INTEGRATION OF SODIUM ALGINATE WITH *BACILLUS SUBTILIS* AS MICROBIAL COMPOSITE FOR FERTILIZER COATING TOWARDS GREEN AGRICULTURE

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
$CaCl_2$	Calcium Chloride
DSC	Differential Scanning Calorimetry
TGA	Thermogravimetric Analysis
SEM	Scanning Electron Microscopy
EDX	Energy-Dispersive X-Ray Spectroscopy
FTIR	Fourier-Transform Infrared Spectrometry
AAS	Atomic Absorption Spectroscopy

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INTEGRASI NATRIUM ALGINAT DENGAN *BACILLUS SUBTILIS* SEBAGAI KOMPOSIT MIKROB UNTUK SALUTAN BAJA KE ARAH PERTANIAN HIJAU

ABSTRAK

Salutan adalah bahan pelindung yang disalutkan pada permukaan sesuatu objek seperti baja untuk pelbagai tujuan. Dalam penyelidikan ini, filem komposit mikrob telah dibuat dengan menyepadukan natrium alginat dengan *Bacillus subtilis* pada jisim yang berbeza. Filem telah diuji pada sifat fizikal, mekanikal, kimia dan mikrobnya. Kemudian, kajian telah dijalankan untuk meningkatkan kekonduksian, kandungan lembapan dan penambahan mikronutrien filem komposit mikrob untuk tumbuhan sebagai nutrien tambahan. Selepas itu, baja kambing organik sebagai baja disalut dengan lapisan salutan filem komposit mikrob yang berbeza melalui teknik pengeringan yang berbeza. Seterusnya, analisis pertumbuhan tumbuhan dan analisis nutrien tanah dijalankan dengan menyalutkan baja tanpa salutan, salutan natrium alginat, dan salutan komposit mikrob (salutan 1 lapisan dan salutan 2 lapisan) kepada tumbuhan untuk melihat kesannya terhadap pemindahan nutrien. Tambahan pula, pemodelan matematik telah dibangunkan untuk analisis nutrien tanah. Didapati bahawa 0.5 g jisim sel bakteria dari fasa log mempunyai kesan yang paling ketara terhadap sifat filem. Kemudian, dengan mencampurkan larutan komposit mikrob dengan ion kuprum 40 ppm dan 1% gliserol memberikan kekonduksian tertinggi kepada filem yang membantu dalam pertumbuhan tumbuhan. Juga didapati bahawa kedua-dua teknik pengeringan selama 30 minit dan pengeringan 24 jam tidak memberikan perbezaan yang ketara pada ketebalan filem dan

oleh itu, teknik pengeringan selama 30 minit dipilih kerana ia menjimatkan masa dan kos dalam proses tersebut. Seterusnya, bayam air dengan salutan komposit mikrob 2 lapisan mempunyai hasil terbaik terhadap pertumbuhan tumbuhan dan *Bacillus subtilis* terdapat di dalam tanah selepas disiram. Akhir sekali, model matematik bukan linear telah berjaya disepadukan, disahkan dan digunakan untuk mewakili proses pembebasan nutrien. Kebaharuan penyelidikan ini memberi penekanan kepada salutan baja organik yang dihasilkan bukan sahaja mengawal kadar pelepasan baja, malah ia turut digabungkan dengan mikrob berfaedah menguatkan filem dan mampu bertindak sebagai agen kawalan bio dan mikronutrien kepada tumbuhan.

INTEGRATION OF SODIUM ALGINATE WITH *BACILLUS SUBTILIS* AS MICROBIAL COMPOSITE FOR FERTILIZER COATING TOWARDS GREEN AGRICULTURE

ABSTRACT

Coating is a protective material that is applied to an object's surface such as fertilizer for various purposes. In this research, microbial composite film was fabricated by integrating sodium alginate with different mass of *Bacillus subtilis.* The films were tested on its physical, mechanical, chemical, and microbial properties. Then, study was conducted to improve the conductivity, moisture content and added micronutrient of microbial composite films for plants as supplement. Subsequently, organic goat manures as fertilizers were coated with different layers of microbial composite films coating via different drying techniques. Next, plant growth analysis and soil nutrients analysis were conducted by applying fertilizers of no coating, sodium alginate coating, and microbial composite coating (1-layer coating and 2-layer coating) to the plants to see its effect on the nutrients transfer. Furthermore, mathematical modelling was developed for soil nutrients analysis. It is found that 0.5 g bacterial cell mass from log phase had the most significant effect on the properties of the films. Then, by mixing microbial composite solution with 40 ppm copper ions and 1% glycerol render the highest conductivity to the films which is helpful in plant growth. It is also found that both 30-minutes drying and 24-hour drying technique did not give significant difference on the thickness of films and hence, 30-minutes drying technique was chosen as it saved time and cost in the process. Next, water spinach with 2-layer microbial composite coating had the best results on plant

growth and *Bacillus subtilis* was present in the soil after watering. Lastly, a non-linear mathematical model was successfully integrated, validated, and was used to represent the process of the nutrients release. The novelty of this research emphasizes on the organic fertilizer coating produced does not only control the rate of fertilizer release, but it is also incorporated with beneficial microbe strengthen the films and able to act as biocontrol agent and micronutrient to the plants.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Materials are the foundation for delivering human growth milestones and enhancing human lives and production standards. The human society will achieve new heights with enhanced production whenever a new epoch-making material is introduced. This reflects people' ability to comprehend and modify nature through the application of socially productive forces, science, and technology. From the Stone Age to the Iron Age, and ultimately to the current day, human society has encountered and used a variety of materials. This advancement rendered all the materials identified as a marker of human civilization's growth (Wang et al., 2011).

