MJoSHT
Malaysian Journal of Science, Health & Technology
[mjosht.usim.edu.my]

*Article*

# Advanced Development of Bio-fertilizer Formulations Using Microorganisms as Inoculant for Sustainable Agriculture and Environment – A Review

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*Article Info*
*Article history:*
*Received: 19 August 2021*
*Accepted: 27 January 2022*
*Published:31 March 2022*

*Academic Editor:*
*Shahrina Ismail*

*Malaysian Journal of Science, Health & Technology*

*MJoSHT2022, Volume 8, Issue No. 1*
*eISSN: 2601-0003*

*https://doi.org/10.33102/2022228*



***Abstract***— **Conventional types of fertilizer such as chemical and synthetic fertilizers have demonstrated various adverse effects on the environment, crops, and humans. The utilization of plant probiotics as a bio-fertilizer in agriculture has been recognized to benefit the growth of the plant and inhibit the activity of plant pathogens. Traditional formulations of bio-fertilizer have provided insight into the beneficial use of microorganisms in crops. Despite its advantage to the environment, the effectiveness of traditional bio-fertilizer is common as compared to chemical fertilizer. Thus, a variety of bio-fertilizer formulations have been developed to improve the success rate of bio-fertilizer in increasing plant productivity. This review was focused on the development of bio-fertilizer formulation and the potential of bio-fertilizer to substitute chemical fertilizer application. In addition, this research review was also undertaken with a great demand on producing low cost and highly effective fertilizer without harming the environment and humans. Thus, the advantages and disadvantages of each formulation type have also been reviewed, emphasizing the perspective of bio-fertilizer and their suitability as bio-fertilizer as a substitute for chemical fertilizers in sustainable agriculture.**

***Keywords***— **Bio-fertilizer formulation, plant probiotic, sustainable agriculture**

## I. INTRODUCTION

The crop is one of the main food sources for humans in order to survive. Therefore, the abundant yield of crops such as rice is crucial in maintaining food supply and demand all over the world. However, some crops are likely to suffer from disease or cannot consistently grow due to several factors such as unsuitable environment, lack of nutrients, pest, infection, and climate change [1, 2]. Rice, which is known as a staple food, is consumed all over the world, facing critical threat diseases such as blast, blight, brown spot, narrow brown spot, bacterial leaf streak, and rice ragged stunt virus disease [3,4]. In tomato crops, the most common disease detected using machine learning is the yellow leaf curl virus, bacterial spot, late blight, Septoria leaf spot, spotted spider mite, early blight, and mosaic virus [5]. Meanwhile, the disease that causes gall formation in root tissue in *Brassicae* crops, including *Arabidopsis thaliana* and

Chinese cabbage, is known as clubroot disease, which is caused by *Plasmodiophora brassicae* [6].

Most plants or crops require extensive care to prevent them from developing the disease symptoms caused by pathogen infection. As a part of infection strategy, many pathogens modify plant development which causes a broad spectrum of impacts from subtle manipulation of host metabolism to extensive tumor formation. For instance, *Blumeria graminis* lives in the host epidermis and shows only the subtlest effects on the plant, such as barley, while *Ustilago maydis* cause a strong effect on its host by developing tumors on above-ground tissues of crops such as corn [7, 8]. Crops rely on macronutrients such as nitrogen, potassium, phosphorus to boost productivity. Therefore, chemical fertilizers have been widely used to overcome these problems. The chemical fertilizer is commonly used in the agricultural industry as it contains more nutrients and can channel nutrients instantly to the crop compared to the organic fertilizer [9, 10]. However, it has been reported to cause adverse effects [11].

The pressure to produce more crop yield has led to the uncontrolled application of fertilizer and pesticides in agriculture. Farmers tend to over-apply chemical fertilizer such as nitrogen, which can cause softening of plant tissue and therefore make the plant more susceptible to disease and infection [12]. In addition, the active ingredients contained in pesticides and chemical fertilizers often leached into the water system, causing water pollution, which could endanger the ecosystems. This issue was supported by a previous study that stated cultivation of rice had been one of the main causes of polluting the environment [13, 14]. Other than that, spraying various types of pesticides without the proper protection may also harm the farmer as they are exposed to toxic chemicals. Later, this may cause skin allergy, breathing difficulties, and other health problems [15]. Microorganism populations in the soil make an important contribution to supply required nutrients to plants and increase resistance toward infection and pathogens [16].

