THE ANTIMICROBIAL EFFICIENCY TEST FOR SOLUTION OF GRAPHENE OXIDE AND CALCIUM LACTATE USING PIXEL COUNTING METHOD

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ABSTRACT

This research is a study of the antimicrobial efficacy of graphene oxide and calcium lactate coatings on filter paper, using the Pixel counting method to measure the antimicrobial effectiveness. In this study, the test solutions used to assess antimicrobial efficacy are divided into three types: graphene oxide solution, calcium lactate solution, and a mixture of graphene oxide and calcium lactate solution. In the experiments, 2x2 centimeter filter papers are immersed in the respective solutions and then dried. Subsequently, they are exposed to UV radiation to kill any microorganisms on the filter paper. Following this step, the filter papers are placed in the air for 20 seconds in order to receive the microorganisms. Lastly, the filter papers that have microorganisms from air are placed on a culture medium and incubated at 37 degrees Celsius for 7 days. The Pixel Counting method is employed to evaluate the effectiveness of this research. The distribution of microorganisms is segmented and converted into black and white images, and then a histogram is used to count the pixels of the black area compared to the total area. The results indicate that microorganisms on the graphene oxide solution are distributed at 12.16%, microorganisms on the calcium lactate solution are distributed at 21.08%, and microorganisms on the mixture of graphene oxide and calcium lactate solution are distributed at 26.23%.

Keywords: Graphene oxide, Calcium lactate, Antimicrobial, Pixel Counting

1. INTRODUCTION

The antimicrobial material has more considered, since Covid-19 was spread around the world in 2019. Many researchers try to develop the material that has antimicrobial ability. Especially in food business, the antimicrobial package can keep more shelf life and quality than ordinary package. Moreover, the active package can gain more value in many businesses and reduce spoiled food while transporting.

From the study, it has been found that there is research related to the development of paper that can create the packaging with antimicrobial properties dating back to 1985, primarily for medical purposes. Various antimicrobial substances have been explored, such as halogenated aromatic nitriles, dichlorophene [1], vanillin [2], thymol mixed with vanillin [3], canola oil [4], and extracts from guava leaves [5]. There are some materials that has antimicrobial ability such as silver [6] and copper [7] that favor to use in antimicrobial work.

Furthermore, there is another material extensively discussed in the research field called graphene. Graphene is a new two-dimensional material that composes of pure carbon with exceptional properties. It is atomically thin, exceptionally strong, conducts electricity well and highly flexible. Moreover, graphene has antimicrobial properties, and it is biocompatible with the human body. Researchers interest in graphene utilizing to develop antimicrobial solid paper to enhance its properties and value. Graphene is currently used in various industries such as disease detection sensors, medical nanocarriers, electronics circuits, solar panels, and batteries [10].

According to previous researches, there are some solutions that has antimicrobial properties and no effect to human body such as graphene oxide (GO) and calcium lactate ($C_6H_{10}CaO_6$). There solutions can apply to created antimicrobial material using coated method. This research aims to study the antimicrobial efficiency of these solutions. The pixel counting method is used to show the microbial growth when contact the proposed solution.

2. BASIC THEORIES

This section describes about the detail that related to this research. It consists of Graphene, calcium lactate and Microorganisms.

A. Graphene

Graphene, a two-dimensional allotrope of pure carbon, was discovered in 2004 by Prof. Sir Andre Geim and Prof.

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Konstantin Novoselov from the University of Manchester, United Kingdom [10]. Their groundbreaking discovery earned them the Nobel Prize in Physics in 2010. This achievement prompted significant global interest in this new material. Graphene is a single atom thick and is arranged in a hexagonal lattice, forming a strong and interconnected framework resembling a honeycomb, as depicted in Figure 1. Each carbon atom in graphene is bonded with three neighboring carbon atoms, resulting in unique properties distinct from other carbon allotropes.

