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Efficiency of renewable plastic material degradation using effective microorganisms in aerobic conditions

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Abstract

Studies were conducted to establish a simulated system based on the aerobic biodegradation of renewable plastic materials (cellulose, Poly lactic acid (PLA), and bio-base). The experiment consisted of carrying out biodegradation by using a reactor. The experiment was tested at 28 days under the following conditions: the control parameter of air flow was 60 mL/min, the humidity was 60-80%, and pH ranged from 7.5-8.5. The results indicated the analytical carbon dioxide (CO_2) and weight loss. It was found that the effective microbes had been able to degrade the renewable materials, except for the bio-base. The EM had been able to degrade PLA and Cellulose at $4.51\pm0.50\%$ and 40.53 ± 2.15, while the weight loss of the PLA and Cellulose had been $5.24\pm0.85\%$ and $42.65\pm3.55\%$. The EM had secreted cellulase enzymes to decompose the cellulose, and the enzymes had caused catalytic reactions, which, in turn, had degraded the cellulose. Because cellulose has organic carbon, the cellulose test showed high degradation. The EM had been able to degrade the cellulose because the EM consisted of aerobic and anaerobic microbes that had contributed to the degradation organic compounds. The result of the observations indicated that cellulose had torn after 28 days. However, the PLA had only slightly changed, while the bio-base had shown no change at all. Moreover, SEM observation showed that the PLA and the cellulose had been damaged. The EM were found to be growing on surface of two materials and were also growing inside of the materials. Meanwhile, no damage was found on the bio-base at all. The EM had been able to degrade the renewable materials. Cellulose is the material with the highest degradation. It is, therefore, possible that EM have the ability to degrade biodegradable plastics.

Keywords: Renewable plastic material, Bio-base, Poly lactic acid, Bioplastics, Effective microorganisms

1. Introduction

At present, plastic is a part of our daily lives, and the world's consumption of plastic has been steadily increasing. In 2015, the annual production of petroleum-based plastics exceeded 300 million tons [1]. The properties of plastics, such as their chemical and ultraviolet light resistance, make them very useful. In fact, plastic has become very popular, both in work requiring durable packaging and in the production of single-use disposable materials. Therefore, after the plastic items have been used, much waste is generated.

Burying plastic items in landfills has become a popular method for managing plastic wastes. However, it is currently causing environmental problems and will continue to do so in the future. The plastic being used today is mostly degradable or almost non-biodegradable, and biodegradable plastics can take 70 - 450 years to decompose [2]. Therefore, the extended period of decomposition represents a long-term problem that is affecting landfill sites, which may not be able to support a vast increase in the amounts of plastic waste.

The problem with long-term plastic decomposition is being solved by using renewable materials [3-5]. The renewable materials are of many types, such as cellulose from plants, bio-plastics from plants, or bio-plastics from animals. The cellulose originating from plants can be derived from many types of plants, such as Bagasse, banana leaf, paper, or wood pulp. Packaging can be produced from cellulose. The bio-plastics originating from plant processing have been processed from corn, potatoes, wheat, and wood, etc. The products derived from the plant processing were polyhydroxyalkanoate (PHA), poly butylene adipate-co-terepthalate (PBAT), and poly lactic acid (PLA), etc [3, 4]. Renewable materials are considered to be eco-friendly, but the time required for them to degrade with compost at the appropriate conditions has been found to be 180 days [6]. Therefore, the time of degradation can lead to the conditions of having insufficient landfill areas to accommodate the plastic wastes and of causing CH_4 emissions due to the anaerobic conditions found in the landfills [7, 8].

Recently researchers have been interested in studying bioplastic degradation under different environmental conditions, such as in soil, marine compost, and in other aquatic environments [9-12]. The conditions were both aerobic and anaerobic with the inoculum from municipal solid wastes and wastewater treatment plants under different temperature conditions [13-16].

Aerobic degradation represents a possible way to solve the problem. The condition of aerobic degradation was able to expeditiously increase the numbers of microorganisms and to help to stimulate the performance of the microbes [17]. Effective microorganisms (EM) were used, because they are cheap and easy to find in the locality. Also, there are many types of microbes, such as *Saccharomyces ceareviceae, Lactobacillus fermentum, Bacillus megaterium,* and *Bacillus subtilis*, etc. However, the organic degradation factor of the microorganisms depends upon the types of materials because the structures of the organic compounds in the renewable materials are different [17], and these differences can affect the performance. Therefore, the effects that various microorganisms have on the degradation of different kinds of materials should be studied.