Emerging technology is built on the basis of new materials. There are now more severe and accurate material criteria as a result of the rapid development of modern science and technology that focuses on industrial growth, economy, and environmental protection. Material science is currently developing towards the development of materials that are manufactured according to specific qualities. High-performance composite materials were created to replace or strengthen most of the other materials available in the twentieth century for this purpose. The creation and growth of composite materials over the last few decades is one of the most impressive examples of material design ever exist in human history.

The composite material is a multi-phase multi-component system made up of matrix and reinforcing materials and divided into three phases: matrix, reinforcement, and interphase (Jesson & Watts, 2012). 'Materials Comprehensive Dictionary' gave a more specific and extensive description of composite material: 'Composite materials are new materials made up of a variety of materials, such as organic polymers, inorganic nonmetal or metal, and so on.' (Fazeli et al., 2019). Materials design may allow each component's performance to balance and interact with one another, resulting in a new performance dominance with critical differences from mixed generic materials. It does not only preserve the original product materials' basic function, but also produces outputs not represented by the integrated effects of the original components. Based on the notion, it is evident that composite materials are designable, and their efficiency is determined by the relative content of composite materials, component relationships and configurations, and phase type and layout.

In industry, composite materials are typically created by combining two materials. The matrix or binder is one of the ingredients, and reinforcement fillers are another. Although the two materials have quite distinct properties, they can be combined to create a composite with unique properties. Because the matrix and reinforcing components do not dissolve or mix, they may be easily differentiated within the composite. Sodium alginate was used as the matrix material in this study. In the name of good film forming characteristics, sodium alginate has the potential to be exploited as a source of edible or biodegradable films (Deepa et al., 2016). The initial intention of this research is to incorporate sodium alginate films with *Bacillus subtilis* for the development of microbial composite films.

Bacteria are noted for their small size structure, which is typically a few micrometres in length. Bacteria were among the first forms of life and the simplest organisms to evolve on Earth, and they can be found in every corner of the globe. Bacteria are the most common and ubiquitous life forms on Earth, and they play an important role in both productivity and the cycling of elements that are necessary for all other life forms (Al-mohanna, 2017). *Bacillus subtilis* is a Gram-positive rod-shaped bacteria that can be found in soil, as well as in the gastrointestinal tracts of ruminants and humans. It's a benign organism since it's not pathogenic or toxic, and it doesn't have any features that cause disease in humans, animals, or plants. The risk of this bacterium being used in fermentation facilities is extremely minimal. *Bacillus subtilis* has also been shown to have a major impact on the self-healing of concrete cracks (Shahid et al., 2020). As a result, it's being employed in this investigation to see how it affects the formation of microbial films.

Fertilizer is a natural or synthetic substance that contains chemical elements that aid in the growth and productivity of plants. To meet supply and demand, roughly 200 million tonnes of fertilizer are produced each year around the world. Nonetheless, due to inefficiency, a quarter of the fertilizer applied to soils is lost to the environment. Slowing the release of nutrients from fertilizer can help to prevent inefficiency (Beig et al., 2020). There are many different types of fertilizers on the market currently, but the two most common varieties are organic and inorganic fertilizers. Composted organic materials and animal dung are examples of organic fertilizers that contain animal- or plant-based components that are either a by-product or end product of naturally occurring processes (Wei et al., 2019). While inorganic fertilizers are chemical fertilizers that contain nutrient elements for the growth of crops made by chemical means. However, utilizing inorganic fertilizers had the effect of contaminating ground water and lowering soil productivity, both of which had a long-term impact on crop output (Anisuzzaman et al., 2021). Therefore, organic fertilizers are preferred to be used nowadays. Nevertheless, organic fertilizers that are used without proper plans or management could lead to severe environmental issues as well such as eutrophication that is polluting our lakes and rivers tremendously nowadays. Ergo, the technology of coating is used to coat the organic fertilizer to slow down its nutrients release rate so that the nutrients are released ideally at the rate that the plants use it. This could significantly reduce the occurrence of environmental issues.

A coating is a layer of protective material that is applied to an object's surface, often known as the substrate. The coating can be applied for aesthetic, functional, or both purposes. To manage fertilizer solubility in soil, a variety of coatings have been put to the particles. Controlling the rate of nutrient release has a number of advantages in terms of the environment, economy, and yield. Coated fertilizers provide a continuous supply of nutrients, which renders longer nutrient release and hence, lower the cost involved. This could provide the plants with more uniform plant nutrition, greater development, and increased performance in its growth. To date, a wide range of materials have been used as coatings on fertilizers such as elemental sulphur, resin-based polymers, and polyethylene polymers (Liu et al., 2014). Sodium alginate, as mentioned earlier, an environmentally friendly and biodegradable material, is an excellent substance to be used as coating for the fertilizer.

