Development of immobilized matrix from durian rind waste in cultivation of microalgae for biofertilizer production

A A Roshidi\textsuperscript{1}, S F Mohamad-Fuzi\textsuperscript{1*}, H M Matias-Peralta\textsuperscript{2}, N L Zaidan\textsuperscript{1}, I M Hailan\textsuperscript{1}, F Kormin\textsuperscript{1}, M F Abu-Bakar\textsuperscript{1}, S F Sabran\textsuperscript{1}

\textsuperscript{1}Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Pagoh Higher Educational Hub, 84600 Pagoh, Johor, Malaysia.

\textsuperscript{2}College of Fisheries-Freshwater Aquaculture Center, Central Luzon State University, 3120 Science City of Munoz, Nueva Ecija, Philippines.

\* Corresponding author: fatimahz@uthm.edu.my

\textbf{Abstract}. Conventional fertilizers have a negative impact on environment and are not economically efficient. This is because the fertilizers are lost by volatilization in the atmosphere, leaching into the groundwater or fixation in the soil. The aim of this project is to produce a new generation of biofertilizers, using an eco-friendly coating based on biomass and biopolymers derived from pectin. In this study, pectin from durian rind was obtained after a treatment with an acidified solution followed by purification through alcohol precipitation. The durian pectin was found to be 3.22 $\pm$ 0.065\% methoxyl content which is classified as a low methoxyl pectin. Therefore, it is suitable to be used as a biopolymeric matrix to produce a slow-release soil fertilizer added with microalgal (\textit{Scenedesmus} sp.) biomass residue. The incorporation of sodium alginate as a gelling mixture was found to enhance the formation of rigid bead structure. The morphology of encapsulated \textit{Scenedesmus}-beads were then examined using scanning electron microscopy and the growth of \textit{Abelmoschus esculentus} (Okra plant) was observed for its potential use as biofertilizer. The results obtained for the morphology of the beads shows that the beads have a porous structure. In just two weeks, the significant growth of plant treated with biofertilizer was shown by having a much greater height (7.17 $\pm$ 1.04 cm), number (4 leaves) and size of leaves (3.67 $\pm$ 0.32 cm) compared to the control (5.17 $\pm$ 0.35 cm high, 3 leaves, and 2.67 $\pm$ 0.20 cm in size, respectively). However, further research is needed on the optimal biofertilizer concentration and dosage as well as on the kinetic study of the release compounds from the beads. Overall, this article exhibits a good perspective for alternative fertilizer for agriculture applications and represent an innovative solution for durian rind final disposal.

1. Introduction

Durian is a seasonal fruit which is very well-known in Southeast Asian countries such as Thailand, Indonesia, Philippines and Malaysia [1]. However, for each durian fruit, the pulp, which is edible stands only one third of the entire portion of the fruit while durian seeds and durian rinds are categorized as waste [2]. To overcome this problem, the durian waste can be transformed into something valuable and commercialized such as extracted pectin that can be used in various industries. Pectin have great gelling properties that makes it capable to act as a matrix in immobilization process.
of cell. The immobilization of cells is being used widely due to their ability to provide high biomass, cell reusability, which will reduce the cost for the cell recovery and recycle. The immobilized cells able to provide a suitable micro environmental condition, can be used in improving the genetic stability of the cell, protect the cell from damage, able to withstand high toxicity, pH, temperature, solvents and heavy metals and able to delay maturation time that makes the cell last for a long time [3].

Biofertilizers are living microorganisms, which, when applied through seed or soil treatment, promote growth by increasing the supply or availability of nutrients to the host plant [4]. Microalgae can be employed as biofertilizers and soil conditioners. This is because microalgae have the capabilities of fixing nitrogen and controlling erosion in temperate climate zones. However, like most fertilizers, the applied microalgae can be lost by leaching into the groundwater or fixation in the soil. The main purpose of the current study was to obtain a slow release biofertilizer by incorporating microalgae into a biopolymer derived from biomass (pectin). This study aims to characterize the durian rind pectin and formed immobilize matrix to encapsulate microalgae. The efficiency of the matrix formed as biofertilizer was also investigated in this study through observation of plant growth.