At this time, no work has been published on the degradation of renewable materials using EM under aerobic conditions. This study has focused on the degradation performance over a short time using different materials. The cumulative carbon dioxide (CO_2) and weight lost were examined, and the physical structure of the material was also observed. The results of this work could be applied to investigate the degradation of renewable plastic materials in the future.

2. Materials and methods

2.1 Chemicals

The chemical reagents consisted of sodium hydroxide (NAOH) 98% (LOBA Chemie, India), phenolphthalein and methyl orange indicator (Intereducation Suppliers, Thailand), hydrochloric acid (HCL) 37%, and dichloromethane (ACI Labscan, Thailand) - all of which were of analytical grade, and only deionized water (DI water) was used throughout all of the experiments.

2.2 Materials

The renewable materials used in this study are commercially available. For testing, the renewable materials consisted of cellulose, bio-base, and bioplastics (Figure 1). The cellulose came from a paper cup. The bio-base was identified as having been manufactured from biomass and was labelled as biodegradable. The PLA, which was used as bioplastics, was in the form of Poly lactic acid (PLA) pellets (Nature Works, USA), which were semi-crystalline and had an average D-lactide content of 1.4 wt%, a melting point temperature in the range of 155-170 °C, and a molecular weight of 150,000. The PLA film was formed by melting the PLA pellets with a dichloromethane solution [18].

To perform the biodegradation test in the reactor, the material was prepared at a volume of 1.5 grams (g) for each reactor, and the samples were cut into 5 mm x 5 mm squares.
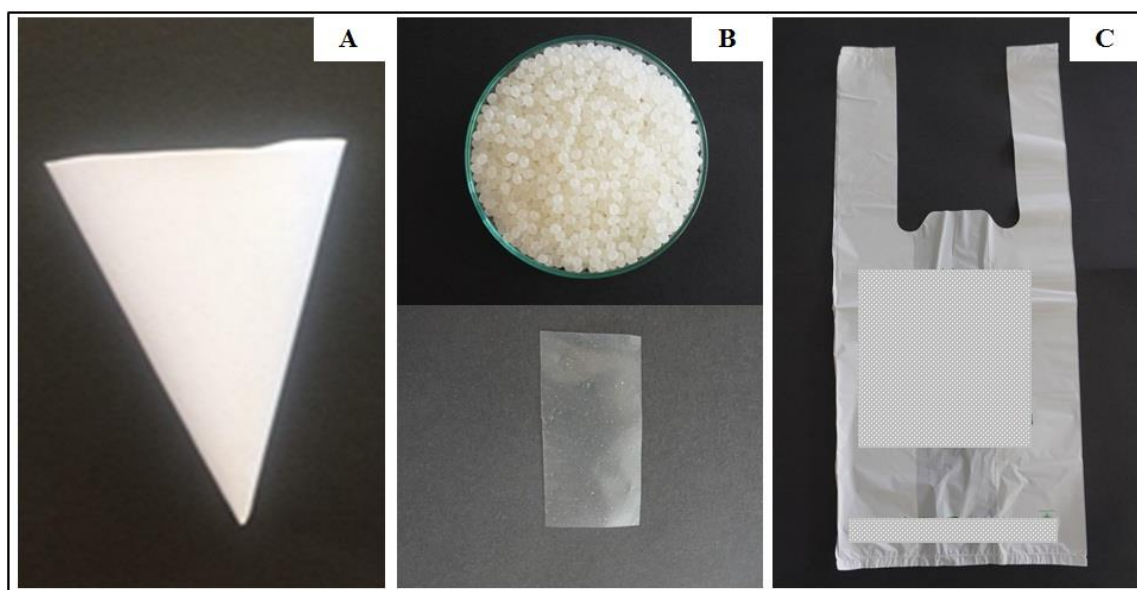

Figure 1 The types of renewable materials used in the experiment (A) cellulose (B) PLA and (C) bio-base

2.3 The microorganisms

The microorganisms used in this study were effective microorganisms (EM) under aerobic conditions. The EM, which was used, was the Nano Industrial Microbial Concentrate brand NBI EV1 (NANOBIO EV1), and the microorganisms consisted of *Bacillus subtilis, Bacillus mesentericus, Pediococcus, Steptococcus fecalis, Candida utilis,* and *Saccharomyces cerevisiae*, which were all of the identified species. At present, the NBI EV1 was used in the wastewater treatment plant. The previous studies used microorganism form wastewater treatment plant to degraded bio-material [13, 14]. So, this research used the NBI EV1 as the degrading material.