The application of bio-fertilizer could be the alternative way to increase crop productivity in the agricultural sector. Thus, this review was focused on the development of bio-fertilizer formulation and the potential of bio-fertilizer to substitute chemical fertilizer application. This research review was undertaken with a great demand on producing low cost and highly effective fertilizer without harming the environment and humans.

## II. BIO-FERTILIZER AND THEIR RELATIONSHIP WITH HOST PLANT

Colonization of plant-associated microorganisms has been recognized to meet the significant functions in crop development and productivity. Microorganisms (bacteria and fungi) are capable of performing various mechanisms, which are directly essential to increase plant growth and restrain the activity of plant pathogens. Therefore, the plant-microbiome interaction has created a great potential in agriculture where it is conceivable to establish a bio-fertilizer for plants [17]. Bio-fertilizer refers to the application of beneficial microorganisms in the fertilizer and is also known as microbial-based fertilizer.

Bio-fertilizers have great potential in maximizing and augmenting crop production. Bio-fertilizer has recently gained popularity due to its promising ability to increase yield and promote the growth of plants, including oil palm, paddy, and wheat [18, 19, 20, 21, 22]. Similarly, the inoculation of bio-fertilizer into crops helps to amend soil fertility and be eco-friendly whilst, at the same time, being cost-effective since the quantity of chemical fertilizer used is minimized [19, 23, 24, 25]. The incorporation of microorganisms as bio-fertilizer has a special impact on the host plant. It is different from any other fertilizer, such as organic fertilizer, as it does not provide nutrients that can be directly taken by the plant. Instead, the bacteria contained in the bio-fertilizer will colonize in the soil and complete certain mechanisms to convert the organic form of nutrient into soluble form for plant uptake.

The concept of beneficial interaction between bio-fertilizer and host plant is important as they promote effective and eco-friendly approaches. Bio-fertilizer can provide essential nutrients for plants and crops such as nitrogen, phosphate, and potassium. For example, nitrogen naturally exists in the atmosphere as nitrogen gas. However, plants are unable to use the natural form of nitrogen directly. Thus, a bio-fertilizer containing nitrogen-fixing bacteria (NFB) will convert it into ammonia form ($NH_3$) for plant uptake. This process also applies to other major nutrients required by the plant. The idea of developing bio-fertilizer was meant to improve crop yield profitability and simultaneously diminish the reliance on chemical fertilizer. It is well known that chemical fertilizer poses a dangerous threat to humans and the environment over the long haul. Thus, commercializing the use of bio-fertilizer would be a better choice to protect the environment, ecosystem, and humans.

Plant growth-promoting rhizobacteria (PGPR), which contain bio-fertilizer, are important for plant health. PGPR acts towards plants by using different mechanisms of action. The simple mechanism of PGPR action is by releasing nutrients such as vitamins, iron, hormones, solubilization of phosphate and ammonia into the soil and then taken up by plants to sustain rapid plant growth [26, 27, 28]. In addition, the interaction between PGPR and plants is supported by cell-to-cell communication, for instance, through quorum sensing, as signaling mechanisms to monitor the surrounding and microbial activities around the rhizosphere [29, 30, 31]. Finally, chemotaxis and chemokinesis reactions through chemotactic agents such as amino acids and sugar are important to increase the mobility of bacteria to establish the interaction between PGPR and plants [26, 32].

## III. THE USE OF MICROBIOME FOR THE DEVELOPMENT OF BIO-FERTILIZER

The role of microorganisms and their interaction with plants are exploited to produce bio-fertilizer. Most microorganisms used in bio-fertilizer are commonly extricated from rhizosphere soil which is in contact with plant roots [27, 32]. A number of studies have been performed to distinguish between beneficial and harmful rhizosphere bacteria according to their impact on soil fertility, plant growth, and productivity [33, 34]. For instance, members of the genus *Bacillus*, which is classified as plant growth-promoting rhizosphere (PGPR) isolated from tomato rhizosphere, could increase the growth of tomato plants [33]. In addition, cover crops such as pea, rapeseed, and wheat could increase the growth of plant-beneficial bacteria, which could restore declining soil nutrients and control bacterial wilt disease in tobacco fields [34]. Beneficial microorganisms, also known as plant probiotic bacteria, are capable of exhibiting beneficial effects in agriculture such as fixing nitrogen, improving the cycling of nutrients, detoxifying pollutants, and generating bioactive compounds such as hormones and enzymes [35, 36, 37]. Furthermore, the application of various beneficial microbiomes can generate 'induce systemic resistance,' which is able to fight against multiple plant pathogens [36].

