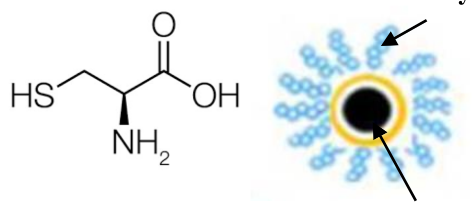


Figure 5: shows the chemical structure of L-cysteine (left) and schematic model of L-Cys grafted Fe NPs

MFC consists of two main parts: cathode and anode part. For anode part, this part which contained bacteria releases and donates electrons and proton to the cathode part [1–5].

Anode: oxidation reaction

- Electrolyte: wastewater, bacteria solution and broth
- Electrode: novel electrode

Cathode: reduction reaction

- Electrolyte: tap water
- Electrode: stainless steel

Stainless steel was used as cathodic electrode because it has their own properties to enhance oxygen reduction reaction (ORR) which can step up the level of the generated electricity. Moreover, tap water was used as cathodic electrolytes because of its non-toxic and availability to the environment.

Glove is one of the materials which are also used in MFCas proton exchange membrane (PEM). PEM is a significant component which play an important role in carrying proton from anode to cathode. Actually, there are lots of material which can be used as PEM, for example, nafion [26-27], j-cloth, nylon [28], and others. However, these kinds of materials have too high cost to be used in actual usage. Therefore, glove which have a moderate ability to carry proton and low-cost are chosen to be components of MFC.

In order to evaluate the efficiency of MFC, Coulombic efficiency [29,30] is a parameter which can indicate how electron transfer in the system. Coulombic efficiency is the efficiency with which electrons are transferred in a system to carry out an electrochemical reaction. This is an important measure of the microbial fuel cell efficiency as it measures the number of coulombs recovered as electrical current. The coulombic efficiency is dependent on two major factors firstly it depends on microorganism carrying out the electrochemical reaction and secondly the substrate used by the bacteria to generate current.

Columbic Efficiency (CE) was determined by integrating the current measured over time (t), and compared with the theoretical current on the basis of chemical oxygen demand (COD) removal and calculated as followed equation:

$$\epsilon_{Cb} = \frac{M \int_0^{t_b} I\,dt}{Fbv_{An}\Delta COD}$$

where M = 32 g/mol, the molecular weight of oxygen, F is Faraday's constant, b = 4 is the number of electrons exchanged per mole of oxygen, V_{An} is the volume of liquid in the anode compartment, and ΔCOD is the change in COD over time t_b

Escherichia coli (E. coli) [31] – gram-negative bacteria in coliform group has its own ability to transfer electron in the system. *E. coli* which is an anaerobic bacterium can convert organic waste to be other forms and release electron in the same time under anaerobic condition. Therefore, *E. coli* is one of the efficient bacteria that can utilize organic wastes in wastewater as a substrate in cellular respiration process for producing electricity efficiently. In addition, *E. coli* are usually found in household wastewater because it is mostly released out from human activities

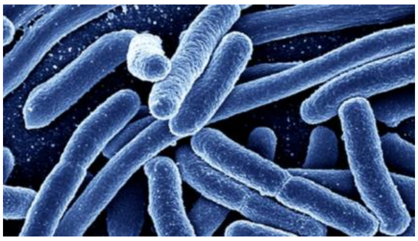


Figure 6: shows the morphology of *Escherichia Coli*

Objective

The specific objectives are as follows: (1) To develop and synthesize the modified graphene oxide with L-cysteine deposited on iron nanoparticle from bamboo for being as an electrode used in MFCs, (2) To determine the rate of electricity production and the efficiency of wastewater treatment from MFC with novel electrode, including the factors of effected parameters, and (3) To improve the energy production and wastewater treatment efficiency of MFC

Hypothesis

The specific hypotheses are as follows: (1) modified graphene oxide with L-cysteine deposited on iron nanoparticle can be successfully synthesized and characterized from bamboo., (2) novel electrode will exhibit high bacterial adsorption ability, high electricity production efficiency and high wastewater treatment efficiency., and (3) MFC with utilizing of novel electrode can be applied for actual usage in everyday life.

Methodology

Materials

Synthesis of modified graphene oxide with L-cysteine deposited on iron nanoparticle and electrode fabrication

Dry bamboo, conc. sulfuric acid, H_2SO_4, potassium nitrate, KNO_3, potassium permanganate, $KMnO_4$, hydrogen peroxide, H_2O_2, hydrochloric acid, HCl, ethanol, C_2H_5OH, distilled water, H_2O, Ferric chloride hexahydrate, $FeCl_3 \cdot 6H_2O$, ferrous chloride tetra hydrate, $FeCl_2 \cdot 4H_2O$ L-Cysteine, sodium hydroxide, NaOH, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate, PEDOT:PSS, and graphitic electrode

Microbial Fuel Cells Construction

Screws, nuts, silicone, glue, acrylic plate, rubber gloves, novel electrode, laser cutting machine, CNC milling and stainless steel

Bacteria culture and wastewater collection

Wastewater from septic tank, nutrient agar, nutrient broth, *Bacillus Subtilis* stock, *Escherichia Coli* stock, distilled water, ethanol, PBS buffer, straining cloth, cotton wool, incubator, and Aluminum foil

Electricity production test

MFCs, wastewater, broth, bacterial solution, tap water, copper wires, and multimeter

Methodology (fig.18)

Synthesis of modified graphene oxide with L-cysteine deposited on iron nanoparticle and electrode fabrication