The EM was activated with culture medium (molasse) and DI water (Figure 2) with a ratio of EM 1.5 mL: molasse 1 mL: DI water 1,000 mL. Initially, the pressurized air of the air pump aerator was 30 L/min for aeration throughout a period of 46 hours, and then aeration was stopped for 2 hours while sedimentation took place. Next, the DI water was changed by draining the water out and then filling with 3,000 mL of DI water. Then, the molasse was filled to 3 mL and was once again aerated for 46 hours and was once again stopped for 2 hours while sedimentation took place (Figure 3). This process continued for 28 days. The duration of the activity of the EM was 28 days, and the EM were examined with SS analysis in order to achieve a 500 mL/L EM concentration. Preparation of the EM at 500 mL/L was carried out by centrifugation at 4,500 rpm for 10 minutes.
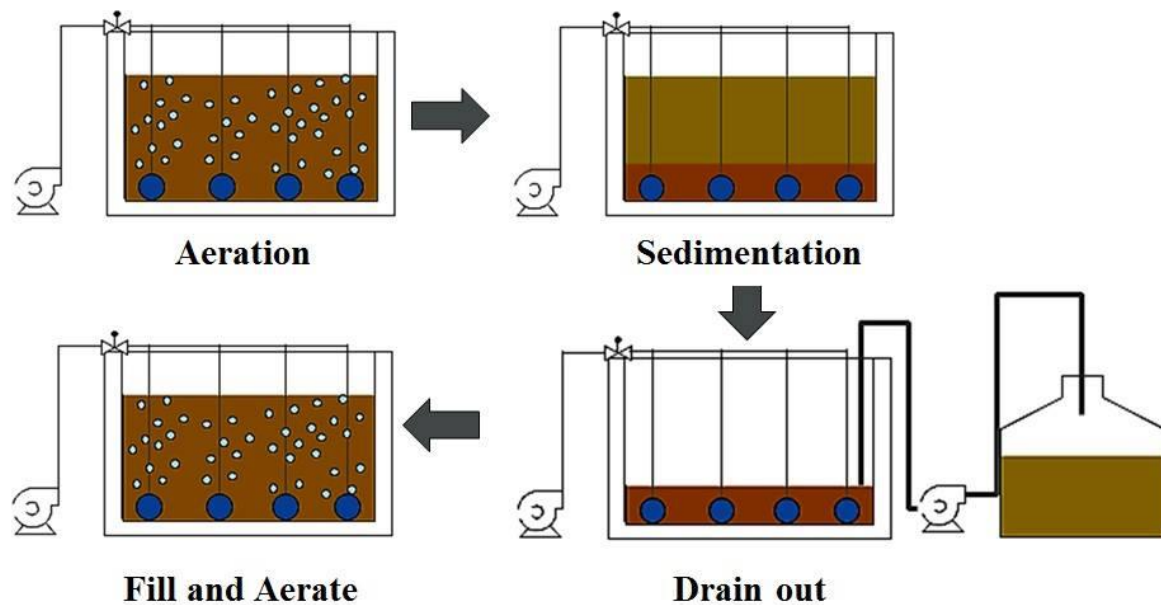
Figure 2 The apparatus for preparing the effective microorganisms

2.4 Material component analysis

Total solids (TS) analysis was carried out using a hot air oven at 103-105 °C. Volatile Solids (VS) were found to be the components (largely carbon, oxygen, and nitrogen), which had already been burned off in a dry sample in a laboratory furnace at 500-600 °C [19]. The percentage of carbon (%C) was based on the calculation method of the VS content [20]. The formula was as follows:

$$\% \text{ Carbon} = \frac{\% \text{ VS}}{1.8} \tag{1}$$

For calculated the theoretical amount of carbon dioxide (ThCO$_2$) in grams per vessel, which can be produced by the test material using equation below [21]:

$$\text{ThCO}_2 = g_{material} * \frac{\% \, C_{material}}{100} * \frac{44}{12} \tag{2}$$

in which

$g_{material}$	=	the mass of the renewable plastic material (g)
% C$_{material}$	=	the percentage of organic carbon content of the renewable plastic material
44 and 12	=	the molecular mass of carbon dioxide and atomic mass of carbon respectively.

2.5 The biodegradation experiment

This study conducted a biodegradation test on renewable materials using mixed EM under aerobic conditions at room temperature. There were 2 types of experiments. In the first experiment, respirometry was used to measure the amount CO$_2$ and to gauge the biodegradation. For this study, standard testing for ISO 14855-1 was applied [22]. In the second experiment, the weight loss caused by the biodegradation was measured.