The development of bio-fertilizer requires extensive research and proper experimental design to establish high-quality bio-fertilizer. As reported in [38], there are five fundamental procedures involved in developing carrier-based bio-fertilizer (Figure 1). The first step is the isolation and identification of plant-associated microorganisms. The second is the preparation of starter culture and inoculum by cultivating the microorganisms, followed by preparation of carrier materials and incorporating microorganisms into carrier materials. The final step is the packaging and storing carrier-based bio-fertilizer [38].

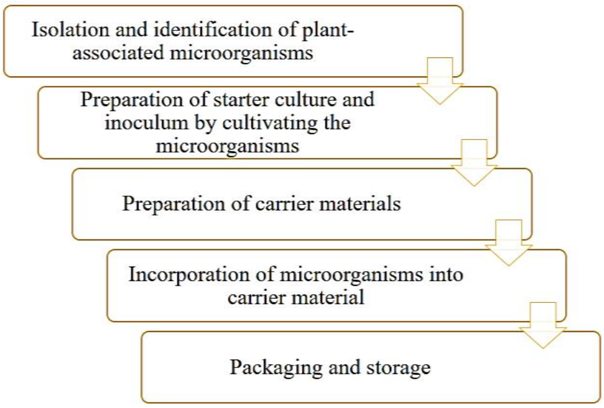


Figure 1. Procedures in developing carrier-based bio-fertilizer

### A. *Selection of Plant Probiotic Strains*

Selection of the right strain of bacteria is crucial as it is the primary step in producing bio-fertilizer. A suitable strain of plant probiotic bacteria must be carefully selected to ensure their effectiveness to the host plant. Several criteria need to be considered when selecting the microorganisms. As stated in [24], some of the essential requirements in selecting plant probiotic strains to be used in bio-fertilizer are effectiveness (provide nutrients for plants), competitiveness, being able to initiate plant defense and showing resistance traits against pathogens. In addition, plant probiotic bacteria can be classified based on their functions and nature.

*1) Plant Growth-Promoting Bacteria*: This type of plant probiotic comprises free-living bacteria that colonize in soil and certain parts of plant tissue to develop a special symbiotic interaction with a host plant. In the interaction phase, the bacteria may carry out direct or indirect mechanisms to boost plant productivity. They can either directly promote the growth by providing the available nutrients or regulating the levels of plant hormones, or indirectly by combating pathogenic agents that have an inhibitory effect on plant development. Plant growth-promoting bacteria (PGPB) have the capacity to synthesize various kinds of antibiotics to inhibit plant pathogens [39]. Among the bacteria that pose functional properties of PGPB are *Rhizobium*, *Azospirillum*, *Anabaena*, *Acetobacter*, *Bacillus megaterium*, *Azolla*, *Pseudomonas*, and *Bacillus polymyxa* [40]. *Pseudomonas putida* strain, for instance, has effectively improved the wheat yield by inhibiting negative impact and reducing stress on the host plant [41].

*2) Nitrogen-Fixing Bacteria:* Nitrogen fixers are the bacteria that fix atmospheric nitrogen and have interchangeable beneficial interactions with the host plant. The natural form of nitrogen must be converted into ammonia for plant absorption through a process of nitrogen fixation. This process is frequently carried out by nitrogen-fixing bacteria (NFB) that are present in the soil. In this process, nitrogen from the atmosphere enters the soil, and nitrogen fixers fix nitrogen into ammonium ions by releasing nitrogenase enzymes. Several strains of nitrogen-fixing bacteria include *Azotobacter sp.*, *Rhizobium sp.*, *Cyanobacteria*, and others [42, 43]. Besides that, *Azotobacter* also can fix a considerable amount of nitrogen in the atmosphere and be able to generate antifungal compounds against plant pathogens. Inoculation of nitrogen-fixing bacteria as bio-fertilizer is important to supply adequate nutrients to plants and maintain the fertility of the soil. For instance, the *Azotobacter tropicalis* strain contributed to a positive impact on maize crops by stimulating a four-fold increase in crop growth [44]. Thus, plants can minimize their dependence on chemical fertilizer due to nitrogen fixer's ability.

*3) Phosphate-Solubilizing Bacteria:* Phosphorus solubilizers are microorganisms that are able to solubilize insoluble phosphorus to supply soluble phosphorus that can promote plant growth. Phosphate-solubilizing bacteria can perform specific mechanisms to solubilize phosphorus by producing organic acid such as citric acid. This acid induces low pH conditions and therefore results in the dissolution of bound phosphate [42]. The conversion of insoluble phosphorus was mainly performed by carboxyl and hydroxyl groups present in organic acid that chelate the cations bound to phosphate. Phosphorus has an important effect on the photosynthesis, maturity, and disease resistance of the plant. As reported in [45], inoculation of *Bacillus megaterium* can increase the uptake of phosphorus and tuber size in potato crops. The identified strains of microorganisms that exhibit the ability of solubilizing phosphate are *Bacillus subtilis*, *Pseudomonas sp*., *Agrobacterium sp*., *Acetobacter sp.*, and *Azotobacter sp.* [40, 46]. Therefore, inoculation of these bacteria will effectively maintain the supply of soluble phosphorus for the plant.