Dry bamboo was first burnt at 550 °C for 3 hours under the condition of N_2 gas. Concentrated H_2SO_4 (69 mL) was added to a mixture of burned bamboo (3.0 g) and $NaNO_3$ (1.5 g), and the mixture was cooled to 0 °C. $KMnO_4$ (9.0 g) was added slowly in portions to keep the reaction temperature below 20 °C. The reaction was warmed to 35 °C and stirred for 30 min, at which time water (138 mL) was added slowly, producing a large exotherm to 98 °C. External heating was introduced to maintain the reaction temperature at 98 °C for 15 min, then the heat was removed, and the reaction was cooled using a water bath for 10 min. Additional water (420 mL) and 30% H_2O_2 (3 mL) were added, producing another exotherm. After air cooling, the mixture was purified by filtering through polyester fiber. The filtrate was centrifuged (4000 rpm for 4 h), and the supernatant was decanted away. The remaining solid material was then washed in succession water, HCl, and ethanol. The solid obtained on the filter was vacuum-dried overnight at room temperature.

Figure 7: shows the washing process of graphene oxide

L-cysteine functionalized Fe_3O_4 NPs were synthesized using the chemical precipitation method. In this procedure the stock solution of $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ with 0.1 M and 0.2 M concentrations, respectively, were added and homogenized in 10 ml of deionized water. After the vigorous mixture was allowed at 80°C for 10 minutes at the magnetic stirrer hot plate. Then added 0.1M of L-cysteine as a surfactant to prevent from the agglomeration. After the completely mixing process, the 0.5 M NaOH concentration was added dropwise until the pH of the solution reached in the range of 9–12. The mixture was allowed until the precipitate formed. Subsequently, the precipitated L-Cyst Fe_3O_4 NPs has appeared in black. The permanent magnet collected the resulting precipitates and washed three times with deionized and ethanol to remove impurities. Finally, it was dried in an oven at 80°C for 3 hours.

Figure 8: synthesis process of L-cysteine deposited on iron nanoparticle

After that, the mixing between GO and L-cysteine deposited FeNPs was well prepared, then, washing and drying were provided respectively.

Figure 9: shows the synthesis process of modified graphene oxide with L-cysteine deposited on iron nanoparticle

In order to fabricate electrode, 0.5 g modified graphene oxide with L-cysteine deposited on iron nanoparticle powder was immersed in 2 ml distilled water, then, mixing it by magnetic stirring. After that, adding 50 drops and mixing under room temperature for 24 hours. Lastly, coating electrode with this solution and dried it

Figure 10: the process of electrode fabrication

Microbial Fuel Cells Construction

MFCs model was prototyped by Solidwork program. Then, Acrylic plate was cut by laser cutting machine followed the designed model. After that, all components including rubber gloves as PEM was assembled together by glue and silicone for preventing the water leakage. The assembled invention is created for further usage as a prototyped model (fig.11) and actual prototype (fig.12).

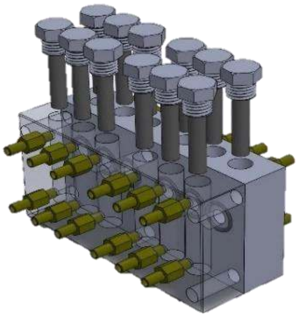


Figure 11: shows the prototyped model of MFCs

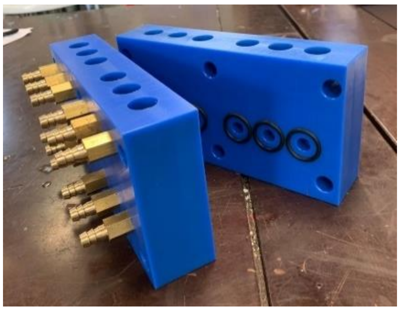


Figure 12: shows the completed cells of MFCs

Bacteria culture and wastewater collection

Bacterial strains were grown on nutrient agar at 37°C for both cultured *Escherichia Coli* and *Bacillus Subtilis* (represented positive- and negative-gram bacteria respectively). For bacterial growth, bacteria were isolated from agar plates into nutrient broth, then, cultures of 300 ml in 500 ml flasks were shaken continuously in an incubator (160 rpm, 37°C). After 18 h of growth, the cells within the broth solution were used in anode chamber of MFCs for generating electricity.

For wastewater collection, wastewater was collected from the school's septic tank which contained lots of bacteria from human activity.

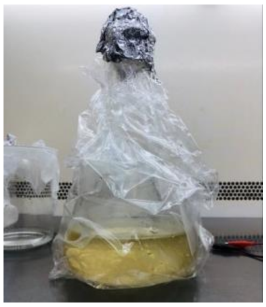


Figure 13: bacterial solution in nutrient broth

Electricity production test

Firstly, all of the required chemicals and solutions are added into assembled MFCs. For cathode part, tap water and stainless-steel electrode was utilized as a part of it. For anode part, wastewater, broth, and bacteria solution were separately used as an anode electrolyte for figuring out the most optimum condition of MFCs. Finally, measuring voltage between anode and cathode by multimeter is required for further calculation of electricity production. The experiment starts up with 3 different conditions which are broth, bacteria solution and wastewater condition in order to figure out the optimum condition to generate the highest levels of electricity.