2.5.1 The experiment to measure the amount CO$_2$

The aerobic conditions tests utilized the standard ISO 14855-1 procedure [22], the aerobic condition test wherein aeration and humidity were closely monitored and controlled. The renewable material and EM were stirred in the closed bottles of the respirometer. The evolved carbon dioxide (CO$_2$) was absorbed by a suitable absorber (NAOH solution) in the headspace of the test bottles.

Using the apparatus, the biodegradation test was performed under aerobic conditions at room temperature. The setup is shown in Figure 3. Initially, the pressurized air from the air pump was passed through the 5N NaOH solution to remove the CO$_2$ in the air. Later, the air was passed through DI water to increase the humidity and to maintain an uniform moisture of between 60-80% throughout the experiment. The air was divided and passed through the flow meters for each reactor at a flow rate of 60 mL/min. Then, the air was passed through reactor. The renewable materials (1.5 g of sample and 3 g of EM) were mixed in the 50 mL reactor. (The ratio between the material and the EM, EM control, and material control are shown in Table 1). A solution of 0.1N NaOH with 100 mL was used to trap the CO$_2$ from the reactors, while the volume of CO$_2$ content in the solution was calculated using the titration method. Every time that the CO$_2$ was calculated (2 days/time), the NaOH solution was changed. The biodegradation tests were performed at room temperature for 28 days. In almost all studies, the number of experimental replicates of the blanks or the samples was duplicated.
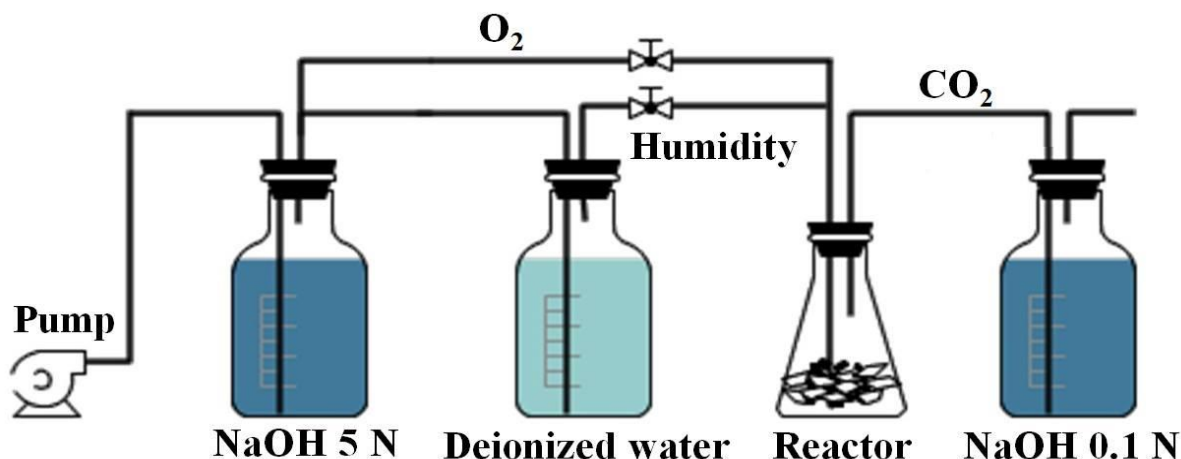
Figure 3 The setup of the biodegradation arrangement

Table 1 The experiment for measuring CO_2

No	Materials (g)				
	EM	Cellulose	PLA	Bio-base	NAMETEST
1	3	0	0	0	CTRL
2	0	1.5	0	0	CELR
3	3	1.5	0	0	CELT
4	0	0	1.5	0	PLAR
5	3	0	1.5	0	PLAT
6	0	0	0	1.5	BIOR
7	3	0	0	1.5	BIOT

To determine the analytical amount of CO_2 derived from degradation, 10 mL samples were removed from the bottles of the 100 mL trapping solution (0.1N NaOH), and the samples were titrated with 0.1N HCl solution in order to obtain the value of CO_2 in the solution. The CO_2 content in the 10 mL NaOH solution was correlated to the 100 mL NaOH solution, and then the actual amount of CO_2 was determined.