This research focused on the utilization of *Bacillus subtilis* as the reinforcing material to fabricate microbial composite films as coating for fertilizer. Firstly, investigation was carried out to study the optimum conditions for the integration of sodium alginate with *Bacillus subtilis* in reinforcing the microbial composite films as fertilizer coating. Analyses performed included physical, mechanical, chemical, and microbial properties. Next, experiments were carried out to study the conductivity, moisture content and added micronutrient of microbial composite films for plants as supplements. Then, the coating of fertilizer with sodium alginate coating and microbial composite films coating via different drying techniques was studied. Subsequently, the plant growth analysis and soil nutrients analysis of plants by applying uncoated and coated fertilizers to the plants was evaluated. Lastly, mathematical simulation was developed for soil nutrients analysis on the rate of fertilizer and micronutrient release to soil and plant.

1.2 Problem Statements

At the present time, most of the composites used for engineering application of composite materials are inorganic. This is because organic composite materials are relatively less in strength and not durable. These inorganic composites are not biodegradable and hardly decomposed even after a long period of time (Dufresne & Castaño, 2017). The leftover non-biodegradable inorganic composites will cause adverse environmental impacts when the composite materials are disposed into the environment. Also, the production of these inorganic composites is highly dependent upon various kinds of chemical methods and processes. These methods not only require relatively high production costs but at the same time, due to the materials, chemicals and scientific approaches used, these methods also cause environmental pollution, safety, and health problems (Manisalidis et al., 2020).

Composites are normally made from a polymer matrix that is reinforced with an engineered, man-made, natural, or other reinforcing material. The size of the reinforcing materials plays a crucial role in producing a high-quality composite film. In most cases, nanoscale reinforcing materials are preferable, but nanocomposites will in fact face processing difficulties and have not proved valuable, at least in an industrial context (Lau et al., 2009). Thus, bacteria of sub-micron size could be an alternative option for reinforcing composite films. Besides, environmental issues have been a continuous problem since decades ago. These environmental issues included climate change, waste problem, and pollutions especially water pollution. One of the biggest reasons that caused water pollution is fertilizer runoff from agroindustry (FAO & IWMI, 2017).

Furthermore, conventional slow-release fertilizer coating such as coating made from sulphur could cause other environmental problems as well. It accounted to both air and water pollution (L. Zhang et al., 2022). Sulphur coated fertilizers are also costly and the coating cracks easily due to its friability (Ibrahim et al., 2020). In addition, most of the conventional coated fertilizer focused only on fertilizer release rate. On that account, sodium alginate which is easily biodegradable and environmentally friendly plays an important role in this case. Moreover, with added beneficial bacterium that acts as both reinforcing material and biocontrol agent, the microbial sodium alginate coating could be the perfect alternative to the current conventional fertilizer coating.

1.3 Objectives

1) To investigate the optimum conditions such as physical, mechanical, chemical and microbial properties for the integration of sodium alginate with *Bacillus subtilis* in reinforcing the microbial composite films as fertilizer coating.

2) To investigate the interaction and amount of the conductivity, moisture content and added micronutrient (Cu^{2+}) of microbial composite films for plants as supplements.

3) To coat the goat manure with microbial composite films via different drying techniques.

4) To evaluate the plant growth analysis and soil nutrients analysis of plants by applying uncoated and coated goat manure to the plants.

5) To develop mathematical simulation for soil nutrients analysis on the rate of nutrients and micronutrient release from goat manure fertilizers to soil and plant.

1.4 Scope of Study

The scope of this study will be mainly focusing on the utilization of *Bacillus subtilis* as the reinforcing material to fabricate microbial composite films as coating for fertilizer. Coated fertilizers can reduce the requirement for several fertilizer applications, resulting in lower labour and application costs. It also delayed nutrient release, which could lead to more consistent plant nutrition, faster development, and improved plant performance. Other than regulating the rate of fertilizer release, the fertilizer coating developed in this study also contains a helpful microbe that strengthens the qualities of films while simultaneously acting as a biocontrol agent for the plants, with added micronutrient that aids in plant growth. Therefore, investigation was carried out to study the optimum conditions for the integration of sodium alginate with *Bacillus subtilis* in reinforcing the microbial composite films as fertilizer coating. Then, study was conducted on the conductivity, moisture content and added micronutrient of microbial composite films for plants as supplements. Next, the coating of fertilizer with sodium alginate coating and microbial composite films coating via different drying techniques was studied. Subsequently, the plant growth analysis and soil nutrients analysis of plants by applying uncoated and coated fertilizers to the plants was evaluated. Lastly, mathematical simulation was developed for soil nutrients analysis on the rate of fertilizer and micronutrient release to soil and plant.