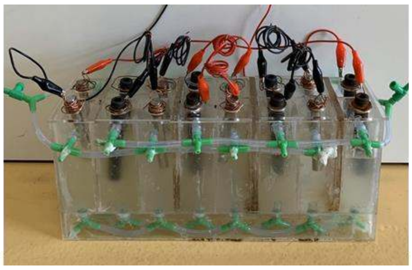


Figure 14: shows the process of electricity measurement of MFCs

Bacteria Adsorption ability test

After 18 h of growth, the cells of both *Bacillus Subtilis* and *Escherichia Coli* (represented positive- and negative-gram bacteria respectively) were harvested by centrifugation (5000 rpm, 5 min, 25°C) and washed three times in a PBS buffer. Then, cell pellet from centrifugation process was inoculated into 100 ml PBS buffer solution. There are 2 condition which are graphitic electrode and novel electrode condition in order to affirm the bacteria trapping efficiency of novel electrode. The experiment starts up with separately immersing each types of electrode into both *Bacillus Subtilis* and *Escherichia Coli* solution. Then, solution was collected every 6 hours for 6 samples for measuring the concentration of bacteria by utilizing UV-vis spectrophotometry. Moreover, the powder sample was characterized by many techniques, for example, SEM, FTIR and zeta potential in order to confirm the attachment of bacteria on novel electrode

Figure 15: shows the experiment of bacteria adsorption ability testing in different condition

Wastewater Treatment efficiency test

Samples from MFCs which are collected before and after testing was measured for chemical oxygen demand (COD) values of each sample to investigate the efficiency of organic waste reduction in wastewater. These are also provided for 2 condition which are graphitic electrode and novel electrode.

Results & Discussion

Results can be classified into 4 parts which are the characterization of L-Cys grafted Fe NPs on GO, the bacteria adsorption test, the electricity production test, and wastewater treatment test

1. Characterization

1.1 Scanning Electron Microscopy; SEM

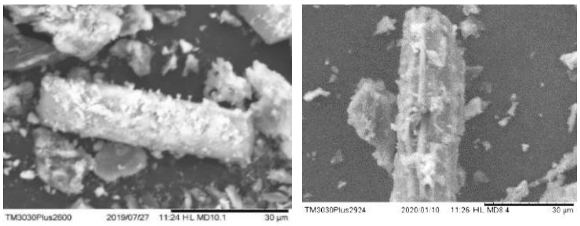


Figure 16: shows the presence of iron nanoparticles grafted on exfoliated graphene oxide sheet using SEM

The SEM image shows the iron nanoparticles attached to the surface of graphene oxide sheets synthesized from bamboo. The iron nanoparticles are small round white objects that are bright and clear uniformly distributed on a gray sheet graphene oxide. The morphology preliminary indicates that iron nanoparticles are successfully grafted on graphene oxide sheets. However, the picture cannot identify the presence of an L-cysteine on the iron nanoparticles, because the structure of L-cysteine is too small to be observed by SEM. The confirmation of the L-cysteine amino acid in the structure can be examined using the FT-IR technique. In addition, EDS, the quantitative elements analysis by Energy dispersive Spectroscopy, is able to identify the elemental composition analysis of the sample in order to confirm the chemical characteristics of L-Cys grafted Fe NPs on GO.

1.2 Energy Dispersive X-ray Spectroscopy; EDS

The results of elemental analysis indicate that the amount of carbon content is up to 92% of the total sample (graph 1) and the presence of oxygen, which can indicate the chemical characteristics of graphene oxide.

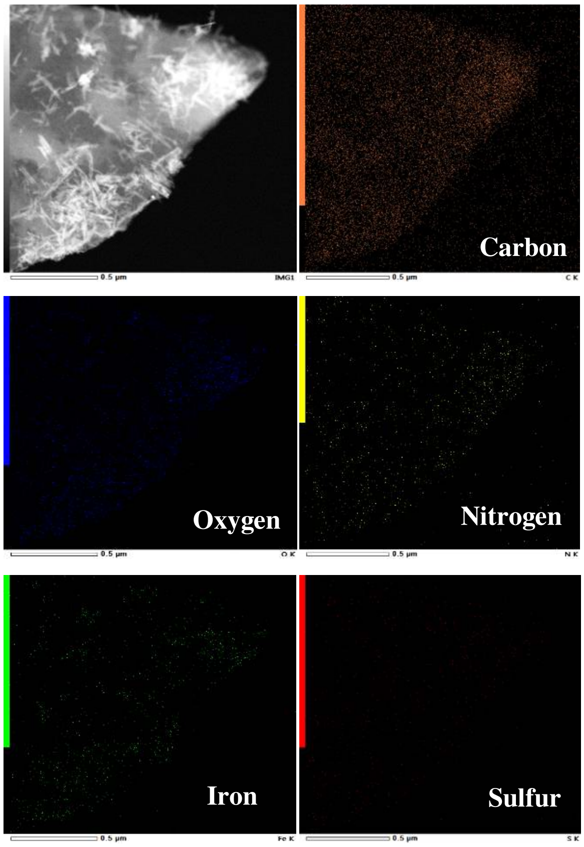
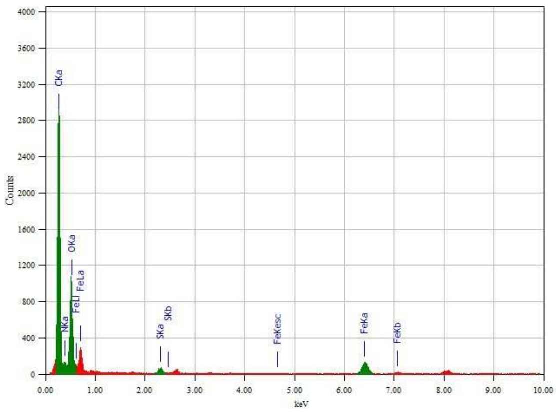


Figure 17: shows the elemental analysis of L-Cys grafted Fe NPs on GO

From the elemental analysis of graphene oxide, large amounts of carbon and oxygen are distributed evenly on the sample, which can indicate the characteristics of graphene oxide.