Initially, for titration method, one drop of phenolphthalein indicator was added to the 10 mL sample, and then the sample was further titrated with 0.1N HCl solution until it turned from pink to colorless. After that, three drops of methyl orange indicator were added and titrated with 0.1N HCl solution till the solution turned from orange to red. The CO_2 trapping reaction was carried out in a two-step reaction [23]. The amount of CO_2 was determined by the following equation:

$$gCO_2 = \frac{V*C*44}{1,000} \tag{3}$$

in which

gCO_2 = the amount of evolved CO_2 in grams (g)
V = the volume of HCl consumed in the second reaction
C = the concentration of the HCl solution

The biodegradation of the renewable material was calculated based on the amount of CO_2 in the degradation as follows [24]:

$$\% \text{ Biodegradation} = \frac{gCO_2\text{ materail + inoc} - gCO_2\text{ inoc}}{g_{material}(\frac{\% C_{material}}{100})(\frac{44}{12})} * 100 \tag{4}$$

in which

$CO_2\text{ materail + inoc}$ = the amount of CO_2 in the renewable plastic material and EM (g)
$CO_2\text{ inoc}$ = the amount of evolved CO_2 in the EM (g)
$g_{material}$ = the mass of the renewable plastic material (g)
$\% C_{material}$ = the percentage of organic carbon content of the renewable plastic material

2.5.2 The experiment to measure weight loss

The weight loss test was performed using the apparatus under aerobic conditions at room temperature. Initially, the pressurized air from the air pump was passed through the DI water to humidify and maintain an uniform moisture of between 60-80% throughout the experiment. The air was divided and passed through the flow meters of each reactor at a flow rate of 60 mL/min. Then, the air was passed through reactor. The renewable materials (1.5 g of sample and 3 g of EM) were mixed in the 50 mL reactor (the ratio between the material and the EM is shown in Table 2) and then passed through deionized water. The weight loss tests were performed for 14 and 28 days. In almost all studies, the number of experimental replicates of the blanks or the samples was duplicated.

Table 2 The experiment for the measurement of the percentage of weight loss

No.	Materials (g)				
	EM	Cellulose	PLA	Bio-base	NAMETEST
1	3	1.5	0	0	CELT_14
2	3	1.5	0	0	CELT_28
3	3	0	1.5	0	PLAT_14
4	3	0	1.5	0	PLAT_28
5	3	0	0	1.5	BIOT_14
6	3	0	0	1.5	BIOT_28

The weight loss was measured by weighing the renewable material before and after the experiments at 14 days and at 28 days. The renewable material was washed by DI water in order to wash out the microorganisms using a 1-sieve. Dehydration took place for 24 hours in a hot air oven at 30 °C, and then the samples were placed in a desiccator for 8 hours. The remaining material from the digestion was taken and weighed, and after that, the obtained value was recorded. The percentage of weight loss for each of the renewable materials was calculated by using the following equation:

$$\% \text{ Weight loss} = \frac{W_{(before)} - W_{(after)}}{W_{(before)}} * 100 \qquad (5)$$

2.6 Scanning electron microscopy

For the SEM analyses, the changes in the structures and surface areas of the renewable materials were observed after aerobic degradation. The structures and surface areas were investigated using the SNE-4500M (SEC, Korea). When operated at an accelerating voltage of 20kV, Scanning Electron Microscopes (MiniSEM) can magnify up to 100,000x. The samples were prepared by removing moisture in a desiccator for 24 hours. Prior to observation, the surfaces of the samples were coated in a thin layer of gold.

3. Results and discussion

3.1 Material composition

The material compositions of the EM, cellulose, PLA, and the bio-base were checked (Table 3). The composition analysis of the material was tested in 1.5 g. The results showed that the TS of EM, cellulose, PLA and bio-base had been 0.69 g, 0.99 g, 1.00 g, and 0.99 g, respectively. In addition, the volatile solids (VS) testing found that the VS of EM, cellulose, PLA, and bio-base had been 0.18 g, 0.99 g, 0.99g, and 0.78 g, respectively. The findings also showed that the EM (7.71%) had exhibited the lowest carbon percentages when compared to the PLA, cellulose, and bio-base at 56.04, 55.34, and 43.43%, respectively. The theoretical amount of carbon dioxide (ThCO$_2$) in the cellulose had been 3.14 g-CO$_2$. The ThCO$_2$ in the PLA had been 3.04 g-CO$_2$, while in the bio-base, the ThCO$_2$ had been 2.38 g-CO$_2$ (Table 3). ThCO$_2$ from the calculation can be used as a default to monitor the degradation of bio-material. The amount of ThCO$_2$ showed the chemical differences of bio-materials [3, 4, 17].

