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Production of biofertilizer using *Lactobacillus* inoculants and glycerin pitch from oleochemical industry

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Abstract. Production of glycerol waste from oleochemical industry in the form of glycerin pitch poseses risk when disposed to the environment. Conventional methods to recover glycerol are infeasible due to the cost constraint, which has urged the exploration into alternative methods by converting glycerol waste into a valuable product at low cost. The glycerol has a potential to be utilized as a cheap carbon source in fermentation attributed to its high availability. This study focuses on the use of *Lactobacillus* inoculant and glycerin pitch as a medium for the production of biofertilizer and the effectiveness of the biofertilizer application in promoting the growth of cucumber plant. The results found that biofertilizer with the ratio of 2:1 in the volume of *Lactobacillus* to glycerin pitch had high effectiveness in promoting the height of cucumber plant by up to 40 cm in 18 days which is almost 2-fold compared to the commercial fertilizer. The produced biofertilizer contained a formulation of pH 5.11, 3.78 Megapascal second (MPas) viscosity and carbon to nitrogen ratio (C/N ratio) of 38.85 after 14 days of storage.

1. Introduction

The abundance of palm oil and palm kernel oil have provided the oleochemical industry with a reliable supply of raw material sources. Oleochemical demand is expected to increase as the end-use industries expand rapidly, such as food and beverage, personal care, and pharmaceuticals [1]. Attributed to the overwhelming demand for the products of oleochemical industry, large amount of waste and effluent containing hazardous materials are produced by the industry.

Glycerin pitch is one of the waste products, which is generated by the industry's fatty acid plant in the residual receiver. Glycerin pitch is one of the scheduled waste products that is gazetted by the Malaysian Department of Environment (DOE). It is listed in the First Schedule of the Environmental Quality (Scheduled Wastes) Regulations 1989. The properties of glycerin pitch vary in different plants. Glycerin pitch is very alkaline (pH>10) which exhibits viscous gel properties with a color ranging from brown to dark brown, as shown in Figure 1. Glycerin pitch comprises of glycerol (55 to 65%), diglycerol (<10%), fatty acids (<10%), and inorganic salts (15 to 25%) [2].

Figure 1. Glycerin pitch.

Glycerol derived from glycerin waste can be used in the direct fermentative substrate as it is abundantly produced by palm oil plants [3, 4]. Research conducted by Ramachandran and Amirul (2013) has utilized glycerin pitch (10 g/L) and 1,4-butanediol (5 g/L) to produce 2.91 g/L of P(3HB-co-40%4HB) copolymer [5]. Meanwhile, Aziati and Sakinah (2017) indicated that the glycerol residue has high potential of becoming a good source of carbon in fermentation [6]. The authors reported the production of succinic acid from glycerol residue through the use of immobilized *Escherichia coli* in a batch fermentation process.

Successful selection of carrier substrate can be developed, whereby, a good carrier substrate can support the growth of the target organism and maintain a high number of inoculant bacteria. Carrier material combined with microorganisms promote an easy-handling, long-term storage and high effectiveness of biofertilizer [7]. In general, peat is the best carrier of choice, but in most cases, it is not commercially distributed and often requires readily available substrates. In literature, alternative carrier materials, including various clays, animal manure such as poultry manure (PM), composted plant materials, and other complex organic matrices have been utilized as carriers in biofertilizers [8]. Waste by-products containing valuable sources that can help the biofertilizer maintain the structural composition could also be considered as alternative materials, particularly, as they are readily available such as the by-products discharged by palm oil plants [9, 10]. Thus, the glycerin pitch containing glycerol has the potential to be used as a fermentative carbon source to produce biofertilizer.

Liquid biofertilizers have been widely manufactured to boost the soil ecosystem and to provide supplementary nutrients for plants [11]. Commercial biofertilizer referred to as fertilizer contains living microbes or inoculants, which when applied to seeds, plants, or soils, promote growth through the supply of essential nutrients, such as N, P, K, and other mineral nutrients [12]. There are several factors that need to be considered in the production of biofertilizer, such as carriers and inoculants. In biofertilizer production, the carrier material needs to be fermented with the inoculant bacterium to produce either liquid fertilizer or solid fertilizer.