1.5 Thesis Outline

This thesis comprises of five chapters. In Chapter 1 Introduction, it discussed about the background for the research was outlined together with the problem statements and objectives of this research project. In Chapter 2 Literature Review, the content of this research was discussed in detailed that comprised of full information about composite materials, bacteria, *Bacillus subtilis,* coating, fertilizer as well as the analyses involved in this study. In Chapter 3 Material and Methods, the overall experimental design was outlined. Also, all the methods and materials involved in this research were described in detailed. In Chapter 4 Results and Discussion, all results and discussion about the effect *Bacillus subtilis* cell mass on the development of microbial composite films were discussed, in terms of their physical, mechanical, chemical, and microbial properties. Then, the added micronutrient to the plants, the techniques involved in the fertilizer coating process, plant growth analysis and soil nutrients analysis of plants were also discussed. Lastly, the mathematical modelling on the rate of fertilizer and micronutrient release to soil and plant was also discussed. In Chapter 5 Conclusion and Recommendations, the findings from the research project were concluded and the recommendations for further research regarding this study were stated.

1.6 Novelty of Research

Coated fertilizers can be found in a wide range of agricultural and horticulture applications. They provide a continuous supply of nutrients, which may have several advantages, including reduced leaching and gaseous losses. Coated fertilizers can minimize the need for multiple fertilizer applications and hence, labour, and application expenses could be decreased. It also prolonged the nutrient release that may result in more consistent plant nutrition, accelerated growth, and better plant performance. Also, the duration of nutrient release with the periods of plant growth could also be synchronized that will help greatly in crops yield. However, the fertilizer coating produced in this research does not only control the rate of fertilizer release, but it is also incorporated with beneficial microbe that is able to strengthen the properties of the films and at the same time, act as biocontrol agent to the plants. More than that, it also supplies micronutrient to the plant that helps in its growth.

CHAPTER 2

LITERATURE REVIEW

2.1 Composite Material

A composite material is a multi-phase combination material created by combining two or more component materials with different shapes and qualities (Jesson & Watts, 2012). In addition to preserving the original component's basic traits, the materials combine to create a new character that none of the original components had. The individual materials in the composite are easy to detect since they do not dissolve or mix together.

Matrix phase, reinforcement phase, and interphase are the three basic physical phases of composite materials (Wang et al., 2011)). The type, composition, configuration, structure, interaction of these phases, and relative content are the keys to determining composite materials' performance and quality. Composite materials' matrix is made up of polymer matrix composites made up of diverse matrix components, metal matrix, and non-metallic inorganic matrix. Fibrous materials such as organic fibre, glass fibre, and others are commonly used as reinforcing materials (Buckner et al., 2016). The fibrous materials played the role in the composite material as the main load-bearing component, since the fibre modulus and fibre strength were far higher than the matrix content. However, Romanenko et al. (2012) stated that the properties of the matrix material will determine the characteristics of the composite materials. Any matrix materials with strong adhesion properties had to bind reinforcing fibre tightly together.

2.1.1 Classification of Composite Materials

There are many different classifications of composite materials. The classification was based on the type of matrix material, the dispersed phase morphology, and the type of reinforcing fibres used (Wang et al., 2011).

Polymer matrix composites, metal matrix composites, and inorganic non-metallic matrix composites are the types of composite materials classified according to the kind of matrix material, according to Rajak et al., 2019. The six types of composite materials classifications based on the form of dispersed phase are continuous fiber-reinforced composite materials, braid, fibrous fabric reinforced composite materials, sheet reinforced composite materials, whisker or short fibre reinforced composite materials, particle reinforced composite materials, and nano-scale particle reinforced composite materials. Carbon fibre, glass fibre, organic fibre, boron fibre, hybrid fibre, and other types of reinforcing fibres were used to classify composite materials in some circumstances (Buckner et al., 2016).

However, Wang et al. (2011) explained that composite materials can also be classified based on some different criteria (Wang et al., 2011). These included optical functional composite materials, thermal functional composite materials, electrical functional materials, and other materials were available, depending on the purpose and function. It could be classed as particle-strengthened composite materials, fibre-enhanced composite materials, or composite materials strengthened by diffusion based on the reinforcing principle. There are also laminated composite materials, winding structural composites, textile structural composite materials, and so on, depending on the preparation procedure. Furthermore, structural and functional composite materials were categorised according to their intended use.

2.1.2 Characteristics of Composite Materials

The characteristics of composite materials can be distinctive in many ways, however, they should have following characteristics generally. Firstly, the products can be microscopically non-homogeneous, with a distinct interface. Secondly, in terms of initial performance, the component materials are different, but it can lead to enhanced performance for composite materials produced (Wang et al., 2011). Other than this, there are also some common characteristics shared by different types of composite materials.

First, the characteristic of the high specific modulus and specific strength. The specific modulus is the modulus-density ratio while the specific strength is the load-density ratio. For both the modulus and the intensity the proportions or units are length. These characteristics are measures of the calculation of the rigidity and the bearing capacity of the material under the assumption of equal weight. Secondly, composite materials require a good resistance to fatigue and a high tolerance for damage. George et al. showed that the matrix-fiber interface can prevent crack propagation. Compared to the damage of traditional materials that instantaneously occurred due to unstable crack propagation, composite materials will develop a series of damage such as matrix cleavage, interfacial debonding and fibre breakage or splitting (Michael W. Irvin, Andries Zijlstra, 2014).