Table 3 The characteristics of the renewable materials and EM.

Composition	Results of the analyses ($\bar{X}\pm$S.D.)			
	EM	Cellulose	PLA	Bio-base
Total Solids (g)	0.69±0.08	0.99±0.08	1.00±0.02	0.99±0.04
Volatile Solids (g)	0.18±0.05	0.99± 0.09	0.99±0.05	0.78±0.04
Carbon (%)	7.71±0.92	56.04±0.06	55.34±1.43	43.23±1.88
ThCO$_2$ (g)	0.85±0.07	3.14±0.11	3.04±0.68	2.38±0.88

3.2 Biodegradation of material using EM

3.2.1 Biodegradation of renewable materials from the CO$_2$ measurements

Measuring the degradation of cellulose, PLA, and bio-base was determined by the amount of CO$_2$ that had been emitted, while the biodegradation of the renewable material from CO$_2$, which had accumulated in 28 days. The CO$_2$ was measured 2 days/time in all replicates and duplicated samples. The cumulative CO$_2$ of the cellulose, PLA, and bio-base had been 1.23±0.01 g-CO$_2$, 0.14±0.01 g-CO$_2$, and 0 g-CO$_2$, respectively. The findings suggested that the cellulose had degraded more than the PLA and the bio-base. There was no degradation of materials in all experiments of non EM. Therefore, Figure 4 shows the biodegradability of renewable materials and the delta between them and non-EM experiments. The cellulose material was beginning to degrade on the second day (0.024 ±0.001 g-CO$_2$ degradation of 0.77%) with a reaction rate of 0.043 g-CO$_2$/day. The EM could degrade the cellulose because the cellulose had been from a natural material-wood. The EM (some species) could produce the enzyme, cellulase, to degrade the cellulose, which was similar to findings from past studies in which the cellulose had been degraded by microorganisms [17].

The results of the efficiency of degrading cellulose was from the microbes that had produced the enzyme, cellulase, which had degraded the cellulose. Meanwhile, PLA had only been digested a little (0.14±0.01 g-CO$_2$ and degradation of 4.56±0.10%) with a reaction rate of 0.004 g-CO$_2$/day. The degradation process of PLA stemmed from microbes, which produced enzymes (protease, esterase, lipase, and cutinase) that were used to degrade the PLA [25, 26]. This caused the structure of PLA to be damaged. Similar previous research studies found that the PLA could be degraded by microorganisms in a composting condition at 98 days (10% degraded), and incubation at 28 days (3.7% degraded). The action of the microbes produced enzymes that degraded the PLA [14, 27]. But, the previous studies, PLA degradation efficiency was found to be up to 60% in 30 days. The higher temperatures effect to increasing efficiency of degradation [28]. The results from the cellulose and PLA showed that the EM had been able to degrade both

materials. However, the EM had been unable to degrade the bio-base because the bio-base had a composition that could not be degraded. The bio-base was a commercially renewable material, but the material could not be specified. The bio-base is produced from bio-materials, such as corn, bagasse, or cassava, etc. In some cases, bio-base has been produced from mixed materials. These could include glue, wet powder, and other materials that are used to integrate the structure. In addition, chemical coatings that are difficult to decompose could also be used. Even though, the bio-base is a natural material, some of its components are not able to degrade.
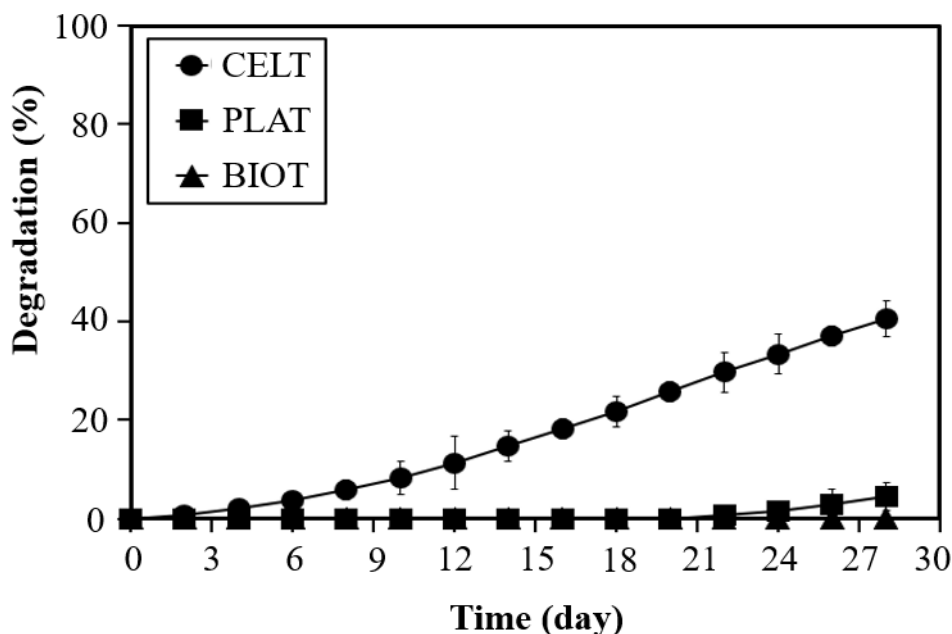