In producing liquid biofertilizer, there are several microbial inoculants that can be considered, based on the role and ability in influencing the plant growth, when infused with soil. The microbial inoculants are particularly useful in helping to boost growth activities in plants through direct application onto the plants. Microbial inoculants can be categorized based on three roles, which include N-fixer, P-solubilizer, and K-mobilizer. N-fixer bacteria that have been identified include *Azotobacter Chroococcum*, *Azotobacter Vinelindii*, *Azotospirillum Lipoferu*, *Rhizobium* and *Acetobacter Xylinum*. *Rhizobium* is one of the inoculants that can utilize glycerol as a carbon source [16]. Specifically, glycerol can maximize rhizobia cell count. Indirectly, a high quantity of rhizobia cell count can promote a long shelf-life of biofertilizer. Common bacteria of P-solubilizer include *Bacillus Megaterium* and *Bacillus sp.* Research conducted by Jena and Rath (2013) observed the

highest phosphate solubilizing activity compared to other phosphate solubilizing isolates with the optimum condition of three days of fermentation, at a temperature of 36ºC and pH 7 [17]. In producing Phosphate Solubilizing Biofertilizer (PSB), glycerol serves as the carbon and energy source and also as a part of the formulation procedure. Glycerol also has the ability to maintain a high population density in the fermentation process [18-20].

This study focuses on the conversion of glycerol; with the use of *Lactobacillus* inoculant and glycerin pitch as a medium for the production of liquid biofertilizer, which can benefit the growth of crops. The production of biofertilizer were optimized by the *Lactobacillus* inoculant and glycerin pitch which were varied by volume ratio of 1:1, 1:2, 2:1. The produced biofertilizers were characterized by determination of organic and carbon content, viscosity and alkalinity. The cucumber seeds were used for the biofertilizer plant test. The present study showed the potential of glycerin pitch as medium and carbon source for stimulating the growth of *Lactobacillus*. The biofertilizer with ratio 2:1 (volume of *Lactobacillus*: glycerin pitch) gave an almost 2-fold increase of height in the growth of plant as compared to the commercial fertilizer which showed an ideal nutrient for enhanced and promoted the growth of cucumber plant.

2. Experimental section

2.1. Material

Samples of glycerin pitch were collected from Natural Oleochemical Sdn. Bhd., Johor, Malaysia. Concentrated nitric acid 65% (Merck) and hydrochloric acid 37% (Sigma-Aldrich), distilled water, nutrient agar (Sigma-Aldrich), iodine solution (Sigma-Aldrich), safranin solution 1% (Sigma-Aldrich), cucumber seeds were also used in this work.

2.2 Instrumentation.

Analytical instrumentations and labwares used in this work were ICPMS (AGILENT 7500 SERIES), digestion tube, mason jar, compost bin, microscope (National 163), autoclave, petri dish, crucible, commercial refrigerator and incubator, furnace, viscometer (Digital Model DV II), pH meter (Mettler Toledo).

2.3 Procedure

2.3.1 Characterization of glycerin pitch. All collected samples of glycerin pitch were preserved under a cold temperature at 4 °C to avoid bio-degradation by microorganisms. Furthermore, all raw materials were autoclaved prior to utilization. Micro and macronutrients in the samples were analyzed using a nutrient test kit as described in table 1.

Table 1. Test methods for micro and macro nutrients.