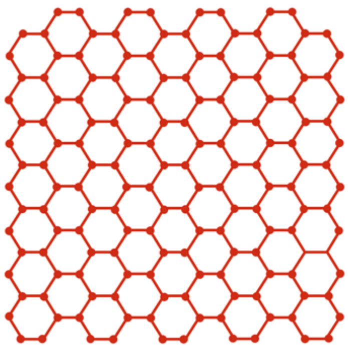

Figure 1 Structure of carbon atoms of single-layered graphene.

If multiple layers of graphene are stacked on top of each other, it results in the well-known graphite structure, resembling a pencil lead shown in Figure 2. The initial separation of graphene layers can be achieved using a simple technique called micromechanical cleavage or the "Scotch tape method." This involves placing a piece of adhesive tape onto a graphene sample and then peeling it off. The process is repeated by placing another piece of tape onto the peeled graphene layer and peeling that off as well. With each iteration, the thickness of the graphene layers on the tape becomes thinner gradually until obtaining a single-atom-thick layer, as illustrated in Figure 3.

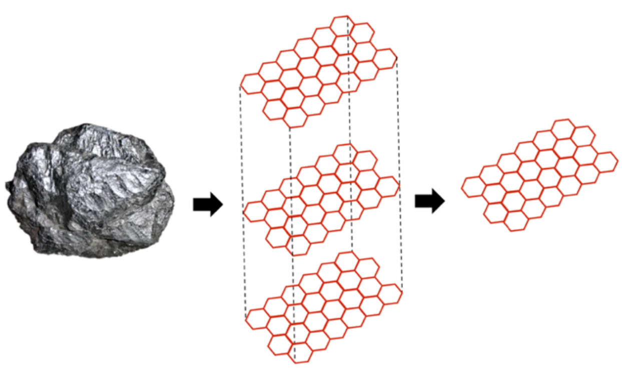

Figure 2 Structure of graphene crystal.

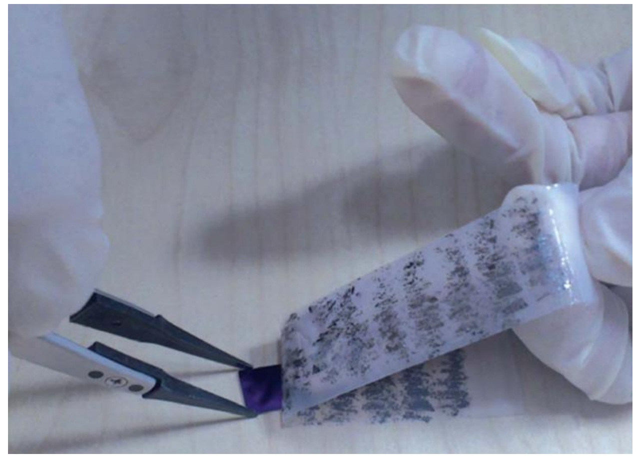

Figure 3 Scotch tape method of graphene synthesis from graphite block [11]

In the initial stages, the sheets of graphene obtained were mere small flakes with sizes not exceeding 1 square millimeter, and their shapes were irregular. Scientists endeavored to devise methods for producing larger-sized graphene with controlled properties through the control of structure and synthesis techniques suitable for various industrial applications.

B. Calcium Lactate ($C_6H_{10}CaO_6$)

Calcium lactate is a salt composed of two lactate ions for every one calcium ion (Ca^{2+}). It is commercially prepared by balancing lactic acid with calcium carbonate or calcium hydroxide. It has received FDA approval as a direct food substance recognized as generally safe and is used as a firming agent, flavoring agent, leavening agent, stabilizer, and thickener. Calcium lactate is also present in daily dietary supplements as a source of calcium. Additionally, it is available in the form of liquid solutions, with calcium lactate pentahydrate being the most commonly encountered form in liquid solutions. Calcium Lactate has antimicrobial properties like graphene [10]–[11].

3. METHODOLOGY

This research has 2 parts of experiment which are Testing the Antimicrobial Properties of Graphene Oxide and Calcium Lactate Solution and Pixel Counting.