Figure 4 The biodegradability of renewable materials by measuring the amounts of CO_2

3.2.2 Biodegradation of renewable material from weight loss measurement

The renewable material degradation, which was measured by weight loss 14 days and 28 days, found that percentage of degradation was less than the measurement by CO_2. For degradation of renewable material in 14 days, the result found that the PLA and bio-base did not degraded while the cellulose was found to be 12.16% degraded. At 28 days, the PLA was at 5.42% degradation, the cellulose was at 42.65% degradation, and the bio-base had not degraded (Table 4).

The results of the weight loss monitoring indicated that at 14 days, weight loss had only begun in the cellulose, because cellulose is easily digested. Meanwhile, both of renewable materials did not show any weight loss. The result of weight loss caused by the EM could degrade both of the bio-plastics. The enzyme that had been produce from the EM was degraded [29, 30].

Table 4 The percentages of the measurement of renewable material degradation by weight loss

Types of bio-plastic	%Weight loss ($\overline{X}\pm$S.D.)	
	14 days	**28 days**
Cellulose	12.16±1.05	42.65±3.55
Poly lactic acid	0.00±0.00	5.24± 0.85
Bio-base	0.00±0.00	0.00±0.00

3.2.3 Biodegradation of renewable material from physical measurements

The physical appearance of the renewable materials were observed. The physical structure of 3 renewable materials found that the cellulose had undergone more structural damage than PLA and bio-base. Results of the observations found that the cellulose had torn after 28 days. The PLA had only slightly changed, while the bio-base had not changed (Figure 5). The PLA had changed shape, but the plastic before test had had a smooth and clear shape. After 14 days, the plastic was in a bent shape [24]. After 28 days, the plastic was in a bent shape and was also white opaque. In addition, SEM observation found that the PLA and cellulose had been damaged. The EM were growing on the surface of two materials and were growing inside of the material (Figure 6). Moreover, the microorganisms, which were found were of many types (observed by the shape of microbes) and were growing in micro-colonies (There were many microbes grouped together) [14]. Meanwhile, no bio-base damage was found at all. Therefore, the result of the biodegradation of the bio-plastics from the physical measurements indicated that EM could degrade cellulose and PLA. The bio-materials structure was damaged as similar to the previous studies [14, 30].