Graph 1: illustrates the elemental composition analysis test by EDS

From the results of EDS, it was found that L-cysteine grafted iron nanoparticles on GO contains a high amount of carbon and oxygen distributing uniformly, which can indicate the characteristics of graphene oxide. There is also the presence of iron nanoparticles from the presence of iron on the sample. In addition, sulfur which shows the existence of the sulfhydryl group in the L-cysteine was also found. To conclude, synthesis of iron nanoparticles on GO does not cause both chemical and physical properties of GO changes. The results can also confirm that Iron nanoparticles containing L-cysteine are successfully synthesized on graphene oxides.

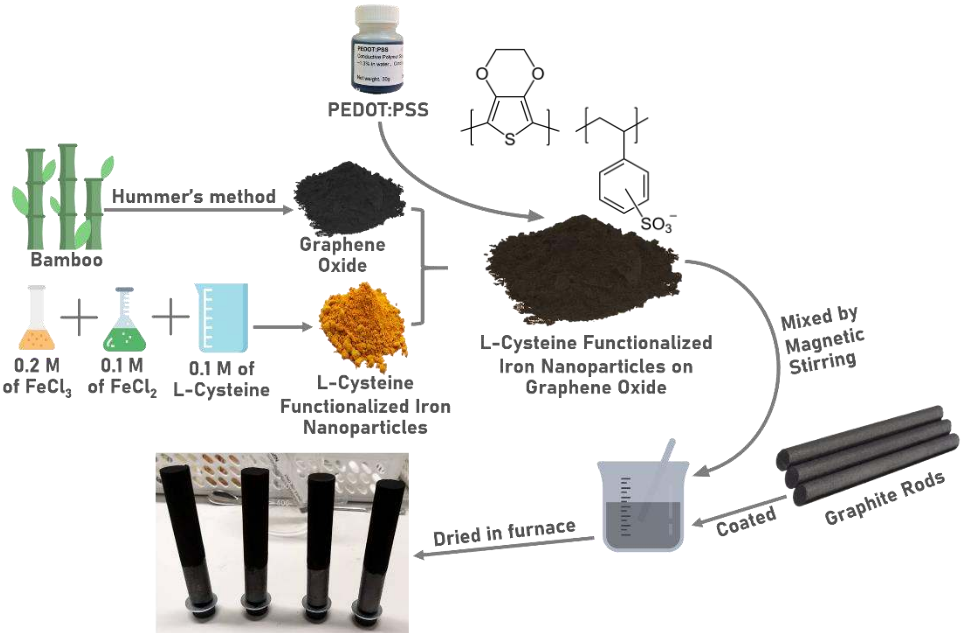


Figure 18: shows schematic of experimental processes

1.3 Transmission Electron Microscopy; TEM

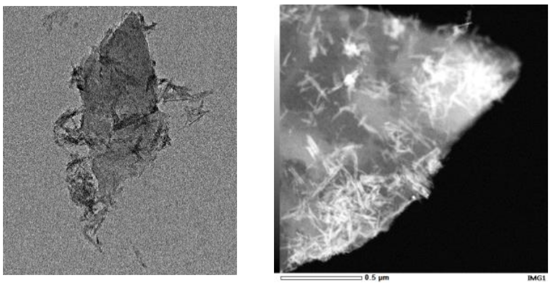


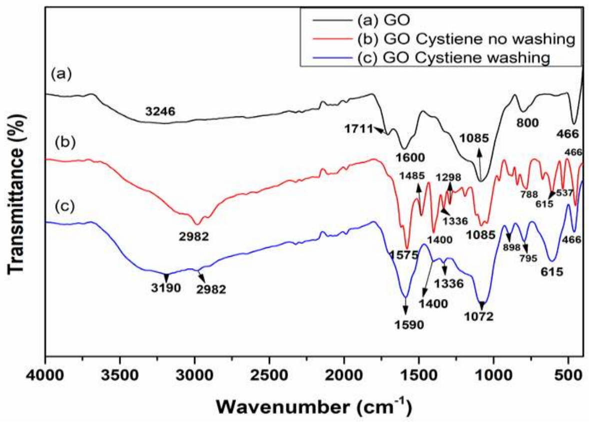
Figure 19: shows the morphology of L-Cys grafted Fe NPs on GO characterized by TEM

Result from TEM shows a small sheet of GO. The morphology can be a preliminary indication that Carbon-based graphene oxide can be successfully synthesized from bamboo. It can also indicate the presence of iron nanoparticles on the structure of graphene oxide, which appear like a clear white-gray needle objects Evenly distributing on GO. As a result, the synthesis of iron nanoparticles on graphene oxide is successful.

To ensure that the images from the TEM follow the assumptions. The chemical characteristics of a sample from an Energy Dispersive X-ray (EDX) device is one of the methods that can be used to indicate where each element is found, also the presence and the number of other compounds containing a certain element.

From the elemental analysis of Graph, it can indicate that the sample contains a high amount of carbon and oxygen, which can indicate the characteristics of graphene oxide. There is the presence of iron nanoparticles from the presence of iron on the sample. Sulfur is also present in the structure, which shows the existence of the sulfhydryl group in the L-cysteine amino acid.