A. Testing the Antimicrobial Properties of Graphene Oxide and Calcium Lactate Solution

In this section, the evaluation of the antimicrobial properties of 3 solutions which are graphene oxide, calcium lactate and graphene oxide mixed with calcium lactate coated on filter paper. The graphene oxide solution (GO) is used as an initial substance for microorganism eradication. Additionally, the solution of calcium lactate ($C_6H_{10}CaO_6$) and graphene oxide mixed with calcium lactate are also tested. These solutions act as binders between graphene oxide and filter paper. These binders are safe and non-toxic to humans. The experimental steps are as follows:

- Mix the solvent of graphene oxide with deionized water that has passed through the deionization process, or DI (Deionized water), in a ratio of 20 ml: 100 ml to use as a starting solution.
- Mix calcium lactate powder with DI water in a ratio of 10 mg: 100 ml to obtain a calcium lactate solution.
- Mix the graphene oxide solution with the calcium lactate solution in a 1:1 ratio.
- Immerse 3 filter papers (2 cm. x 2 cm.) into the prepared solutions (Figure 4) and bake them until dry (Figure 5).

Figure 4 Immerse filter paper into the prepared solutions.

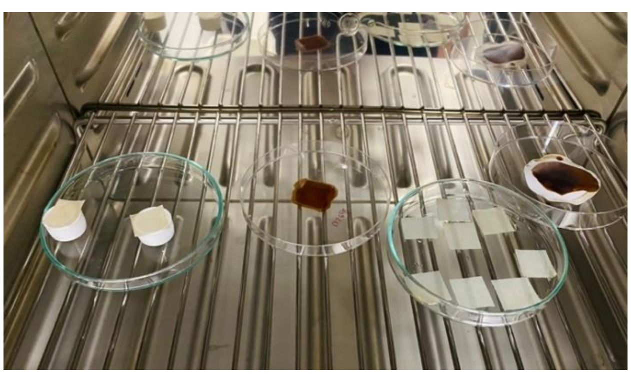

Figure 5 Bake the immersed filter papers until dry.

- Disinfect filter papers using UV radiation to prove that they are no microorganisms on the filter papers.
- Place each filter paper to receive microorganisms into the air for 20 minutes (Figure 6).

Figure 6 Place each filter paper to receive microorganisms.

- Place them on the nutrient agar and store them in an incubator at a temperature of 37 degrees Celsius for 7 days shown in Figure 7.

Figure 7 Cultivate and nurture microorganisms in an incubator at a temperature of 37 degrees Celsius for 7 days.

B. Pixel Counting

In microbial distribution measurement, it usually us ruler to measure the diameter of microbial distribution in culture disk and calculate to area. This method causes error when the microbial distribution is non-geometrical distribution shape. Therefore, the pixel counting method can solve the problem of non-geometrical distribution shape of antimicrobial distribution.

This step is about determining the antimicrobial effectiveness of all three types of solutions in inhibiting microorganism growth. Experimental results will be obtained by calculating the percentage of the area covered by microorganism compared to the total area of the culture dish. This calculation will be done using image processing principles, where the image of the region of interest will be converted into a black and white image, and a histogram will be used to count the pixel points. This will be used to calculate the percentage of the area covered by microorganism in the culture dish. The pixel counting process is as follows:

- Take the original image of the culture dish and perform segmentation to separate between the culture dish and the area of microorganism distribution.