*4) Arbuscular mycorrhizae*: *Mycorrhizae* is basically a fungus that establishes symbiotic relationships with the root of the host plant. They attach to the plant root system to absorb nutrients and form a mild type of parasitism that is symbiotic, in which both organisms (plant and fungus) obtain benefit from the association. This can be explained as most plants depend on mycorrhizal fungi to channel moisture and essential nutrients required by the plant, while in return, the plant helps supply the fungus with substances and nutrients produced by the plant. They provide various benefits to the host plant, such as aiding in the major nutrient uptake and reducing the vulnerability to water stress [47]. Certain mycorrhizal fungi are capable of forming a protective cover around the root, making the plant more tolerant to drought, extreme temperature, extreme soil acidity, and infection by pathogenic fungi. As reported in [48], they discovered *Rhizophagus fasciculatus* as the best strain to enhance salinity stress of *Casuarina equisetifolia L.* and increase the nutrient content in the plant.

### *B. Selection of Carrier Material*

Bio-fertilizer can be classified into several types, such as peat formulation, liquid formulation, and granules formulation [40]. Bio-fertilizers are typically supplemented with the carrier material. However, it can also be formulated without the inclusion of carrier material such as liquid bio-fertilizer. Carrier materials are commonly added into the formulation to improve the potency of bio-fertilizers. The primary role of carrier substances is to provide the inoculated microorganisms with a suitable and appropriate environment to increase bio-fertilizers shelf life and effectiveness. The inclusion of carrier materials can facilitate the handling and application of bio-fertilizer as well as enhance the storage condition. In order to develop good quality bio-fertilizer, a proper selection of carrier material is required. In selecting the most suitable carrier material, the following properties need to be considered such as prices, organic matter quality, contaminants, water retention capacity, processing method, friability, and vulnerability. Ideal carrier material should be readily accessible, lower in prices, free from hazardous contaminants, have a water retention capacity of more than 50%, simple to process, be non-clumping material, and be easy to sterilize [49]. In nature, carrier materials may be organic or inorganic. Followings are the types and examples of carrier [50]:-

1) *Soils*: Inorganic soil, peat, clays, coal, lignite.

2) *Plant waste materials:* Composts, cellulose, farmyard manure, charcoal, peanut oil, wheat bran, corn cobs, press mud.

3) *Inert materials:* Vermiculite, talc, rock phosphate, perlite, calcium sulfate, alginate beads, polyacrylamide gels.

4) *Plain lyophilized microbial cultures and old dried bacteria:* Can be readily used or may be integrated with a solid carrier.

The selection of carrier substance depends on the desired function and aims to produce the bio-fertilizer formulation, either in granule, pellet, immobilized, or other forms. During the preparation of the carrier, the sterilization process is one of the critical parts to ensure the bio-fertilizer produced is safe from any pathogenic organisms. The type of sterilization methods may be variant depending on the type of carriers used. Gamma-irradiation and autoclaving are the common methods used for carrier sterilization.

## IV. HISTORY ON THE APPLICATION OF BIO-FERTILIZER

Traditional agriculture was practiced a thousand years ago. Traditional farming is based on the thousand years of experience and knowledge practiced by local farmers. The traditional practices have provided insight and basic knowledge in developing scientific expertise and advanced application in agriculture. Traditional formulations made from organic materials or wastes are used as bio-fertilizer to supply essential nutrients to plants [51]. The application of traditional formulations helps to conserve the environment, soil quality as well as microbial diversity in soil. It is important for soil health as it facilitates the colonization of microorganisms and enhances microbial diversity. In most Asian countries, the local farmers tend to create their own traditional bio-fertilizer formulations by incorporating various organic compounds, which then undergo fermentation or composting to boost their activities and functions (Table I).