2. Materials and method

2.1. Materials
Durian rinds were obtained from the fruit stall at Muar, Johor, the chemicals (brand Fluka, Honeywell) such as 1 N hydrochloric acid, 95% ethanol, sodium chloride, phenol red indicator, 1 N sodium hydroxide, sodium alginate, calcium chloride and the freeze dried Scenedesmus sp. powder and the seeds of plant vegetable, Abelmoschus esculentus (Ladies finger), were obtained from UTHM (Universiti Tun Hussein Onn, Pagoh, Johor).

2.2. Extraction of pectin
The extraction process began with stirring the dried durian rind powder (900 g) in a mild acid aqueous solution (2 mL of 1 N hydrochloric acid in 900 mL distilled water) and pH was adjusted to 2.5. Then, the solution was incubated for 4 hours at 85°C and the resulting slurries was filtered through cheesecloth and cooled at room temperature. Acidified ethanol was prepared in which 25 mL of 4% hydrochloric acid was then added in 100 mL of 95% ethanol. The filtrate pectin was mixed with acidified ethanol and incubated for 1 hour at room temperature. After that, it was centrifuged in a bench-top centrifuge (Kubota 5100, Fujioka, Japan) for 15 minutes at the speed of 4700 rpm. The supernatant was washed two times by using 95% ethanol and being centrifuged again for 15 minutes at the same speed. Then, the precipitate was collected and dried in a hot-air oven at 55°C until constant weight was achieved [13]. Then, the dried pectin was grounded, placed in sealable plastic bags and then stored in desiccator until next experiment is being conducted.

2.3. Characterization of pectin by titration method
The characterization of pectin started by preparing the neutral solution which 0.5 g of the sample was weighed and 5 mL of ethanol was added in 250 mL of conical flask. Then, 1 g of sodium chloride with 100 mL distilled water was added into the mixed solution. A 6 drops of phenol red were then added in the mixture as the indicator and the mixture was titrated against 0.1 N sodium hydroxide. The titration points of purple or pink color indicated that the neutral solution has formed. After that, 25 mL of 0.25 N sodium hydroxide was added to the neutral solution and the mixture was stirred thoroughly. It was then kept at room temperature for 30 minutes. After 30 minutes, 25 mL of the 0.25 N hydrochloric acid was added in the solution and titrated against 0.1N sodium hydroxide [5]. The methoxyl content of the pectin was calculated by multiplying the mL of alkali used with the Normality of alkali, which will then be divided with the weight of the sample.
2.4. Formation of biofertilizer by immobilizing microalgae with durian rind pectin

The immobilization of the microalgae was done using extracted pectin from durian rinds. Sodium alginate solution was prepared in another beaker by mixing 0.2 g of sodium alginate powder in 10 mL and mixed thoroughly using a magnetic stirrer. Then, the gel beads were prepared in another beaker by dispersing 0.5 g of dry pectin into rehydrated freeze-dried microalgae powder (0.25 g of microalgae powder in 50 mL of distilled water allowed to stand for 2 hours). After 2 hours, the pectin solution containing the microalgae was added into the sodium alginate solution and were mixed for 15 to 20 minutes to form a homogenous solution. The beads were formed by expelling the mixture using syringe from a height of 5 cm into a beaker filled with 6% w/v solution of calcium chloride. The forming beads immersed in the sodium chloride solution were continuously stirred by a magnetic stirrer for 20 minutes to prevent the beads from clumping together at the bottom of the beaker. After that, the sodium chloride solution was removed by filtration and the beads were washed using distilled water. They were then immersed in distilled water and kept at 4\(^{0}\) C before used in the next test [6].

2.5. Morphology analysis of pectin beads

The beads formed were oven dried at 40\(^{0}\) C. The dried sample was then kept inside a desiccator to prevent moisture until further use. The morphology of the dried beads was obtained using scanning electron microscopy (SEM, COXEM EM-30AX, Korea). Prior to scanning, the beads were coated with a thin layer of gold in a vacuum by using Sputter Coater Quorum Q300T D. Then, the images of the dried beads were obtained at an excitation voltage of 20 kV at magnification from 500 to 1000 [7].