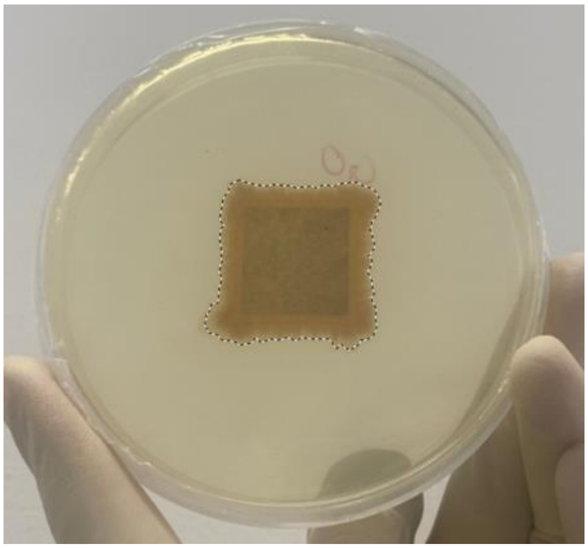

Figure 8 Segmentation of distributed microorganism.

- Convert the segmented image to black and white image shown in Figure 9.
- Use the histogram to read the pixel values in the black areas, representing the regions with microorganism distribution, and read the white pixel values along with the black areas, representing the area of the culture dish.
- Compare the percentage ratios of the results from each experiment to observe the distribution of microorganisms in different solvent solutions.

Figure 9 Convert to black and White image.

4. RESULT

This section will discuss the testing of the effectiveness of coated filter paper with graphene oxide, calcium lactate and graphene oxide mixed with calcium lactate solutions in inhibiting microorganism growth. In this research, the percentage of microorganism distribution is compared to find the most effective solvent solution for inhibiting microorganism growth. This information will be used to determine the coating material for future paper applications. The percentage of microorganism distribution can be found by the following equation.

$$\%\ \text{distribution} = \left(\frac{Black\ pixels}{Black\ pixels + White\ pixel}\right) \times 100 \qquad (1)$$

According to (1), The percentage of microorganism distribution of each sample are shown in Figure 10 – 13.

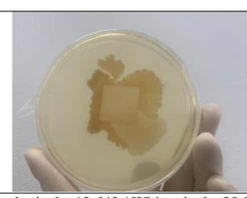	
Black pixels: 13,642 / White pixels: 38,920 White pixels + Black pixels: 52,562	The percentage of bacterial distribution: 25.95%

Figure 10 The 25.95% of microorganism distribution of control.

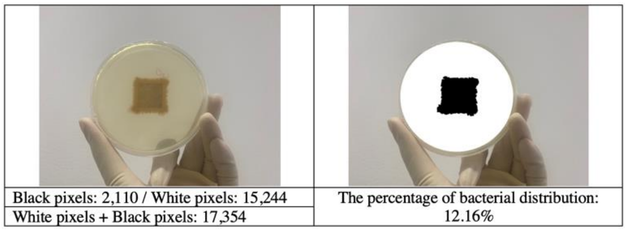

Figure 11 The 12.62% of microorganism distribution of graphene oxide solution.

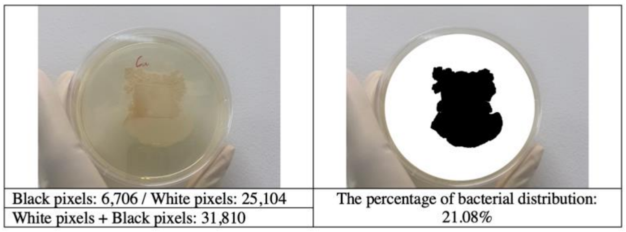

Figure 12 The 21.08% of microorganism distribution of calcium lactate solution.

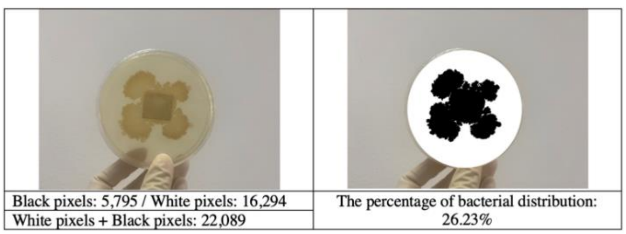

Figure 13 The 26.23% of microorganism distribution of graphene oxide mixed with calcium lactate.