1.4 Fourier Transform Infrared Spectroscopy (FT-IR)



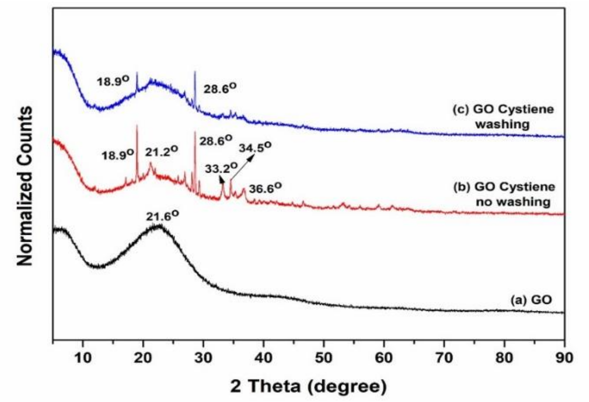
Graph 2: illustrates the functional analysis test by FTIR

From the results of FTIR spectra, it shows the presence of some functional groups contained in L-Cys, such as N-H and -SH which represented by peaks located at 2982 and 2650 cm-1 respectively. Therefore, L-Cys is successfully grafted on Fe NPs which is synthesized on GO. Consider the presented peaks of GO and L-Cys grafted Fe NPs on GO, FTIR spectra at peaks 3246, 1711, 1600, 1225, and 800 cm-1 are indicating -OH, C=O, C=C, C-O, and C-H functional groups respectively. Thus, the synthesized sample has a typical characteristic of GO, functional groups.

Samples	Main functional group	Wave number (cm^{-1})
Graphene oxide (GO)	-OH, C=O, C=C, C-O,	3246, 1711, 1600, 1225,
L-Cys grafted Fe NPs on GO	N-H, -SH	2982, 2650

Table 1: shows the functional analysis test by FTIR

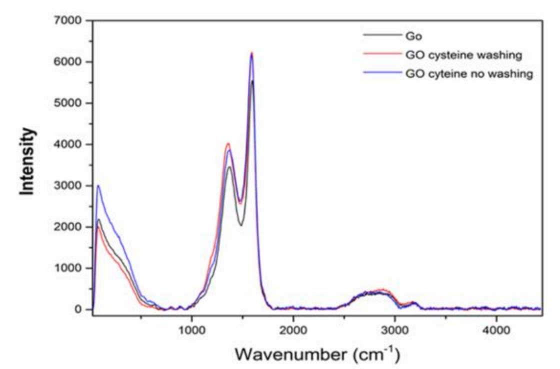
1.5 X-ray Diffractometer, XRD



Graph 2: illustrate the crystalline structure analysis of L-Cys grafted Fe NPs on GO by XRD

From the crystal structure testing of graphene oxide by using X-ray diffraction meter, the pattern of XRD at the vertex with diffraction angle is 21.6 degrees, which indicates the graphene oxide characteristics. In addition, when the graphene oxide is grafted by the iron nanoparticles, two peaks were found from the graph which had the diffraction angle at the peak of 18.9 degrees and 28.6 degrees. These peaks can indicate the appearance of Fe_2O_3 (220) and Fe_3O_4 (210) crystals, respectively, showing the presence of iron nanoparticles on graphene oxide. Therefore, the results of XRD presents the successful synthesis of iron nanoparticles on graphene oxide.

1.6 Raman Spectroscopy

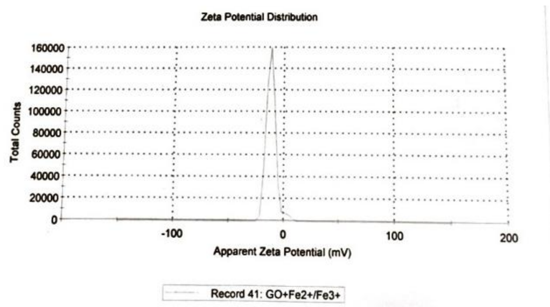


Graph 3: illustrate the Raman Spectroscopy characterization L-Cys grafted Fe NPs on GO

From the Raman spectroscopy graph, found that graphene oxide is one form of carbon that undergoes oxidation, Causing the insertion of oxygen atoms into the structure of carbon atoms. Then a separate double bond resulting in resonate of the G band peak in graphene oxide at the wavenumber higher than the G band peak of graphite or graphene. Moreover. It has broader peak than that of graphite. In addition, the disorder of the structure or the defects will reduce the sp^2 hybridization.

Normally, D Band peak of graphite or graphene is not present. However, the graph shows that the sp^3 hybridization of the product is reduced, or the sp^2 hybridization of the product increased due to the insertion of oxygen atoms. It can be concluded that the sp^2 hybridization of the product increased by the addition of oxygen atoms by oxidation, which changed the carbon atoms hybridization from sp^3 to sp^2. Therefore, the results from Raman spectroscopy technique can indicate that graphene oxide has successfully been synthesized. In addition, it can be confirmed from the analysis results that the synthesis of iron nanoparticles onto graphene oxide does not affect the appearance of graphene oxide.