2.6. Testing the biofertilizer efficiency

The efficiency of the biofertilizer was tested by monitoring the growth of a plant vegetable, *Abelmoschus esculentus* (ladies’ finger). The two treatments (with and without application of biofertilizer) with three replicates were utilized for this experiment. The plants were provided with the same amount of water, and sunlight throughout the growth experiment. The growth of the plants was evaluated for 18 days. The height of the plant (cm) for both treatments was recorded for 18 days.

3. Results and discussion

3.1. Methoxyl content of durian pectin

In this study, the methoxyl content of pectin obtained from durian rind was determined by titration method. The result obtained from the methoxyl content was presented in Table 1. The result showed that the methoxyl content of pectin extracted from durian rind is 3.22 ± 0.065% which is categorized as low methoxyl pectin. According to Indriani, Legowo & Susanti [8] pectin with methoxyl content ranging from 2.50 % to 7.12 % is classified as low methoxyl pectin whereas those containing more than 7.2 % is classified as high methoxyl content [8].

A study by Lara-Espinoza et al. 2018 stated that, pectin with a high methoxyl content has been used to form a matrix for drug delivery, but due to their high molecular weight and poor solubility in water, it gives affects of the encapsulated drug which lead to drug migration, early release and erosion of the cover [9]. On the other hands, study by Maciel et al. [10] stated that, the used of low methoxyl pectin for encapsulation of bioactive compounds is more preferable due to its low molecular weight and ability to form water-insoluble cross-linked polymer. These characteristics can help in form stable matrix that are able to control the release of bioactive compound efficiently. In this study, the pectin obtained from durian rinds showed potential as an encapsulation matrix for *Scenedesmus* sp. biofertilizer.
Table 1. Methoxyl content of durian pectin.

| Sample | Volume of NaOH used (mL) | Methoxyl content (%) | Average + SD |
|--------|--------------------------|----------------------|--------------|
| 1      | 5.1                      | 3.16                 |              |
| 2      | 5.3                      | 3.29                 | 3.22 + 0.065%|
| 3      | 5.2                      | 3.22                 |              |

3.2. Morphology of pectin beads

The morphology of microparticles that undergo encapsulation can provide a suitable effect on the release behavior of the encapsulated ingredients [11]. In this study, the surface morphology of the dried pectin bead using SEM with x500 and x1000 magnification was observed. Figure 1 shows that the beads formed have porous structure. It was stated by Jaya, Durance & Wang (2010) that the porous structure of encapsulated ingredients is helpful for the release of the bioactive compound [12]. Therefore, the porous structure of the pectin beads formed shown that they have a good potential for the formation of biofertilizer since the pore will help control the release rates of the bioactive compound from microalgae.

Figure 1. The morphology of encapsulated microalgae-pectin bead at magnification of x500 and x 1000.

3.3. Effect of the biofertilizer on plant growth

The effectiveness of the pectin encapsulated *Scenedesmus* sp. was done by periodically observing the growth of the plant vegetable, *Abelmoschus esculentus* (ladies finger) provided with biofertilizer. Results obtained show that there is significant improvement in the plants’ growth rate. In terms of their height, plants provided with biofertilizer grow higher compared to the control (Table 2; Figure 2 and Figure 3).

The results show that both treated and untreated plants started to germinate on day 6 with only small differences in height, which 0.46 cm for plant treated with biofertilizer while 0.37 cm for the control (Table 2). Differences in height of plants started to show on day 12 with 4.90. The maximum height was achieved on day 18, showing 7.17 cm for treated plant and 5.17 cm for the control, respectively (Table 2). The growth pattern shows by both plants indicate that the plant treated with the biofertilizer has a much faster growth rate compare to the control. Khan, Singh, & Jat [13] reported that, the application of biofertilizer on the plant will help in the better growth and production during the later part of the growth period as the nutrients and active compounds were slowly but continuously released throughout the growing period [13]. This is supported by another finding that stated, the growth of plants can be improved when biofertilizer is being incorporated, as they have an ability to fix and replace the nitrogen and phosphorus fertilization by the application on soil [14]. Therefore,
based on the results obtained, it was found that the encapsulation of *Scenedesmus sp.* with pectin from durian rind has a great potential to act as biofertilizer to enhance the growth of plants.