Nutrients	Test Method
Nitrogen	In House AOAC 988.05
Phosphorus	In House MERCK NOV A60 (method no. 055)
Potassium	In House MERCK NOV A60 (method no. 103)
Magnesium	In House MERCK NOV A60 (method no. 158)
Calcium	In House MERCK NOV A60 (method no. 165)
Iron	In House MERCK NOV A60 (method no. 037)
Manganese	In House MERCK NOV A60 (method no. 159)
Boron	In House MERCK NOV A60 (method no. 168)
Molybdenum	In House MERCK NOV A60 (method no. 175)
Sodium	In House MERCK NOV A60 (method no. 164)

Identification of metal content was analyzed by using ICPMS. The procedure started with the digestion of the samples by using EPA Method 1638. Firstly, 50 mL of an aliquot from a well-mixed acid-preserved sample was transferred to a 50 mL digestion tube. Subsequently, 0.5 mL of concentrated nitric acid and 0.25 mL of hydrochloric acid were added. The samples were heated for 2 hr until a temperature of 85 °C was obtained. The samples were then allowed to cool. Then, the samples were analyzed with a blank rinse was flushed for 30 sec between the samples during the analytical run.

2.3.2 Preparation of inoculant and biofertilizer. Strain of *Lactobacillus* was used in this work. At first, one-fourth or 62 mL of rice and a cup of water were transferred into a Mason jar and shaken vigorously until the water formed a cloudy white emulsion. Subsequently, the rice kernels were strained and transferred into a tour compost bin. A cap was placed loosely on the bin and stored in an enclosed cabinet for 5-7 days. Then, the top layer of the fermented rice was lifted and the liquid was strained. Next, the rice liquid was measured and a ratio of one-part of fermented rice to ten-part of rice milk was mixed, and subsequently cultured in a jar for 5-7 days with a lid on top.

The materials that used were included glycerin pitch and starter culture (*Lactobacillus*). Three samples were prepared with varying ratios of glycerin pitch to *Lactobacillus* volume (1:1, 1:2, 2:1). Formation of *Lactobacillus* was confirmed through observing under a microscope. 10 mL of the biofertilizer sample was inoculated on an agar plate prior for observation under the microscope. The agar plate was prepared by mixing of 1 L distilled and 20 g of nutrient agar. The mixture was heated until the agar nutrient was well dissolved. Afterward, the agar mixture was autoclaved for 1 hr. The agar mixture was then poured into a petri dish and was subsequently allowed to cool to room temperature under a fume board before refrigerated for 24 hr. 0.1 mL of the inoculant sample was spread onto the surface of the agar and incubated for 24 hr at 37 °C.

The bacterial smear was prepared prior to observe under a microscope. The bacterial smear was prepared on a clean slide using iodine solution, which was washed afterward. Safranin solution was added to observe the culture under a 40X objective and a 10X eyepiece.

2.3.3 Characterization of biofertilizer. Initial and final of organic matter (OM) and carbon content was analyzed twice after 14 days using the method described in [30]. A dried crucible was weighed prior to the addition of 10 g of the sample. The sample was then oven-dried at 100 °C for 24 hr. Next, the crucible was placed in a desiccator and re-weighed. The sample was then ignited by inserting into a 500 °C furnace for 12 hr. The remaining sample, known as ash, was weighed. The organic matter and carbon content were calculated by using Equation (1) and Equation (2), respectively.

$$organic\ matter\ (\%) = \frac{W_d - W_a}{W_d}\ X\ 100 \quad (1)$$

$$\%\ carbon = \frac{\%\ organic\ matter}{1.8} \quad (2)$$

where, W_d = dry weight of compost, W_a = weight of ash after combustion.

Initial viscosity and final viscosity (after 14 days) of the biofertilizer samples were measured by using a viscometer. Meanwhile, initial pH and final pH (after 14 days) of biofertilizer samples were recorded using a pH meter.

2.3.4 Biofertilizer plant test. Samples of biofertilizer were varied to three ratios of volume of *Lactobacillus* to the volume of glycerin pitch; 1:1, 1:2 and 2:1. Three samples of cucumber seeds were used for this experiment. A control was planted in a natural soil while the rest were planted in a natural soil amended with the samples of the biofertilizer. Firstly, a cucumber container was basked under the sunlight for 8 hr, where the organic soil was then added into the container. Next, the cucumber seeds were immersed into a distilled water for 4 hr to ensure that all seeds' shells were smoothened. The

cucumber seeds were planted 2.5 cm deep into the soil. Further, 2 mL of each biofertilizer sample was mixed with water and subsequently used to flush the cucumber seeds. An optimum ratio of the volume of *Lactobacillus* to the volume of glycerin pitch was determined by the height of the the cucumber plant growth. The commercial biofertilizer (AB powder fertilizer) was also applied on the plant for comparison.