TABLE I
EXAMPLES OF TRADITIONAL BIO-FERTILIZER FORMULATION

| Countries | The traditional formulation of bio-fertilizer |
|---|---|
| Indonesia | Bio-fertilizers containing inoculants of symbiotically nitrogen fixer bacteria (*Bradyrhizobium japonicum*) were used to substitute chemical nitrogen fertilizers for soybean cultivation [52]. |
| South Korea | Wild weed wineberry extract with indigenous microorganisms was used by combining the extract of wild weeds, molasses, salt, and extract of decomposed leaf soil [53]. |
| Thailand | The extract of the fermented plant was used to stimulate plant growth and serve as a bio-control agent [54]. |
| India | Panchagavya, the traditional bio-fertilizer formulation, is prepared by fermenting cow-based products such as milk, urine, dung, curd, and clarified butter [55]. |
| Japan | Traditional formulation known as Bokashi in the 1980s contained effective microorganisms (EM). It has been used as a starter to ferment raw materials such as residue from plant or animal materials and then produced into bio-fertilizer [56]. |

A group of researchers [57] has reported a study on a traditional organic formulation that uses organic fertilizer extract with 2% yogurt. The finding showed that the type and concentration of components used directly contribute to the variation of the microbial population on the host plant. The application of traditional formulation has shown a good effect on plant growth. Chemicals are not utilized in this type of traditional farming. Thus, the quality and pH balance of the soil can be maintained. In contrast, it yields an indifferent impact on crop productivity. This may be due to limited information available on the microbial and chemical components contained in it. Following the two world wars, traditional agriculture has become ordinary and indifferent, as, during that period, the expertise in chemistry has significantly improved. Modern formulations of fertilizer such as synthetic fertilizers, chemical fertilizers, and pesticides have been commercialized due to their promising effects on crop production and yield. However, the application of modern fertilizer may cause long-term adverse effects on the environment and diminish the supply of natural resources and soil microbes in agriculture. Therefore, in the past decades, many researchers have formulated various types of bio-fertilizers with extensive and advanced effects on crop growth to preserve the environment and ecosystem.

## V. ADVANCED DEVELOPMENT OF BIO-FERTILIZER FORMULATION USING BENEFICIAL MICROORGANISM

### A. *Liquid Formulation Bio-fertilizer*

A liquid bio-fertilizer is a bio-fertilizer that comprises viable microorganisms in liquid form. This liquid inoculant grows selected microorganism strains without the inclusion of solid carrier material. After screening and identification of effective microbes to be used as bio-fertilizer, the cells will be cultured in a broth medium rich in nutrients [58]. Once the cells have been cultured and propagated on a larger scale, the liquid inoculants are ready to be applied to the seed or host [59].

There are several formulations of liquid bio-fertilizer that have been established by previous researchers (Table II). Liquid bio-fertilizer seems to be an effective bio-fertilizer formulation for many crops, especially for the cultivation of crops without using soil (hydroponic). The development of low-cost bio-fertilizer yet effective is favorable in the agricultural industry. As stated [60], a simple liquid inoculant in sterile water was able to improve the quality and antioxidant level in strawberry fruits. Researchers of the Malaysian Nuclear Agency, as in [61], studied the ability of four broth mediums (sterile distilled water, nutrient broth, Luria-Bertani broth, and Tryptic soy broth) in retaining the viability of plant probiotic cells to produce high shelf life of liquid bio-fertilizer with low production cost. Based on the result, the nutrient broth is the best medium to retain the survivability of bacteria (Phosphate solubilizing bacteria and plant growth-promoting bacteria (PGPB)) compared to other mediums. Similarly, a liquid formulation of several strains of bio-fertilizer with added nutrients such as rock phosphate, K-feldspar, and natural mineral fertilizers has shown extensive growth on vegetables [44].

Apart from that, [62] proposed that treatment of bacterial cells with polymeric additives helps to maintain the viability of bio-fertilizer over long periods of storage. The additives help to enhance the shelf life and adherence of bio-fertilizer to seed [63]. Furthermore, [62] found that the development of liquid bio-fertilizer using a combination of glycerol and polyvinyl pyrrolidone has the longest shelf life and excellent efficiency over 180 days of storage.

### B. *Granules Formulation of Bio-fertilizer*

Granules formulation is a type of carrier-based bio-fertilizer formulated in the form of small like-grain particles. Granules form was first invented in the 1990s [64]. The main principle of this formulation is to create dust-free bio-fertilizer without having concern about powder segregation [65]. Besides that, this formulation plays an important role in ensuring the uniformity of the bio-fertilizer content in each granule particle.