Composite materials also have multifunctional efficiency in various manufacturing techniques. Seymour was able to manufacture fibreglass reinforced plastics with good instantaneous temperature resistance, high frequency dielectric properties and exceptional electrical insulation properties (Manisalidis et al., 2020). Generally, composite content can be constructed depending on the condition and performance specifications of product. The manufacturing techniques, primarily moulding processes, can also be selected according to the form of fillers and matrix as well as the shape, size and product number (Wang et al., 2011). The flexibility of design of composite materials gives advantages in producing a product that is reliable, economical, safe and reasonable.

The first modern composite material was glass fibre that is still commonly used in sporting equipment, vehicle bodies, boat hulls, and concrete panels today. Glass fibre is made of plastic as matrix and glass as reinforcement. Nowadays, some advanced composites use carbon fibre instead of glass fibre, since carbon fibre is safer and lighter but needs higher manufacturing costs. These components are used in expensive sporting uniforms, especially golf clubs and aircraft frames.

Composite technologies were first used in aerospace industry. Composite materials are primarily used in this industry on the adiabatic shell structures of solid rocket engine combustion chamber, liquid hydrogen tank structure, module structure of apparatus, missile inter-segment structure, and various satellite structures. Similar to the aerospace industry, the use of composite materials in the aircraft industry is designed to reduce aircraft weight, which in turn reduces costs and improves aircraft performance (Maria, 2013).

2.1.3 Important Roles of Composite Materials in 21st Century

The world's growth pattern in 21st century is unpredictable at many extents as environmental problems are moving to a severe condition. Raw materials are also facing shortages and severe depletion. These will undoubtedly give composite materials significant development roles and opportunities. Composite materials with distinctive features such as light weight, high strength, and noise reduction or insulation improve the quality of human life through numerous construction and transport applications, enhancing houses and transport tools comfort (Alberto, 2013). Besides, composite materials also solve the problems of energy crisis and resources depletion (Wang et al., 2011). In terms of energy conservation, products made or reinforced by using composite materials have been found to be lighter in weight, consume less energy, resistant to corrosion and longer lifetime. Composite products may substitute the raw materials or undeveloped resources for various forms of applications or product production, thereby preventing the depletion of the resources.

There were composite materials produced from waste in the role of environmental protection, using the waste and turning the harm to benefits, at the same time, creating green composite materials that could be easily biodegraded. This scenario could also be related to waste to wealth. As a result, composite materials are extensively used in industries such as the construction, shipbuilding, automotive manufacturing, electrical and electronic, sporting equipment, agriculture and fisheries, mechanical engineering, etc. (Wang et al., 2011). At the present time, all these applications are becoming more prevalent, mainly because of the price and performance advantages of composite materials.

2.1.4 Applications of Composites as Fertilizer's Coating

Composites are very useful in many applications in our daily life. The main benefit of composite materials is the combination of strength and rigidity with lightweight. Properties that meet the needs for a particular structure with particular purpose can be generated by selecting an appropriate combination of reinforcement and matrix material. One of the useful applications of composites is as the coating of fertilizer. The first instance of utilizing composites as the coating of fertilizer is the biodegradable polymer composites that consist of poly(vinyl alcohol), horn meal, rapeseed cake, glycerol, and phosphogypsum as coating materials for granular fertilizers. As a binder, poly(vinyl alcohol) was utilised. The other components, which accounted for roughly 70% of the composites' bulk, were waste materials or by-products. This fertilizer contains plant-friendly nutrients such as phosphorus, nitrogen, calcium, potassium, and sulphur. Fertilizers were encapsulated using the composites created. It was discovered that encapsulation increased the time it took for fertilizers to be released. Encapsulation also improved the fertilizer's mechanical qualities. The fertilizer granules were covered with composite sheets and put to the test in tomato sprout culture. They had a significant positive impact on the development of the plant's roots (Treinyte et al., 2018).

Next example is the degradable slow-release fertilizer composite prepared by ex situ mixing of inverse vulcanized copolymer with urea. It is a slow-release urea composite fertilizer (SUCF) created by employing inverse vulcanised copolymer with enhanced biodegradation and nutrient release lifetime to improve crop production and nitrogen absorption efficacy. The leaching test demonstrated that after 16 days of incubation in distilled water, only 70% of the total nitrogen of SUCF made from 50% sulphur copolymer was released, however after 20 days in soil, only 35% nitrogen was released. This shown that the composite coating exhibited good slow-release properties on the fertilizers (Manzoor Ghumman et al., 2022).

The composite coating for fertilizer can also be produced from combination of epoxy resin (ER), bio-based polyurethane (BPU), and polyolefin wax (PW). The coatings included, firstly, the use of PW as a modified inner coating that improved fertilizer surface performance and reduced urea surface roughness, secondly, the degradable BPU film was synthesised with liquefied starch (LS) as the outer coating material, and thirdly, an epoxy resin protective layer that improved the coated urea's hydrophobicity for controlled release. It is shown that the PW increased the uniformity of urea heating by optimising the fluidity, thermal insulating characteristics, and microscopic surface of the particles. Also, the release period of the fertilizers was also proven to be extended (Tian et al., 2019).