3. Results and discussion

3.1 Characterization of Glycerin pitch, biofertilizer, and soil

Characterization of glycerin pitch was conducted to determine the characteristics of the glycerin pitch in the production of biofertilizer. Biofertilizer quality can be examined through the microbial population. However, the major contributor to the growth of the cucumber plant was attributed to the content of macronutrients, such as N, P, and K. The cucumber plant depends on the nutrients to promote the growth, in addition to the glycerol content inside the glycerin pitch, which functions as a major substrate to the *Lactobacillus*, thus, boosting the plant growth. A high density of microbial population encourages a long shelf-life of the biofertilizer, which, therefore, allows the biofertilizer to possess a longer optimum effect, prior to the production time, on the growth of the plant. Based on the analysis, the micronutrients and macronutrients in the glycerin pitch are quite low with NPK about 0.12-0.15-0.91, as shown in table 2 and table 3, respectively.

Table 2. Glycerin pitch micronutrients.

Micronutrient	Content
Magnesium (mg/L)	5.590
Calcium (mg/L)	1.500
Iron (mg/L)	0.230
Manganese (mg/L)	0.012
Boron (mg/L)	>0.200
Molybdenum (mg/L)	0.039
Sodium (mg/L)	2.400

Table 3. Glycerin pitch macronutrients.

Macronutrient	Waste	
	Glycerin Pitch	Food Waste
Nitrogen (%w/w)	0.120	3.56 ±5.65
Phosphorus (%w/w)	0.148	1.12 ± 5.53
Potassium (%w/w)	0.910	2.03 ± 12.87
Reference	This work	[22]

Based on table 2 and 3, contents of macronutrients and micronutrients in glycerin pitch are quite low. Low content of macronutrients has a low yield in promoting plant growth. Therefore, glycerin pitch should not be amended to the soil directly without pretreatments or combination with inoculants. A study conducted by Qian *et al* has shown that the direct amendment of glycerol to the soil is not entirely feasible, as an addition in low rate resulted in a low uptake of the N, P, and K by the canola [24]. The study recorded an uptake rate was 3.01 mg kg^{-1} of N, 1.15 mg kg^{-1} of P and 7.01 mg kg^{-1} of K, with the rate of glycerol added at 100 kg ha^{-1}. When the rate of glycerol is above 100 kg ha^{-1}, the uptake was low attributed to the changing of C:N ratio, leading to microbial immobilization. In general, a direct usage of glycerin pitch does not relatively promote plant growth, instead, the glycerin pitch solution should be combined with other carrier materials that could help in promoting the plant

growth. Micronutrient analysis detects certain metals that can affect the soil ecosystem, which is essentially the micronutrients that indirectly help the growth of plants. Micronutrient shortage can limit plant growth. However, the high content of NPK can adversely affect the plant, related to microbial immobilization in the soil.

Table 3 also shows the NPK contents of waste from industry and food waste. These wastes that have been tabulated has a potential to be converted to the fertilizer. Table 3 clearly shows that it is much different in NPK contents of industrial waste and food waste. NPK in glycerin pitch was low because most of NPK is being filtered and purified in producing glycerol. Conversely, in the food waste most of the NPK is being preserved and if loss, it may due to the degradation process. The pulp is the by-product from the fermentation of food waste in producing liquid fertilizer. This pulp has NPK that in range of NPK from glycerin pitch. Liquid fertilizer from glycerin pitch and food waste has its own impact in reducing the environmental problem. Moreover, segregation of food waste can decrease the greenhouse gas emission at the landfill [23]. This conversion to fertilizer relatively can increase the practice of segregation at the landfill. Glycerin pitch as liquid fertilizer has a potential to decrease the cost of disposing of the glycerin pitch and at the same time decreasing the emission of carbon caused by the burning of glycerin pitch for disposing of. In comparison to other liquid fertilizer, there is also liquid fertilizer that has high NPK such as from Water Hyacinth, pig weed and Russian Comfrey as shown in table 4. High NPK may be contributed by the nutrient contained in these wastes and fewer nutrients loss after decomposition by the microbes [34].