TABLE III
LIQUID FORMULATIONS OF BIO-FERTILIZER

| **Bio-fertilizer/ Strains** | **Formulation** | **Result** | **Ref.** |
|---|---|---|---|
| Phosphate solubilizing bacteria (PSB), Plant growth-promoting bacteria (PGPB) | Broth media: Sterile distilled water, Nutrient Broth (NB), Luria-Bertani Broth (LB), Tryptic Soy Broth (TSB) | NB formulations are effective for microbial survival. | [61] |
| *Azotobacter tropicalis*, *Burkhoderia unamae*, *Bacillus subtilis* | Addition of rock phosphate and K-feldspar | Vegetables' growth improved by seven times. | [44] |
| *Bacillus amylolequefaciens* (BChi1), *Paraburkholderia fungorum* (BRRh-4) | Sterile water (nutrient-free bacterial suspension) | Enhanced strawberry quality. | [60] |
| *Rhizobium, Azotobacter, Azospirillum, Bacillus megaterium* | Cell protectants: Glycerol, Polyvinyl pyrrolidone (PVP), Polyethylene glycol (PEG), Gum Arabic (GA), Sodium alginate (SA) | Glycerol and PVP are the best cell protectants. | [62] |

Granule formulation is composed of carrier materials that are moistened with an adhesive and blended with powdered form inoculum [66]. Followings are the basic process in producing granules formulation. After preparing powdered inoculum from the efficient strains of the microbiome, the ideal carrier material is processed into fine powder. The carrier material will be mixed with bacteria inoculum using a mechanical mixer or manually until both components blend homogeneously. In order to produce a grainy-like structure of bio-fertilizer, the homogenous mixture is loaded into a rotary drum granulator, and the particles will be air-dried upon storage. Granules formulation of bio-fertilizer is developed due to its reasonable function and association with the host plant. This formulation can be modified in various ways based on the function and characteristics that one desires to convey (Table III). According to [67], a granular formulation made from legume inoculation is better in terms of nitrogen accumulation, $N_2$ fixation, and nodule amount and weight compared to liquid inoculant. Besides that, [68] acknowledged that granular formulation of phosphate solubilizing *Burkholderia* with clay, rock phosphate, and rice bran as a carrier had demonstrated high viability of bacteria during storage. Figure 2 shows an example of urea granule coated with castor oil and starch gel and *Aspergillus niger* as biological activation microorganisms [69].

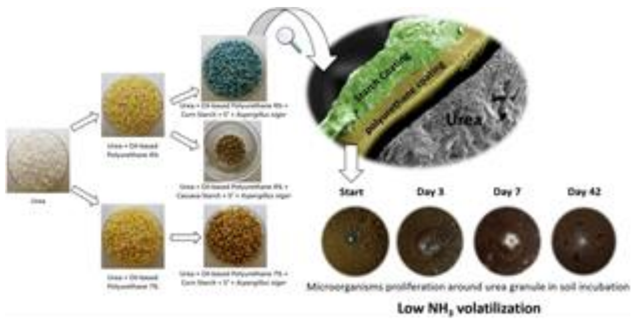


Figure 2. Urea granule bio-fertilizer coated with starch, urethane, and biologically activated by *Aspergillus niger* [69].

TABLE IIIII
GRANULE FORMULATIONS OF BIO-FERTILIZER

| **Bio-fertilizer/ Strains** | **Formulation** | **Result** | **References** |
|---|---|---|---|
| Legume inoculation | Employed as granules formulation | Enhances N accumulation, $N_2$ fixation, total biomass, nodule amount and weight | [67] |
| *Burkholderia* | Combination of substrates clay, rice bran and rock phosphate | High viability of beneficial bacteria. | [68] |

*C. Pelletized Bio-fertilizer Formulation*

Pellet formulation is one of the carrier-based bio-fertilizers. Pellet bio-fertilizer is a solid, compressed, and spherical particle of bio-fertilizer made from a compressed mixture of microorganisms and carrier matter. The principle of pellet formulation of bio-fertilizer is to apply force onto the bio-fertilizer formulation until it forms into pellets [70]. The basic formulation of pellet bio-fertilizer is almost similar to other formulations, starting with the selection and isolation of microbial strains of interest. In this formulation, the microbial inoculants require carrier material to provide solid-state and favorable conditions to the bio-fertilizer. The carrier material and inoculants are mixed, and the mixture is subsequently passed through a pellet press machine to create a small and pellet shape of bio-fertilizer.

Based on research made by [70], three sources of carrier substances which are aquatic weed, biochar, and compost, have been used to evaluate their efficacy in bio-fertilizer. The result showed that the application of pellet bio-fertilizer made from the combination of compost, bioinoculant, and nutrient supplement on paddy plants yielded the highest productivity in terms of shoot height, the number of grains per panicle, and weight of the grains. Also stated in [71], the pellet formulation of *Trichoderma harzianum* in the wheat carrier is effective as it can support the growth and productivity of mustard and tomato.