1.7 Zeta potential

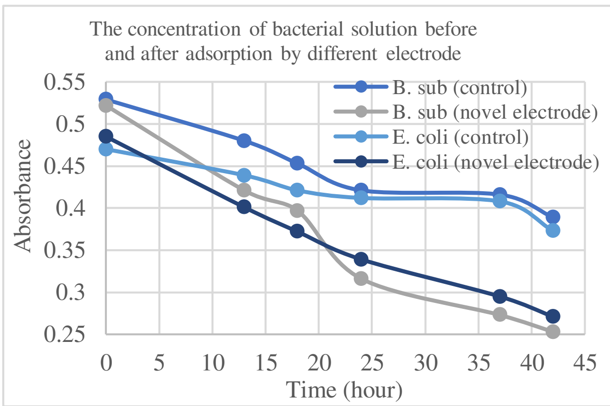


Graph 4: illustration of zeta potential measurement of L-Cys grafted Fe NPs on GO

Zeta potential is used to analyze the charge on the surface of graphene oxide electrode attached to iron nanoparticles. The results show that the zeta potential of L-Cys grafted Fe NPs on GO is -11.3 mV. Therefore, the graphene oxide grafted with L-Cys grafted Fe NPs on GO has negative charge on the surface of the sample, due to the fact that iron nanoparticles in the form of oxides tend to have hydroxyl groups on the surface, causing negative. In addition, carboxyl groups also have a chance to result in more negative effects. However, bacteria are able to bind to the material well, due to the positive charge of the amine group (-NH) in the amino acids, which can bind and bond with the negative charge of the cell walls of bacteria.

2. Bacteria adsorption Test

2.1 UV/VIS spectrophotometer



Graph 5: illustration of *B. Subtilis* and *E. Coli* adsorption by L-Cys grafted Fe NPs on GO

Measuring the concentration of bacterial solution before and after adsorption by electrode using UV/VIS spectrophotometer, adjusted to measure turbidity at wavelength of 600 nm.

Condition % removal	*B. subtilis* (control)	*B. subtilis* (novel electrode)	*E. coli* (control)	*E. coli* (novel electrode)
Initial absorbance	529	522	470	485
Absorbance after 42 hours	389	253	373	271
Removal (%)	26.27	51.53	20.64	44.12

Table 2: Values showing *B. Subtilis* and *E. Coli* adsorption by L-Cys grafted Fe NPs on GO electrode

Note: The values shown on the table are OD 600 or the value of turbidity of the bacterial solution measured at wavelength 600 nm, which has no unit. The values are measured from the UV-Vis Spectrophotometer. It is also found that 1 unit of OD value has about 1x10^8 cells per milliliter.

From the graph that shows the trend of concentration of the bacterial solution before and after use with electrode during a 42 hours' time period, it can be observed that the concentration of the bacterial solution that is tested with L-Cys grafted Fe NPs on GO electrode continuously dropped. However, the concentration of bacterial solution in the control sample also decreased. This is due to the fact that the bacterial solution that is gained from bacterial culture has a low stability and is sensitive to environmental change, and the environment can't be controlled due to the nature of the tests conducted. Despite that, the bacterial solution tested with L-Cys grafted Fe NPs on GO electrode decreases in concentration at a faster rate in both Bacillus subtilis and *Escherichia coli*. From these observations, we can conclude that the new electrode can adsorb Bacillus subtilis and *Escherichia coli*, in both negative and positive gram. The L-Cys grafted Fe NPs on GO electrode can adsorb 25% of *Bacillus subtilis* and 23.48% of *Escherichia coli* after a period of 42 hours.

2.2 Scanning Electron Microscopy; SEM

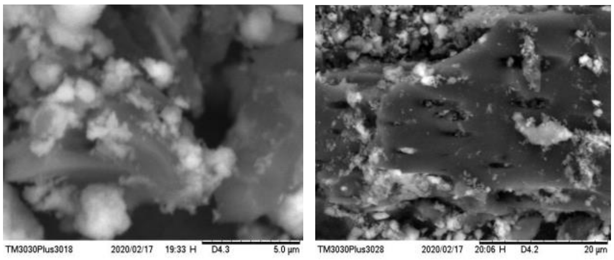


Figure 20: shows the attachment of *B. Subtillis* (right) and *E. Coli* (left) on L-Cys grafted Fe NPs on GO electrode

From analyzing the morphology of the attachment of both *Escherichia coli* and *Bacillus subtilis*, it was found that both the negative and positive gram can attach to the particles of the electrode. The structure of bacteria can be observed on the image as bright white to gray objects with indefinite shapes, since they are coated with platinum to clearly observe the cells.

To ensure that the observed bacteria are not cells that aren't attached sufficiently, the sample is washed with distilled water and the bacteria is filtered out using filter paper with 15-20 micrometers holes, compared to the 4-10 micrometers *Bacillus subtilis* and 0.5 micrometers *Escherichia coli*. This reduce the possibility of observing improperly attached bacteria and only observe those that are truly attached to the sample.

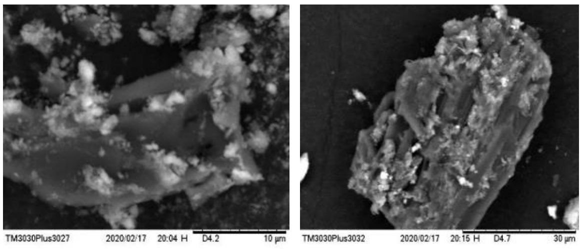
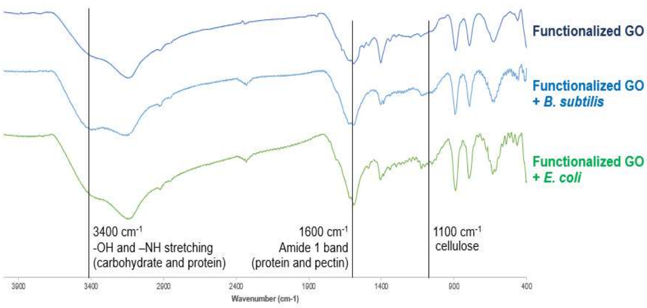


Figure 21: shows the attachment of *B. Subtillis* (right) and *E. Coli* (left) on L-Cys grafted Fe NPs on GO electrode after being washed with distilled water

From observing the morphology of the attachment of both *Escherichia coli* and *Bacillus subtilis* after being washed and filtered, both negative and positive gram bacteria are found on the sample sufficiently, judging from the presence of bright, white to gray objects with indefinite shape, same as the ones observed before washing. However, the number of cells observed was reduced.