Table 4. Macronutrients of other organic liquid manure.

Macronutrient	Waste			
	Water Hyacinth	Pig Weed	Russian Comfrey	Pulp
Nitrogen (%w/w)	3.72±0.33	1.54±0.37	2.90±0.10	0.390
Phosphorus (%w/w)	2.86±0.41	2.98±0.24	2.94±0.05	0.159
Potassium (%w/w)	2.89±0.02	2.01±0.40	3.90±0.06	0.510
Reference	[33]	[33]	[33]	[21]

The result from ICPMS analysis on heavy metal content in glycerol residue or glycerin pitch is tabulated in table 5. These four heavy metals (arsenic, cadmium, lead and mercury) are considered as hazardous to the soil and disturb the growth of plants. Lead and mercury detected in glycerin pitch was about 11.57 ppb (0.012 mg/kg) and 160 ppb (0.16 mg/kg), respectively. The tolerable limit of heavy metal in soil was about 2 mg/kg for lead and 50 mg/kg for mercury [25]. The detected heavy metals complied with the limit of heavy metal in soil but the frequent amendments of glycerin pitch could lead to the accumulation of heavy metals in soil.

Table 5. Heavy metal in glycerin pitch.

Metal	Content
Arsenic (ppb)	ND
Cadmium (ppb)	ND
Lead (ppb)	11.57
Mercury (ppb)	160.00

ND: Not detected

Nutrients available in the soil, in terms of N, P and K were relatively low, thus, justifying the need to add fertilizer to the soil. The analysis of the soil showed 0.5 %w/w of N, 2.0 mg/L of P and 6.1 mg/L of K. The analysis showed a lack of soil nutrients for enhancing the plant growth. Thus, the addition of fertilizer was essential to promote increment in plant growth. High phosphorus content can give a positive impact to the plant as it can enhance the plant growth. Phosphate with $H_2PO_4^-$ and

HPO_4^{2-} always exists when the pH is acidic which is less than pH 7. $H_2PO_4^-$ has higher likely to be uptake by the plant compared to HPO_4^{2-}. It can be associated with the complexity of the molecule to be uptake. If compare to the other liquid fertilizer plant height, direct application of glycerol as fertilizer on the plant had a positive impact on the growth on three different species (‘Chantenay ‘carrot and corn) [35]. Carrot seedlings had a positive impact on its growth after applying the 50 mM of glycerol by spraying method and it enhance up to 105.6%, 158.4% and 53.8% on its fresh weight, dry weight, and taproot diameter, respectively. Meanwhile, on corn seedlings it enhances up to 83.5%, 154.6% and 90.9% of fresh weights, dry weights and shoot length, respectively. This study has increased up to 37.5% of cucumber plants height compared to the commercial fertilizer (AB fertilizer) and showed a positive impact on the plant’s growth.

3.2 Preparation and formulation of biofertilizer

Inoculation of *Lactobacillus* was carried out through the fermentation of rice water with milk. Initially, the color of the rice water was less cloudy. After it was stored in an enclosed cabinet for seven days, the color turned cloudier with a suspended layer formed on top of the liquid. Upon the addition of milk into the fermented rice water, the mixture turned milky white. At the end of the fermentation, a thick curd layer was formed at the top of the mixture. The curd layer was then removed while the serum which contained *Lactobacillus* was used in the preparation of biofertilizer samples.