Furthermore, composite from two comparable polyhedral oligomeric silsesquioxanes POSS with eight same vertex groups can also be used to fabricate bio-based polyurethane nanocomposite thin coating. This is done by two identical POSS, with eight poly(ethylene glycol) (PEG) and octaphenyl groups connected to the cage, were incorporated into thin castor oil-based polyurethane coatings via in situ polymerization on the urea surface. The nanostructure coatings are safe for the environment, simple to make, and have variable properties. It is shows that even with a low coating rate of 2 wt percent, the vertex group of POSS had a significant impact on dispersion level and interaction between polyurethane and POSS, which fine-tuned the release pattern and time of coated urea. This is because the liquid POSS with long and flexible PEG groups had superior compatibility and dispersibility in polyurethane matrix than the solid POSS with rigid octaphenyl groups. The varying degrees of physical crosslinking also resulted in unique characteristics. Therefore, the addition of POSS to a biobased polyurethane coating gave another way of slowing down the release of nutrients from the fertilizer (L. Li et al., 2021). Table 2.1 shows previous research using composite as fertilizer's coating.

Table 2.1: Previous research using composite as fertilizer's coating

Components of composite	**Reference**
Poly(vinyl alcohol), horn meal, rapeseed cake, glycerol, and phosphogypsum	(Treinyte et al., 2018)
Inverse vulcanized copolymer with urea	(Manzoor Ghumman et al., 2022)
Epoxy resin (ER), bio-based polyurethane (BPU), and polyolefin wax (PW)	(Tian et al., 2019)
Polyhedral oligomeric silsesquioxanes POSS with eight same vertex groups	(L. Li et al., 2021)

2.1.5 Advantages and Disadvantages of Fertilizer Coating from Composites

Fertilizer coating can be made from different types of materials. It can be made from materials of single ingredient or composite materials. There are always pros and cons of using the aforementioned materials on its own. It is all depends on the condition that the fertilizers will be used or other factors that have to be taken into account on choosing the right materials as fertilizer coating. However, with recent research and advancement of technology in fertilizer coating, composites coating is preferred in many extents as generally, composites are more durable and versatile compared to coating made of single material (Trenkel, 2010). Table 2.2 shows the advantages and disadvantages of fertilizer coating from composites.

Table 2.2: The advantages and disadvantages of fertilizer coating from composites.

Advantages	Disadvantages
Composite coating is more resistant to chemicals.	Composite materials are generally more expensive to produce.
Composite coating mostly does not require post-treatment finishing efforts.	Highly specialized manufacturing processes are required.
Composite materials are lighter than some typical materials.	
Composite coating can withstand relatively well in harsh environments. This increases the lifespan and strength of the composite materials.	
Most composite coating for fertilizers is biodegradable and environmentally friendly.	

2.2 Sodium Alginate

Sodium alginate is a potential biopolymer film or coating component because of its unique colloidal properties, which include thickening, stability, suspension, film formation, gel development, and emulsion stabilisation. It is a colloidal hydrophilic carbohydrate derived from different types of brown algae (*Phaeophyceae*) with diluted alkali (Norajit et al., 2010). In molecular terms, it is composed of β-D-mannuronic acid units and α-L-guluronic acid units which are linked together by 1-4-linkages. Ikeda et al. (2000) stated that alginic acid is the only polysaccharide that naturally contains carboxylic groups in each residue and possesses various functional material capabilities in different industries (Ikeda et al., 2000).

With its excellent properties, such as readily available, biocompatible, biodegradable, non-toxic and gel-forming properties, alginate has been extensively used in industry as an emulsifier, colloidal stabilizer, and non-toxic food additive, hydrogels or as films (Carneiro-da-Cunha et al., 2010). It was developed as a source for biodegradable or edible films considering the potential amount available as a natural resource as well as the reproducibility of alginic acid (Vivek et al., 2021).

Sodium alginate was produced by first purified and precipitated to form alginic acid. Subsequently, sodium alginate was formed by combining alginic acid with sodium carbonate. Biodegradable or edible film made from sodium alginate has been used as packaging materials to replace conventional plastics in many fields. This was because the biodegradable film produced was colourless or translucent and when imparted with plasticizer had improved flexibility.

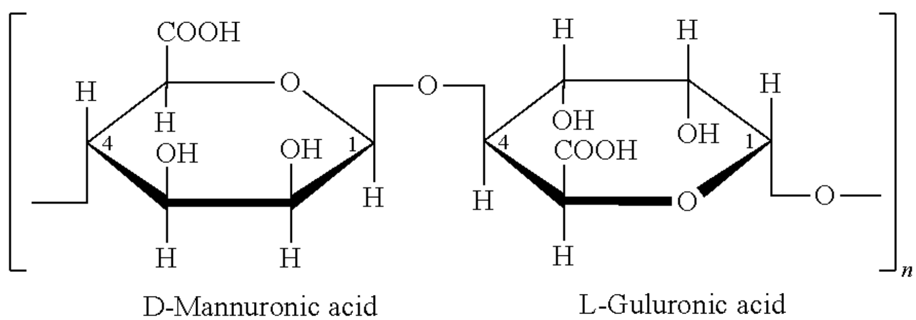

Figure 2.1: Chemical structure of alginic acid. Retrieved from: Rhim, J.-W. (2004). *Physical and mechanical properties of water resistant sodium alginate films*.