![Figure 2. Okra plant treated with biofertilizer after 18 days.](image1)

![Figure 3. Okra plants without biofertilizer (control) after 18 days.](image2)

**Table 2.** The height of non-biofertilized (control) and biofertilized plants.

| Day | Height of plant without application of biofertilizer (control) (cm) | Height of plant treated with biofertilizer (control) (cm) |
|-----|-------------------------------------------------|-------------------------------------------------|
| 2   | $0 + 0$                                         | $0 + 0$                                         |
| 4   | $0 + 0$                                         | $0 + 0$                                         |
| 6   | $0.37 + 0.321$                                  | $0.46 + 0.416$                                  |
| 8   | $1.60 + 0.530$                                  | $1.73 + 0.503$                                  |
| 10  | $3.37 + 0.231$                                  | $3.50 + 0.764$                                  |
| 12  | $3.87 + 0.231$                                  | $4.90 + 0.656$                                  |
| 14  | $4.23 + 0.208$                                  | $5.43 + 0.603$                                  |
| 16  | $4.67 + 0.231$                                  | $6.27 + 1.102$                                  |
| 18  | $5.17 + 0.351$                                  | $7.17 + 1.041$                                  |
4. Conclusion
This study showed the potential of pectin from durian rind as an encapsulation matrix for the microalgae in the formation of biofertilizer. Characteristics of the beads formed, such as having a porous surface structure will help in the release of bioactive compound in order to fertilize plants. It was also found that the incorporation of sodium alginate to form a gelling mixture helped in the formation of strong and rigid bead structure. Finally, better growth of the plant vegetable treated with biofertilizer than the control was evident in the significant difference in the height of the plants.

References
[1] Siriphanich J 2011 *Postharvest Biology and Technology of Tropical and Subtropical*. Cambridge UK: Woodhead Publishing.
[2] Amid B T and Mirhosseini H 2012 Optimisation of aqueous extraction of gum from durian (*Durio zibethinus*) seed: A potential, low cost source of hydrocolloid *Food Chemistry* **132** 1258-1268
[3] Bayat Z, Hassanshahian M and Cappello S 2015 Immobilization of microbes for bioremediation of crude oil polluted environments: A mini review *Open Microbiol.* **9** 48-54
[4] Moinuddin, Dar T A, Sajad, Hussain, Khan M M, Hashmi N, et al. 2014 Use of n and p biofertilizers together with phosphorus fertilizer improves growth and physiological attributes of chikpea *GJAAS* **2** 168-74
[5] Wai W W, Alkarkhi A E and Easa A M 2009 Optimization of pectin extraction from durian rind (*Durio zibethinus*) using response surface methodology *J. Food Sci.* **74** 637-41
[6] Yadav S R, Khan Z H, Kunjwani S S and Mular S M 2015 Extraction and characterization of pectin from different fruits *IJAR* **1** 91-94
[7] Ramana G and Chaitanya A K 2011 Preparation and in-vitro characterization of ethylcellulose coated pectin alginate microspheres of 5-fluorouracil for colon targeting *J. Appl. Pharm. Sci.* **1** 170-76.
[8] Jaya S, Durance T D and Wang R 2010 Physical characterization of drug loaded microcapsules and controlled in vitro release study *Open Biomaterials* **2** 9-17
[9] Indriani R, Legowo A M and Susanti S 2017 Characteristics of pectin isolated from mango (*mangifera indica*) and watermelon (*citrullus vulgaris*) peel *Int. J. Food Sci. Technol.* **4** 31-34
[10] Lara-Espinoza C, Carvajal-Millan E, Balandran-Quintana R, Lopez-Franco Y and Rascon-Chu A 2018 Pectin and pectin-based composite materials: beyond food texture *Molecules* **23** 942
[11] Maciel V, Yoshida C, Pereira S, Goycoolea F and Franco T 2017 Electrostatic self-assembled chitosan-pectin nano- and microparticles for insulin delivery *Molecules* **22** 1-22
[12] Liu Y 2014 Starch-pectin matrices for encapsulation of ascorbic acid 1-90
[13] Khan I, Singh D and Jat B L 2007 Effects of biofertilizers on plant growth and yield characters of *Pisum sativum L.* *Adv. Res. J. Crop Improv.* **8** 99-108
[14] Iwuagwu M, Chukwuka K S, Uka U N and Amandianeze M C 2013 Effects on biofertilizers on the growth of *Zea Mays L.* *AJMBE*, **15** 235-40