Upon mixing glycerin pitch with *Lactobacillu*s in varying ratios; 1:1, 2:1 and 1:2, the samples were left to ferment for 14 days prior to use. Observation of physical changes and measurement were performed on the carbon content, viscosity, and pH. Referring to figure 2, after 14 days, the color of the inoculant changed from dark brown into faded brown. The color change was assumed to be influenced by the gradual decrease in the amount of carbon in the glycerin pitch and reaction of microorganisms through the release of carbon dioxide.

Figure 2. *Bacillus* inoculation after 14 days of fermentation of ratio 2:1 glycerin pitch and *Lactobacillu*s inoculant.

To verify the presence of *Lactobacillus* in inoculant, the formation of *Lactobacillus* was observed under a microscope as shown in Figure 3. The formation of *Lactobacillus* was clearly showed the presence of rod-shaped bacteria that are arranged in chain-like structures. In a study of microbial inoculant production for plant enhancement, Fadhl (2010) has also described *Lactobacillus* as a straight rod-shaped bacteria, measuring approximately 0.5-2.5 x 1.2-10μm and are arranged in a chain with either; rounded tip, or square tip, or in pairs [26].

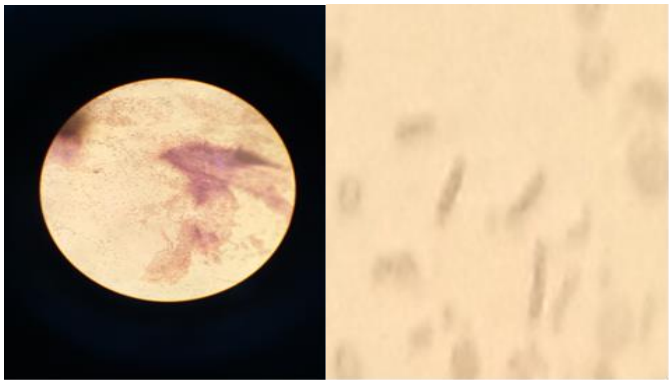

Figure 3. Formation of *Lactobacillus* under microscope after 14 days of fermentation of ratio 2:1 glycerin pitch and *Lactobacillus* inoculant. The microscope image was viewed with magnification of 40X objective and a 10X eyepiece.

The increment in turbidity of the samples was determined based on the increase in the colony forming unit (CFU) and the increase in the dry mass concentration. *Lactobacillus* size is determined in the order of the wavelengths of visible light. *Lactobacillus* scattered light quite well with turbidity in the present study. Light scattering not only refers to the increase in the number of particles, but also refers to bacteria size and form.

Figure 4 shows the growth curve of *Lactobacillus* in relation to time. The curve indicates the presence of two phases, consisting of log phase and stationary phase. During the first 90 min of log phase, *Lactobacillus* exhibited the high metabolism and doubling time. Subsequently, the growth of *Lactobacillus* reached a stationary phase at 120 min to 210 min. This showed that the glycerine pitch used as a fermentation substrate or carbon source has the capability to support the growth of *Lactobacillus*. Meanwhile, figure 5 shows a scattered graph of the log phase growth of *Lactobacillus*. Log phase of the growth is the best way to determine the growth rate of the *Lactobacillus*. According to R^2 of the growth, the log phase showed a relatively high consistency of growth in the presence of glycerine pitch and was able to provide a considerable growth for the *Lactobacillus*.

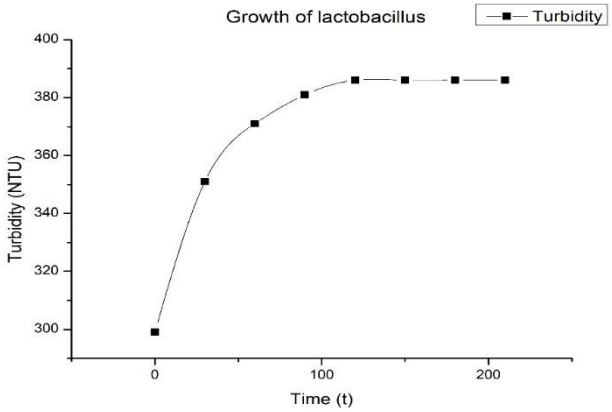

Figure 4. Growth curve of *Lactobacillus* using turbidimeter after 14 days of fermentation of ratio 2:1 glycerin pitch and *Lactobacillus* inoculant.