2.2.1 Water Resistant Sodium Alginate Film

Though edible films created from hydrocolloids like alginate yield exceptionally high-mechanical-strength and transparent films (Moon et al., 2011). The films display low water resistance owing to their hydrophilic nature. According to Yai (2008), the films have low water vapour barrier properties and will dissolve when in contact with water.

(Yai, 2008). Therefore, researchers have designed water-resistant sodium alginate film to tackle this issue (Rhim, 2004).

The mixing of alginate and calcium chloride formed rigid and insoluble gels that produced films with improved properties (Vivek et al., 2021). In most circumstances, however, the rapid gel formation of alginate with calcium ions jeopardises smooth film casting (Bierhalz et al., 2020). There was study suggested a method for the formation of a uniform gel through gradual release of calcium or to immerse the film in aqueous multivalent cation solutions to improve the gel strength. Pavlath et al. (2019) stated that the technique of film immersion substantially enhanced the water resistance capability of alginate films (Pavlath et al., 2019).

To remove the alginate hydrophilic groups, it could be done by reacting the alginates with polyvalent metal ions, and the water solubility properties of sodium alginate films can be reduced. Calcium ions from $CaCl_2$ were the multivalent metal ion used in this research to crosslink the alginates to create a water-insoluble film.

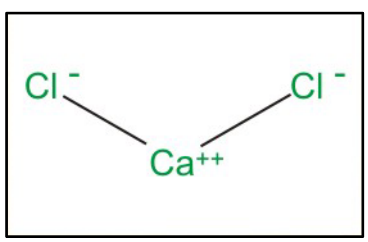

Figure 2.2: Chemical structure of calcium chloride. Retrieved from: Rhim et al., (2003). *Modification of Na-Alginate Films by $CaCl_2$ Treatment*.

According to (Bt Ibrahim et al., 2019)), water-resistant properties of alginate film can be enhanced by using $CaCl_2$ by the crosslinking of calcium ions on the film. There are two approaches for the process of crosslinking. The first approach is done by immersing the alginate film in solution $CaCl_2$, while the second method used mixing of $CaCl_2$ with alginate during the film preparation process. It shows, however, that this latter approach does not significantly enhance the water-resistant properties of films (Rhim, 2004). Therefore, the alginate films in this research were produced by using the preferable first method which is through the immersion of films in $CaCl_2$ solution.

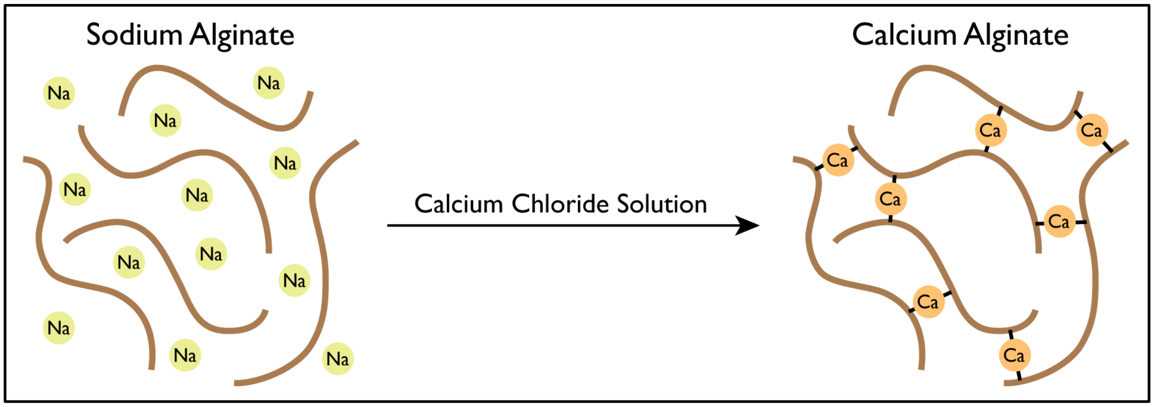

Figure 2.3: Crosslinking process of sodium alginate by using calcium chloride solution. Retrieved from: Rhim et al., (2003). *Modification of Na-Alginate Films by $CaCl_2$ Treatment*. Figure 2, pp 217.

2.3 Introduction to Bacteria

Bacteria, singular bacterium, any of a group of microscopic single-celled creatures found in vast quantities in almost every habitat on Earth. Bacteria are the most common of all species, and they may be found anywhere from deep marine vents to deep beneath the Earth's surface to human digestive tracts (Adam & Perner, 2018). There is approximately 5×10^{30} bacteria on Earth, forming a biomass which exceeds that of all animals and plants.

Bacteria are single-cell organisms that lack a membrane-bound nucleus and certain internal features, making them the only ones with prokaryotic cell organisation. Bacteria may use certain inorganic chemicals and practically any organic component as a food source as a group due to their incredibly diversified metabolic capabilities. Although some bacteria can cause disease in humans, animals, and plants, the vast majority of germs are harmless.