Based on the aforementioned experimental results, a graph illustrating the relationship between the type of solvent and the percentage ratio of microorganism distribution in the culture dish is created.

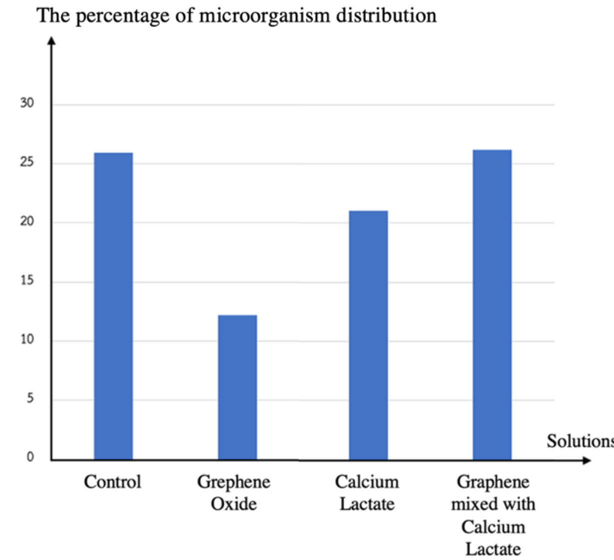

Figure 14 A graph illustrating the relationship between the type of solvent and the percentage ratio of microorganism distribution in the culture dish.

According to Figure 14, each solution can be compared to control that can be shown in Table I.

TABLE I. Antimicrobial Efficiency of each solution comparing with control

Solution	The ratio of the spread area of the microorganism to the area of the culture dish.	Antimicrobial efficiency comparing with control
Control	25.95%	-
Graphene Oxide	12.16%	53.14%
Calcium Lactate	21.08%	18.77%
Graphene Oxide mixed with Calcium Lactate	26.23%	No antimicrobial efficiency

In Table I, the spread area of microorganism (black pixels) in culture disk (white pixels + black pixels) of each sample are compared to control in order to observe the antimicrobial efficiency. Comparing to control, graphene oxide shows high antimicrobial ability which is 53.15%. Calcium lactate shows 18.77% of antimicrobial ability. Finally, Graphene Oxide mixed with Calcium Lactate has no antimicrobial ability.

5. DISCUSSION

From the experimental results, it was found that graphene oxide solution has antimicrobial properties of

53.14% compared to the control, while calcium lactate solution has antimicrobial properties of 18.77% compared to the control.

From the experimental results, it can be observed that graphene oxide possesses antimicrobial properties, which are related to previous research [10]. Additionally, calcium lactate, commonly used in the food industry, also exhibits antimicrobial properties [11]. However, when graphene oxide is combined with calcium lactate, it does not enhance the antimicrobial properties; instead, it leads to the loss of these properties. This may be attributed to some chemical reactions that occur during the mixing of these two substances. Conducting experiments to improve the effectiveness of disease resistance in graphene oxide is therefore an interesting avenue for future research.

Moreover, this research should be noted that this experiment has not tested the adhesive efficiency between the solution and filter paper, which will be investigated in the future.

6. CONCLUSION

This research is an experiment to test the antimicrobial efficiency between graphene oxide solution, calcium lactate solution, and a mixture of graphene oxide solution coated on filter paper. In this experiment, the antimicrobial efficiency is tested using the pixel counting method, which involves counting the number of pixels from the spread of microorganisms in the culture dish images. The experimental results show that graphene oxide solution has the highest antimicrobial properties, followed by calcium lactate solution. On the other hand, the mixture of graphene oxide and calcium lactate solution does not exhibit antimicrobial properties. For future work, the efficiency of adhesion between the solution and filter paper will be tested to facilitate practical applications.

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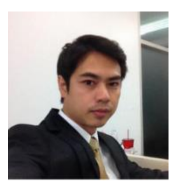

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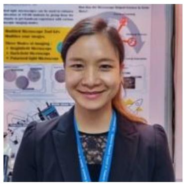

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