2.3 Fourier Transform Infrared Spectroscopy (FT-IR)



Graph 6: illustration of functional group analysis by FTIR of B. Subtilis and E. Coli adsorption by L-Cys grafted Fe NPs on GO electrode

From the graph FT-IR showing the attachment of *B. Subtillis* and *E. Coli* on L-Cys grafted Fe NPs on GO electrode, peaks of sulfhydryl at wave number 2550 cm^{-1} can be observed from both the sample before testing and after testing with

Bacillus subtilis and *Escherichia coli*. Peaks can also be seen at wave number around 1100 cm^{-1}, which shows the existence of cellulose, which might be due to the creation of bacterial cellulose from attachment with bacteria.

Peaks at wave number around 1600 cm^{-1} indicate the presence of protein, which might be protein that is both produced and contained in the bacteria. In conclusion, both *Bacillus subtilis* and *Escherichia coli* can attach to L-Cys grafted Fe NPs on GO electrode. The observed peaks can be ensured that they are purely created by the sample and the bacteria that are sufficiently attached to eh electrode, since the sample had been washed and filtered before the conduction of the test.

2.4 Zeta potential

Sample	1st trail (mV)	2nd trail (mV)	3rd trail (mV)	Average (mV)
GO Fe NPs L-Cys	-11.3	-11.5	-11.3	-11.36
GO Fe NPs L-Cys + Broth	-31.1	-32.8	-32.2	-32.03
GO Fe NPs L-Cys + *B.Sub*	-40.0	-39.4	-40.1	-39.83
GO Fe NPs L-Cys + *E.coli*	-40.1	-40.1	-39.9	-39.73

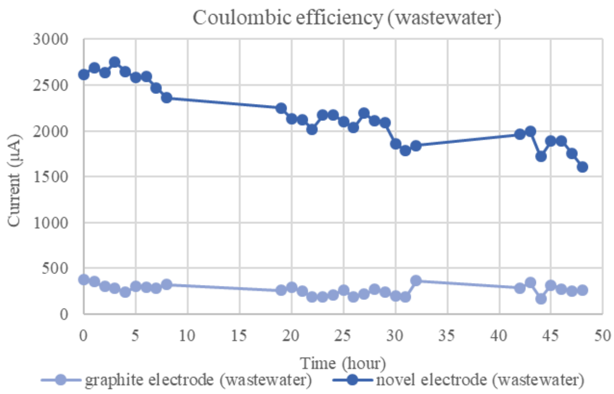
Table 3: Zeta potential in mV of L-Cys grafted Fe NPs on GO in bacterial solution of broth*, B. Subtilis* and *E. Coli*

In measuring the zeta potential, the nutrient broth that was used to culture bacteria was used to set the standard for the measurements. This is done by submerging the L-Cys grafted Fe NPs on GO in the broth and centrifuge it, replicating the process for measuring zeta potential of the bacterial solution samples. The results from the broth show the average zeta potential of 32.03 mV, meaning that the ions in the broth affects the zeta potential. After setting the control standard, the L-Cys grafted Fe NPs on GO powder is tested with Bacillus subtilis and Escherichia coli. The zeta potential is equal to -39.83mV and -39.73mV accordingly. The measured values are different from the sample that is submerged in the nutrient broth, showing the change of charge on the surface of L-Cys grafted Fe NPs on GO. This is due to the creation of biofilm of the bacteria. From the results of this experiment, we can confirm that the bacteria were attached to the surface of the L-Cys grafted Fe NPs on GO particles. Not only that, but it is also found that there is minimal difference in zeta potential between both Bacillus subtilis and Escherichia coli, which are positive and negative gram accordingly. We can conclude both types of bacteria can attach to the L-Cys grafted Fe NPs on GO particles sufficiently.

3. Electricity production

From table 4 the electricity production test of MFC, it can be observed that, in wastewater conditions MFC that use anode electrode as graphene oxide grafted by iron-nanoparticles with L-Cysteine can produce a voltage up to 550 mV, the current of 2.750 mA, and the power of 15.13 W/m^3, which is 28.5 times of MFC using anode electrode as graphite under the same condition. Moreover, it can be found that MFC that uses anode electrode as L-Cys grafted Fe NPs on GO can generate more voltage, current, and power than MFC using anode electrodes as graphite electrodes under all conditions significantly. The maximum electric voltage and current is found in wastewater conditions, which is expected to be caused by more durability of bacteria to the environment and a greater number of bacterial cells than cultured E. coli solution. However, considering the coulombic efficiency, it is found that in the bacterial solution E. coli, bacteria fuel cell Which uses anode as graphene oxide electrode grafted with iron nanoparticles with L-Cysteine has an CE of 2.5%, which is greater than 0.84% of using in the wastewater condition ,which corresponds to the assumption that E. coli solution are more effective in the production of electrical energy than other species of bacteria. Since the wastewater contains many species of bacteria, the CE of MFC is less than those in the E. coli solution. However, the electrode of L-Cys grafted Fe NPs on GO can generate electricity efficiently

5.3 Coulombic efficiency



Graph 7: illustration of coulombic efficiency in wastewater condition utilizing different anodic electrode

The coulombic efficiency (CE) is the efficiency of electrons transfer in a system to create the electrochemical reaction, but it cannot exhibit the electricity production efficiency directly. It pointed out only the ability to create electrons transfer based on the chemical substrates consumed by bacteria under a certain condition. From the results, under wastewater condition, the CE of the MFC using the novel electrode is 0.84%, which is 4.2 times compared to 0.20% of the MFC using ordinary graphite electrode. In addition, from the trend of the graph, it was found that during the final period of 48 hours, the electrical performance tends to slightly decrease. Therefore, it can be concluded that after 48 hours of use, the ability in generating electricity of MFC using electrode of L-Cys grafted FeNPs on GO is still effective continuously.