On the one hand, the formulation of pelletized bio-fertilizer using organic fertilizer substrate from chicken manure mixed with four bacterial strains has shown a significant effect on the host plant [72]. Moreover, as in [72], also proposed that the liquid form of bacteria culture is the most suitable for pellet bio-fertilizer compared to immobilized cells. Therefore, rather than mixing, the pelleted carrier may also be coated with microorganisms by spraying the liquid inoculant after the pelleting process. Figure 3 shows the carrier formulation to produce bio-fertilizer from rubberwood, decanter cake, rice husk ash, and spent coffee ground mixed with purple non-sulfur bacteria *Rhodopseudomonas palustris* and *Rubrivivax gelatinosus* [73]. Table IV summarised the pellet bio-fertilizer formulation.

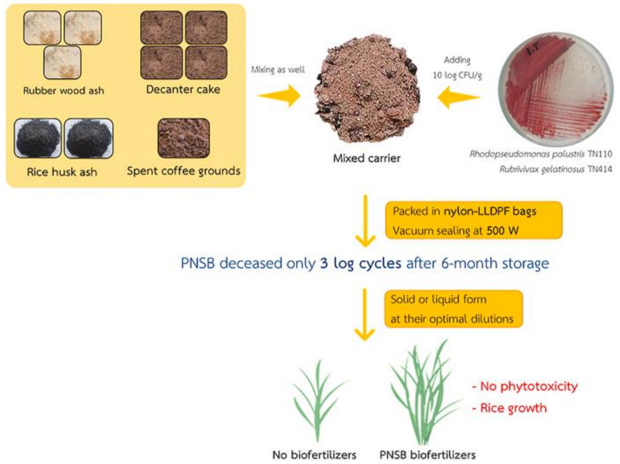


Figure 3. Pellet formulation of bio-fertilizer by using rubberwood, decanter cake, rice husk ash, and spent coffee ground mixed with purple non-sulfur bacteria *Rhodopseudomonas palustris and Rubrivivax gelatinosus* [73].

TABLE IVV
PELLET FORMULATIONS OF BIO-FERTILIZER

| **Bio-fertilizer/ Strains** | **Formulation** | **Result** | **Ref.** |
|---|---|---|---|
| *Azospirillum* sp., *Pseudomonas fluorescens* AMF | Different carrier substances<br>• Biochar<br>• Compost<br>• Aquatic weed | Compost, bioinoculant, and nutrient supplement yielded the highest productivity. | [70] |
| *Serratia marcescens* (P2-2& 8G), *Enterobacter cloacae* (P1-8), *Klebsiella pneumoniae* (CB36) | The bacteria are inoculated as liquid culture and incorporated into organic fertilizer substrates. | Pelleted liquid bio-fertilizer retains the bacteria population and improves the growth of the host. | [72] |
| *Trichoderma harzianum* | Wheat grains (pellet). | Benefit productivity of mustard and tomato. | [71] |

*D. Immobilized/Encapsulated Formulation of Bio-fertilizer*

Immobilized bio-fertilizer is recognized as the advanced formulation of bio-fertilizer nowadays. It is a formulation where the microorganism cells are encapsulated and attached to inert and insoluble material. Encapsulation of bio-fertilizer was invented to boost up the cell's resistance against environmental stress and unfavorable soil condition [74]. As a result, it exhibits higher stability toward pH and temperature and is more resistant to environmental change [75]. Other than that, immobilization assists the gradual and consistent release of the microorganism or enzyme to the soil. Encapsulation may include macro and microform depending upon the intended application. However, the technology of encapsulation and microencapsulation in bio-fertilizer is still new and limited to the experimental in the laboratory.

In this formulation, a prepared culture of bio-fertilizer is mixed with encapsulation matrix formulation (alginate) and formed into rigid capsules by ejecting dropwise of the mixture into calcium chloride. This immobilization technique may be employed to the microorganism itself or the enzyme produced from the fermentation by the microorganism (Table V). As [76] explained, alginate-based macrocapsules of bio-fertilizer offer better function and impact to the host plant compared to other formulations. In addition, the macrocapsules bio-fertilizer is preserved at a high number in field conditions and has a significantly longer shelf than the control without matrix.