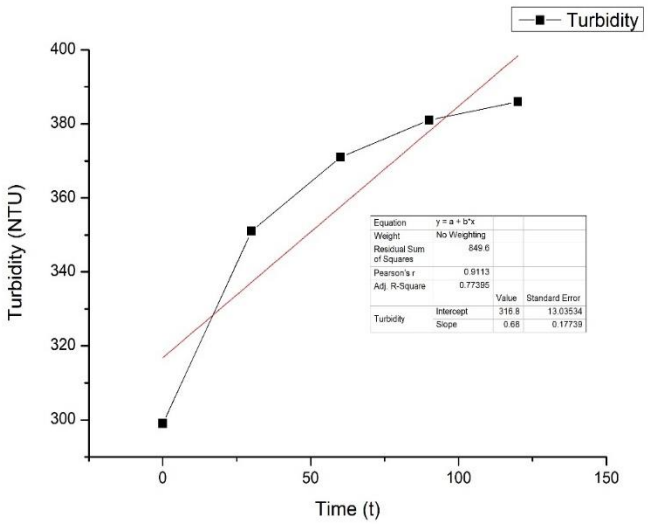

Figure 5. Growth curve of *Lactobacillus* using turbidimeter after 14 days of fermentation of ratio 2:1 glycerin pitch and *Lactobacillu*s inoculant.

From measured pH of the samples, initial pH of glycerin pitch was 11.18, while pH readings of samples that had been added with glycerin according to varying ratios and left to ferment for 14 days, showed the decrement. After 14 days, the obtained pH of samples with ratios 1:1, 2:1, and 1:2, were 7.6, 5.11, and 5.45, respectively. The reduction of pH was associated with the release of the acidic substance. The possible acidic substance was attributed to the secretion of the enzyme from microbes such as phosphatase or the products from the metabolism of *Lactobacillus* such as acetic acid, gluconic acid, citrate and oxalate [36]. Good liquid biofertilizer possesses pH readings between pH 8.20-8.50 [27]. The slight alkalinity in the pH of biofertilizer is beneficial as it can contribute towards the neutralization of acid in soil [28]. Having acidic biofertilizer is not indicative of offering good benefits to plants. Lim and Matu (2015) has revealed that the acidic biofertilizer (pH 4.08) produced with citrus orange can inhibit the growth of plants, while biofertilizer with pH 4.42 and 4.52 produced from pineapple, papaya, and banana promoted the growth of plants [29]. Reduction of pH in this study also substantiated that fumaric acid, lactate and acetate were formed by the *Lactobacillus*. *Lactobacillus* releases organic lactate acid which exhibited a nutrient-solubilizing characteristic, which helped in dissolving hard-to-dissolve nutrients and make them available for plant [26].

Initial and final viscosity of the samples were measured two times after 14 days. Initially, the viscosity of the glycerin pitch reading was 2.35 MPS. An increment in the viscosity was observed after being added with *Lactobacillus* and stored for 14 days. The average final viscosity obtained was 3.75±0.03 MPS. The increment was assumed to be influenced by an increase in microbial density in relation to time. The increment in the viscosity of liquid biofertilizer by Ngampimol and Kunathiga (2018) was concluded to be related to swelling effect [11]. The swelling effect occurs as microbial activity incites the production of carbon dioxide during storage.