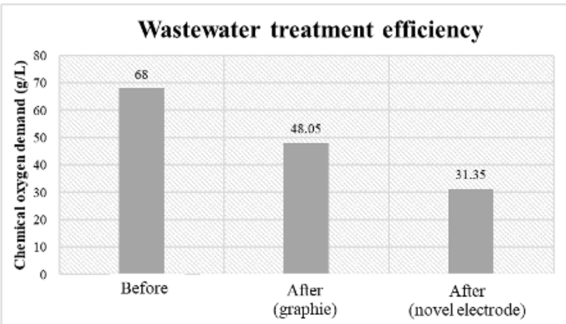
4. Wastewater treatment efficiency

Sample	Maximum Voltage (mV)	Maximum Current (μA)	Maximum Power (μW)	Coulombic Efficiency (%)	Power Density (W/m^3)
Graphite (broth)	66	173.7	115	0.09	0.115
L-cysteine grafted FeNPs GO (broth)	128	640.0	819	0.28	0.819
Graphite (*E. coli*)	99	260.5	258	3.78	0.258
L-cysteine grafted FeNPs GO (*E. coli*)	291	1455.0	4234	2.50	4.234
Graphite (wastewater)	142	373.7	531	0.20	0.531
L-cysteine grafted FeNPs GO (wastewater)	550	2750.0	15,125	0.84	15.125

Table 4: illustration of electricity production in various condition: broths, *E. coli* solution, and wastewater utilizing different electrode

condition	Graphite (broth)	L-Cys grafted FeNPs GO (broth)	Graphite (*E. coli*)	L-Cys grafted FeNPs GO (*E. coli*)	Graphite (wastewater)	L-Cys grafted FeNPs GO (wastewater)
CE (%)	0.09	0.28	3.78	2.50	0.20	0.84

Table 5: illustration of coulombic efficiency in wastewater condition utilizing different electrode



Graph 8: shows the decrease of COD of wastewater, treated by graphite electrode and the novel electrode from L-Cys grafted Fe NPs on GO

From the Chemical Oxygen Demand (COD) results, which illustrates the amount of oxygen that was consumed by microorganism to decompose organic waste to be carbon dioxide and water, suggests that the initial COD of wastewater from a sewer is about 68 g/L. After treating wastewater with the MFC with the ordinary graphite electrode and with the novel electrode, the COD of wastewater become 48.05 g/L and 31.35 g/L respectively. Hence, the COD results indicated that the MFC using the novel electrode can treat wastewater efficiently by highly percentage COD removal, 53.89%.

Conclusion

In this research, a novel electrode was proposed by synthesizing modified graphene oxide with L-cysteine deposited on the iron nanoparticle. Since L-cysteine is one of the amino acids that contained sulfhydryl functional group (-SH) which can pass through the bacterial cell wall because of its strong nucleophilic properties, it was chosen to use as a bacteria trapping part. Nevertheless, the positivity of the amine functional group (-NH) can greatly attach to the negativity of the bacteria cell wall. Moreover, Iron nanoparticles are used for increasing the surface area because of its low-toxicity, availability, appropriate size and shape. Therefore, this novel electrode is one of the noel innovations which can generate green energy to the environment by an eco-friendly method.

According to the results, a novel electrode developed by modified graphene oxide with L-cysteine deposited on iron nanoparticle can be successfully synthesized from bamboo which is one of agricultural wastes of Thailand. The synthesized powder was coated appropriately on the graphitic electrode as electrodes for utilizing in MFCs. Results from SEM, TEM, FT-IR, XRD, EDS, Zeta Potential, and Raman Spectroscopy can depict the presence of iron nanoparticles on exfoliated graphene oxide sheets. Moreover, the appearance of L-cysteine was already confirmed. For the bacteria adsorption ability, the novel electrode can trap bacteria significantly and continuously for about 26% by utilizing UV-vis spectroscopy to measure the concentration of remained bacteria. In the same way, this novel electrode exhibits the high performance of wastewater treatment by decreasing COD values for about 53.89% which are significantly higher than the normal graphitic electrode. For the electricity production test, the results suggested that a novel electrode under condition can show the highest performance which can generate electricity up to 15.13 watts per cubic meter which is 28.5 times compared to the ordinary electrode. Consequently, this novel electrode not only exhibits the high performance for generating electricity but also exhibits the high performance for reducing the toxicity of wastewater. Our MFCs with a novel electrode can improve society's life quality to be better for future generation.

Ideas for Future Research

According to the result, MFCs' generated sufficient voltage for charging a lead acid battery when MFCs were connected in series (8 cells). Therefore, this innovation can probably be used as a huge energy sources for generating electricity to the society if cells was connected in large series of cells. Moreover, this innovation can also be applied in a wastewater treatment application which can be developed for further research.

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