Apart from that, there is also research performed regarding microencapsulation of bio-fertilizer. As [77] suggested, microencapsulation of rhizobacteria with gelatin polymer through spray drying methods is an appropriate formulation to enable efficient release of the bacteria. Furthermore, [75] stated that encapsulated bio-fertilizer could efficiently function in sodium alginate polymer with gelatin concentrations of 91.23% and 87.23% to protect potatoes from *Fusarium solani* disease.

Aside from bacteria, published research on immobilized enzymes for bio-fertilizer has the potential to be commercialized in agricultural practices. For example, according to [78], the immobilized keratinase enzyme can degrade chicken feathers into a bio-fertilizer to enhance the growth and chlorophyll content of *Solanum lycopersicum*.

TABLE V
IMMOBILIZED/ENCAPSULATED FORMULATIONS OF BIO-FERTILIZER

| **Bio-fertilizer/ Strains** | **Formulation** | **Result** | **Ref.** |
|---|---|---|---|
| *Pseudomonas fluorescens* | Encapsulated in alginate beads with different gelatin concentrations. | Optimum gelatin concentration: 91.23% and 87.23% | [75] |
| *Azospirillum* | Macrocapsule<br>• Alginate (3%)<br>• Standard starch (44.6%)<br>• Modified starch (2.4%) | The bacteria have longer shelf life. | [76] |
| *Rhizobacteria* | Microencapsulation within gelatin polymer. | Enable efficient release of bacteria | [77] |
| Immobilized keratinase enzyme | Entrapment in calcium-alginate beads.<br>Chicken feather (organic material) is degraded by the enzyme. | Significantly improved the growth and chlorophyll content in *Solanum lycopersicum.* | [78] |

Each formulation is able to deliver benefits to plant and crop productivity, but it may still have limitations (Table V1). Thus, the selection of suitable bio-fertilizer based on plant conditions will determine the successfulness of the inoculation. Appropriate methodology and detailed process are necessary to produce a good formulation of bio-fertilizer with competent strains of bacteria. Most of the studies have emphasized the advancement of bio-fertilizer formulations. In contrast, very limited work has been carried out on the optimization of bio-fertilizer quality and affectivity that could completely substitute chemical fertilizer in agriculture.

## IV. CONCLUSIONS

Microorganisms have an essential role in promoting plant growth. Plant probiotic bacteria are able to interact with a host by supplementing nutrients and compounds required by the plant. The introduction of bio-fertilizers has shown incredible effects on plants and is able to conserve nature. The selection of effective bacterial strains and suitable carriers is important in developing bio-fertilizers. The technology revolution today has contributed to the advanced development of bio-fertilizer that serves important benefits to the agricultural sector globally.

There are many studies that have been established to create improved formulations of bio-fertilizer. Therefore, this review focuses on an advanced bio-fertilizer formulation that can meet the objective of an effective bio-fertilizer. In the future, a study on the formulation of bio-fertilizer is needed to be carried out. These varieties of bio-fertilizer formulations could then be commercially practiced for more sustainable agriculture and a green environment.

TABLE VI
ADVANTAGES AND DISADVANTAGES OF BIO-FERTILIZER FORMULATION

| Types of bio-fertilizer | Bio-fertilizer formulation | Advantage | Disadvantage |
|---|---|---|---|
| Inoculant suspension | Liquid | • Does not require carrier material<br>• Easy to inoculate.<br>• Suitable for soilless agriculture systems.<br>• Microbial cells survive with the addition of the additive.<br>• Contain a high concentration of cells.<br>• Cost-effective | • Low stability during application (no protection from the carrier)<br>• Complicated storage condition (at 4°C) |
| *Carrier-based bio-fertilizer* | Granular<br>Pellet | • Convenient for storage and application<br>• Easy to handle (dust-free)<br>• Supply nutrients for microorganisms<br>• Provide a suitable environment for microbes<br>• Carrier protect cells from the chemical component<br>• Long shelf life | • Selection of compatible carrier material is difficult<br>• Complex preparation of the carrier<br>• Bulky in size: High cost for storage and transportation |
| | Encapsulated / Immobilized | • Facilitate soil application<br>• Easy to store (room temperature)<br>• Ensure consistent release of microorganisms depending on nutrient availability in soil<br>• Matrix: provide reliable protection against unfavorable conditions | • Expensive method<br>• Require specific equipment<br>• Complicated production<br>• Not commercially available (limited to laboratory) |

ACKNOWLEDGEMENT

Author contribution to this paper: Nur Maisarah Mohamad Sarbani: Writing-original draft preparation, Nazariyah Yahaya: editing, supervision, and project administration. This review is under a research project which is supported by the Malaysian Ministry of Higher Education, USIM/FRGS-RACER/FST/51219.

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