In the analysis of carbon content, it was found that the initial carbon content in 10 g of glycerin pitch was 53.8%. After 14 days, the carbon content in the sample of ratios, 1:1, 2:1, 1:2 were 50.75%, 46.62% and 48.16%, respectively. The percent reduction in carbon source in varying sample ratios of 1:1, 2:1, 1:2 were 3.05%, 7.18%, and 5.64%, respectively. The reduction of carbon source was due to the addition of *Lactobacillus*. The *Lactobacillus* made the glycerin pitch converted to the simple form and beneficial to the plant. It was also associated with the metabolism of microbes to consume the carbon as an energy source in the samples after 14 days period. The result verified that probiotic *Lactobacillus* can utilize the glycerol in the glycerin pitch as a sole carbon source as there was decline number of carbon source analyzed after 2 weeks. Another study by Aziati and Sakinah (2017) found

that 30 g/L of the substrate provided the best concentration for producing succinic acid [6]. While Khanna, et al. (2013) reported that 10 g/L of substrate was the ideal concentration in the production of propanediol [31]. In order to determine the exact amount of glycerin pitch (glycerol concentration), the optimization must carried out to establish the exact ideal concentration.

In this study, the initial C:N ratio was 44.33. After 14 days, the C:N ratio slightly decreased to 42.29 and 40.13 for ratio 1:1 and 1:2, respectively. The initial value indicated that the carbon was in excess and gradually decreased over time. The decrement was attributed to the utilization of glycerol in glycerin pitch. When the *Lactobacillus* was mixed with the glycerin pitch, the *Lactobacillus* consumed the carbon and subsequently decomposed the carbon source to produce the nutrients for soil. The carbon essentially assisted the cucumber plant in terms of plant nutrient uptake. Sample C: N ratio of 2:1 exhibited the highest reduction of 38.85.

3.3 Application of biofertilizer to cucumber plant

There were three ratios of biofertilizer samples that were prepared. The volume of *Lactobacillus* was mixed with glycerin pitch based on varied ratios of 1:1, 2:1, and 1:2. Table 6 shows the results of application of biofertilizer and control (without fertilizer) towards the growth of cucumber plant by using the soil drenching method. The ratio of 1:1 biofertilizer obtained the least growth in plant height after 18 days. The result was supported by the fact that the ratio 1:1 has a high C/N ratio, compared to the other biofertilizer formulations. As a result, the biofertilizer with 1:1 ratio inhibited the plant growth. Meanwhile, the biofertilizer with 2:1 ratio recorded the highest growth in the height of cucumber plant as it has a low C/N ratio compared to other biofertilizer formulations. The C/N ratio ranging from 10 to 30 is considered as ideal but if the C/N ratio is above than 30, it can contribute to immobilization of N in soil [32]. A high growth rate of plant using ratio 2:1 is owing to several other factors as well, such as phosphorus that has been solubilized in the soil and the contribution of micronutrients in the biofertilizer that promoted the growth. This resulted in an almost 2-fold increase of height in the growth of plant as compared to the commercial fertilizer.

Table 6. Cucumber growth.

Sample	Length (cm)						
	Days						
	4	6	8	10	14	16	18
A(ratio 1:1)	7.5	8	8.5	9	11	13	13.5
B(ratio 2:1)	10	12	15	19	25	30	40
C(ratio 1:2)	8	10	11	11.5	13	15	16
D(control)	6	7	8	8.5	9.5	9.7	10
Commercial fertilizer	8	9.5	12	13	16.5	20	25

4. Conclusion

In this study, *Lactobacillus* inoculant and glycerin pitch were successcully used as a medium for the production of liquid biofertilizer. The production of biofertilizer were optimized by the *Lactobacillus* inoculant and glycerin pitch which were varied by volume ratio of 1:1, 1:2, 2:1. The result showed that the glycerin pitch is able to act as medium and carbon source for stimulating the growth of *Lactobacillus*. Furthermore, the combination of the glycerin pitch and *Lactobacillus* as biofertilizer with ratio 2:1 (volume of *Lactobacillus*: glycerin pitch) gave an almost 2-fold increase of height in the growth of plant as compared to the commercial fertilizer. The biofertilizer produced ideal nutrients that enhanced and promoted the growth of cucumber plant.

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