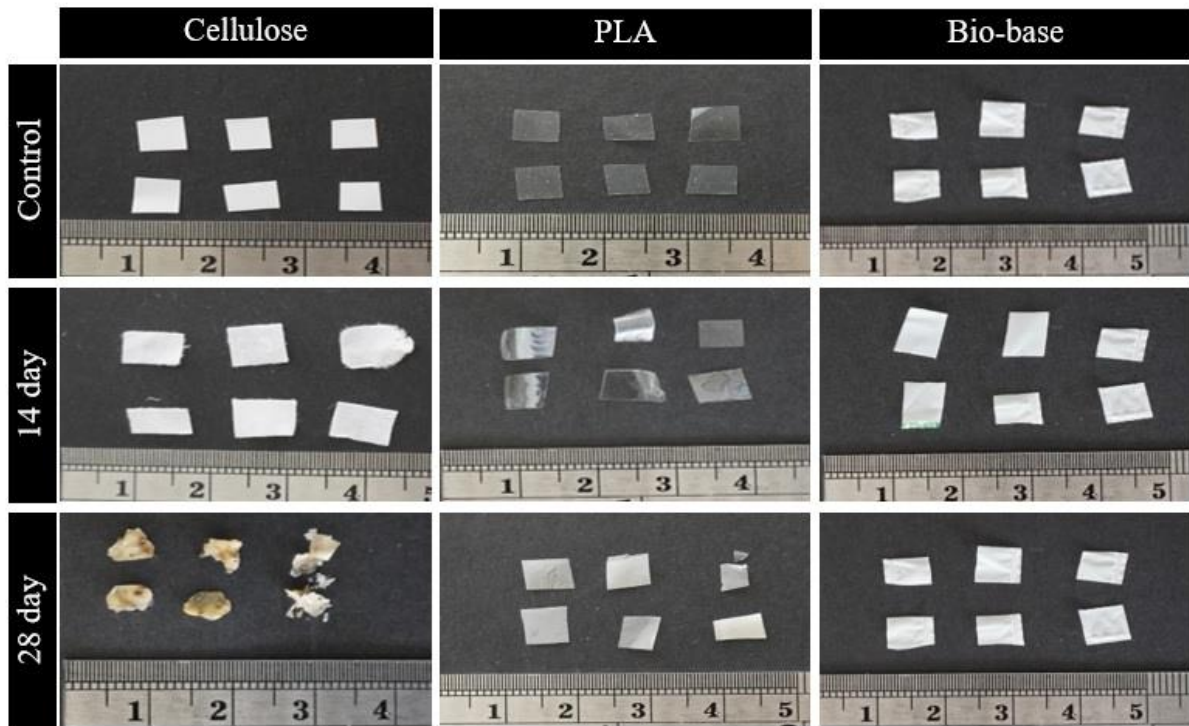
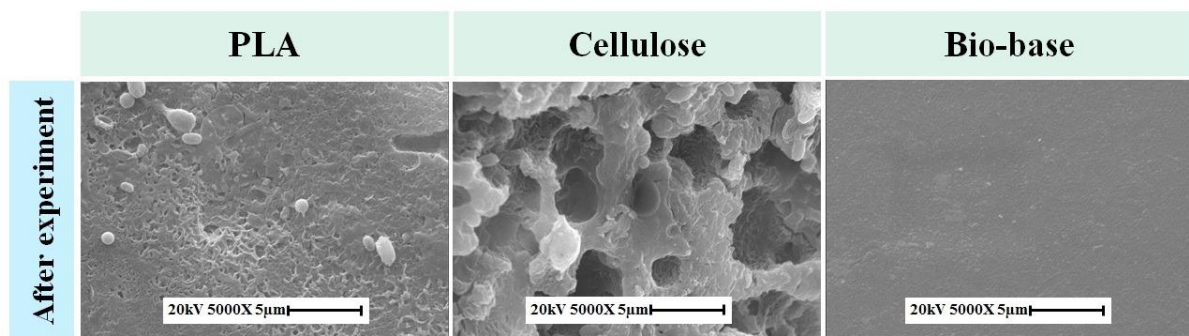**Figure 5** The physical evidence of the renewable material degradation

Figure 6 SEM micrographs of the renewable materials after the experiment at 28 days

4. Conclusions

The problem of plastic degradation was solved by using renewable materials, which means that the degraded renewable material problem can be solved by bio- degradation. This study shows the possibility of utilizing effective microorganisms (EM) in biodegradation processes. The renewable material was produced using natural materials, and natural materials can degrade. The structural differences of the renewable materials depend upon the types of materials used to create them. Difference in structures were found to affect the production of CO_2. Because cellulose is produced from wood, it had produced the highest CO_2 (1.23 ± 0.01 g-CO_2). The performance degradation of renewable materials under aerobic conditions at 28 days had affected the structure and amount of CO_2 in the materials. The degradation of the renewable materials was observed by the amount of CO_2 emitted and the weight loss. The results showed that bio-base couldn't be degraded, because the bio-base had been produced from mixed materials - which are difficult to be decomposed. The cellulose could degrade the most (amount of CO_2 1.23 ± 0.01 g-CO_2 and weight loss of $42.65\pm3.55\%$) and PLA was found to be the second most biodegradable (amount of CO_2 0.14 ± 0.01 g-CO_2 and weigh loss of $5.24\pm0.85\%$), because cellulose and PLA had both been produced by bio-materials. The structures of cellulose and the PLA sheets were damaged by the microorganisms. Microbial cells were found in the structure of cellulose and the PLA sheets, which confirms the results of the biodegradation of the cellulose and the PLA. As for the bio-base, no damage was found at all. The results showed the possibility of using effective microorganisms to decompose cellulose and PLA materials. Therefore, EM has a potential to develop for commercial viability. However, future studies are needed to improve the degradation efficiency of both PLA and cellulose such as a study of the efficacy of individual microbial strains, a study to enhance the efficiency of PLA degradation with the higher temperature.

5. Acknowledgements

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