In terms of biodiversity, illness, genetics, and technology, studies of interactions between distinct species of bacteria aim to provide amazing insights into the processes of evolution and the history of life on Earth. Bacteria serve a significant role as beneficial ecological organisms whose metabolic activity is essential for the survival of higher life forms on Earth, and without them, life on the planet would perish (Cabral, 2010). Bacteria in the ecosystem help to ensure important processes such as cellulose degradation, organic matter decomposition, nitrogen fixation, and photosynthesis. Approximately half of the bacterial phyla can now be cultured in the laboratory, and over 5000 distinct bacteria kinds have been identified. There are certainly several thousands of bacteria that have not been identified and that await their proper identification.

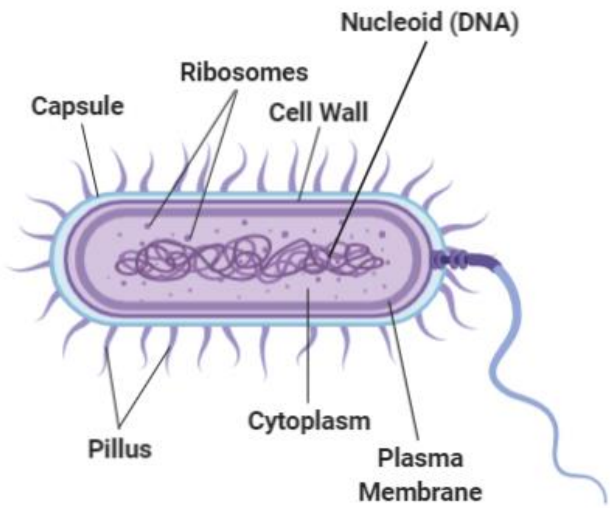

Figure 2.4: Basic structure of bacteria.

2.3.1 Classification of Bacteria

By naming and classifying bacteria's properties, classification aids in the identification of their diversity. Bacteria are classified using genetic methods or cell shape and metabolism. Genetic techniques such as DNA-based systems and 16S rRNA analysis have been used to determine the degree of interconnectedness between bacteria species in order to acquire essential information (Rosselli et al., 2016). Because genetic differentiation emphasises bacteria's evolutionary ties, biochemical and physical traits are also important for their categorization and identification. In actuality, bacteria were classified based on a variety of characteristics, including motility, cell morphologies, spore development, multicell aggregation structure, and Gram stain reactivity. According to Tortora et al. (2004), the morphological characteristics can be affected by environmental factors, including the colour and form of bacterial colonies, which were not always constant (Sousa et al., 2013).

Bacteria can only be properly characterised when grown on a specific medium, because the conditions of their growth influence the changes in their attributes. Bacteria will consume nutrients in the medium and begin to expand, growing from thousands to millions of cells to billions. A bacterial colony is a collection of visible bacterial cells that stems from a single bacterial cell. Diverse bacteria contribute to different colonies; some colonies are coloured, while others are shaped irregularly (Kandi, 2015). Colony morphology is the study of the structure and shape of bacterial colonies, and it is the initial stage in identifying and characterising a bacterial culture. Size, colour, opacity, form, elevation, and margin are common features used to consistently and accurately define colony morphology.

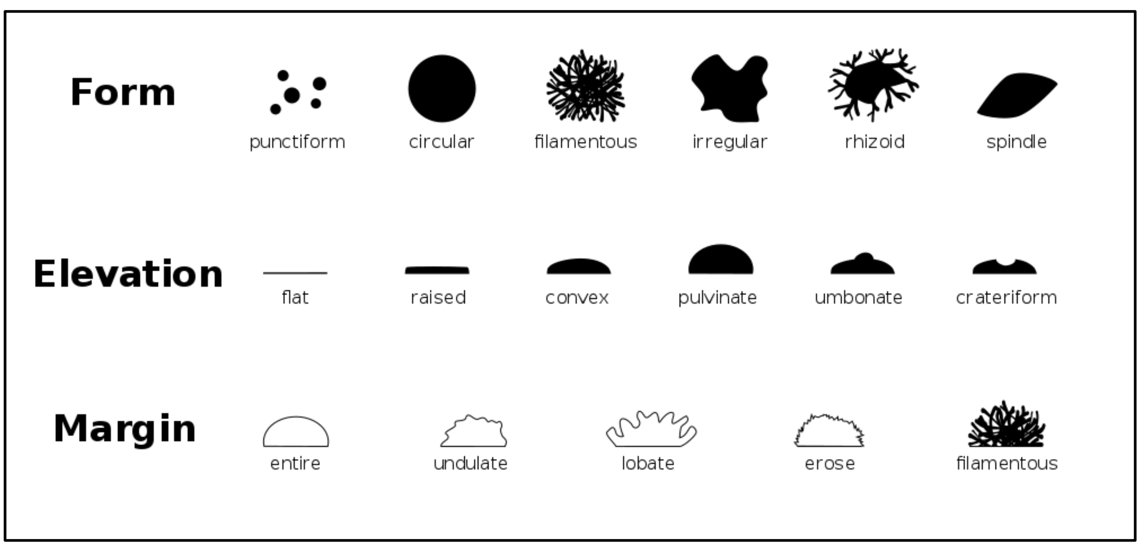

Figure 2.5: Morphological characteristics of bacterial colony.
Retrieved from: Brown and Alfred E (2012). *Benson's microbiological applications: laboratory manual in general microbiology*, 8th Ed. Figure 47.4, pp 160.