A Systematic Review: Current Technology of Solid Carrier Formulation to Improve Viability and Effectiveness of Nitrogen-Fixing Inoculant

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ABSTRACT/ABSTRAK

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Tinjauan Sistematis: Teknologi Terkini Formulasi Pembawa Padat untuk Meningkatkan Viabilitas dan Keefektifan Inokulan Penambat Nitrogen

Keywords: Indonesia, PRISMA, Pupuk hayati, Umur simpan Pupuk hayati merupakan solusi alternatif yang menjanjikan untuk mengurangi dampak buruk jangka panjang dari penggunaan pupuk kimia dan berperan penting untuk mendukung pertanian berkelanjutan. Namun, pupuk hayati memiliki umur simpan yang relatif singkat dan sering terjadi penurunan keefektifan mikroba selama penyimpanan dan aplikasi. Oleh karena itu, diperlukan inovasi mengenai formulasi bahan pembawa pupuk hayati yang berpotensi untuk mempertahankan viabilitas dan keefektifan mikroba selama masa simpan. Kajian komprehensif dilakukan dengan metode Systematic Literature Review (SLR) menggunakan mesin pencari untuk mengevaluasi dan menilai status terkini formulasi pembawa padat untuk meningkatkan viabilitas dan efektivitas inokulan pupuk hayati. Hasil tinjauan sistematis literatur ilmiah diperoleh sebanyak 149 artikel bersumber dari ScienceDirect dan Scopus, sebanyak 10 artikel dipilih untuk ditinjau lebih lanjut. Beberapa bahan pembawa dilaporkan dapat meningkatkan viabilitas dan efektivitas inokulan penambat N. Setiap bahan pembawa memberikan berbagai manfaat yang menguntungkan, seperti peningkatan umur simpan mikroba, aktivitas mikroba, dan pertumbuhan tanaman. Beberapa bahan pembawa berpotensi dikembangkan lebih lanjut di Indonesia.

Kata Kunci: Biofertilizer, Indonesia, PRISMA, Shelf life

Biofertilizers are a promising alternative solution to reduce the long-term adverse effects of chemical fertilizers and important for promoting sustainable agriculture. Unfortunately, biofertilizers have a relatively short shelf life, and microbial effectiveness often decreases during storage and application. Therefore, innovation is needed regarding the formulation of biological fertilizer carriers that have the potential to maintain microbial viability and effectivity during storage. The comprehensive study was carried out using Systematic Literature Review (SLR) method by the search engine to evaluate and assess the current status of solid carrier formulation to improve the viability and effectiveness of biofertilizers inoculants. The results of a systematic review of scientific literature were obtained from as many as 149 articles from ScienceDirect and Scopus, and a total of 10 articles were chosen for further review. Several carrier materials have been reported can increase the viability and effectiveness of N-fixing inoculant. Each carrier material provides various benefits, such as increased microbial shelf life, microbial activity, and plant growth. Some carrier materials have the potential for further development in Indonesia.

INTRODUCTION

Biofertilizer is a material that contains living microorganisms that can increase and restore the availability of nutrients for plants. Microorganisms play an important role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients such as N, P, and K. Furthermore, these microorganisms help to improve plant productivity, nutrient availability in the soil, and fertilization efficiency (Itelima et al., 2018). Soil microbes use various mechanisms to restore nutrient availability in the soil, such as N fixation, P solubilization, K solubilization, and Plant Growth Promoting Rhizobacteria (PGPR). Using fertilizers containing beneficial soil microbes have great potential as an environmentally friendly and sustainable nutrient supply for plant cultivation.

Microbes as active ingredients in biofertilizers are generally formulated carriers as temporary habitats before applying biological fertilizers. Biofertilizer carrier material plays an important role in supporting the survival of microbes during their shelf life and effectiveness of microbes (Aksani *et al.*, 2021). Good carrier material for microbial survival must be able to retain moisture, provide nutrients for microbes (organic matter and nitrogen), have suitable pH buffering capabilities, provide sufficient aeration and micropores, and be non-toxic for

inoculants (Lawal & Babalola, 2014; Wang et al., 2015). The concern of biofertilizer carriers is a short storage life followed by a decrease in the microbial population. The evaluation of Phosphate Solubilizing Microbe (PSM) rhizobium inoculants on solid carriers that had been conducted by Shravani et al. (2019) showed that the carriers of peat, wood charcoal, and lignite were only able to maintain the microbial population (viable count) in the first three months. Afterward, a gradual decrease in the viable microbial population was observed in the biofertilizers evaluation.

Several studies have shown that there are various latest carrier technologies to increase the viability and effectiveness of N-fixing microbial inoculants in biofertilizers, which are presented in the results and discussion of this review article. This review article aims to provide scientific information about the enhancement of solid carrier formulation to improve the viability and effectiveness of N-fixing inoculants.

MATERIALS AND METHODS

The research method applied in this article was a Systematic Literature Review (SLR) based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow guidelines (Figure 1).

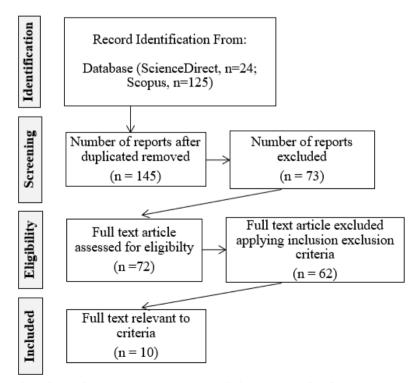


Figure 1. PRISMA Flowchart of Systematic Review on Solid Carrier Technology in N-Fixing inoculant.

The strategy used for the searching in this database was by looking for the terms such as "Solid Carrier Formulation", "Solid Carrier Technology", and "Solid Carrier Innovation" associated with "Nitrogen Fixing Inoculant" and their synonyms. The articles were published between 2012 and 2022. The scope of the study was restricted to documents such as journals, which were fully accessible and indexed by Scopus. As a result, the following search instruction was generated for the database: ("Solid Carrier Formulation" OR "Solid Carrier Technology" OR "Solid Carrier Innovation") AND ("Improve Viability and Effectiveness") AND ("N-Fixing Inoculant" OR "Nitrogen Biofertilizer"). Using of quotation marks ensures that terms containing multiple words are searched simultaneously, preventing individual words from being searched. Articles retrieved from search results are manually reviewed through the title, abstract, and keyword analysis. Based on the review, works are identified to eliminate duplicate articles. Table 1 shows the inclusion and exclusion criteria considered in the selection of the articles as described in Mustapha *et al.* (2022).

Table 1. Selection Criteria

Criteria	Inclusion	Exclusion	Justification
Relevance topics	Journal with focus on solid carrier formulation N- Fixing inoculant technology	Journal without core focus on biofertilizer solid carrier and nitrogen fixing inoculant	To provide information about solid carrier N- biofertilizer formulation technology
Date of publication	2012 - 2022	Years before 2012	To present the latest innovation about N-Fixing solid carrier formulation
Type of Publication	Research article	Books chapter, Encyclopedia, News, Conference abstracts	These were excluded due to limited peer review process
Language of publication	English	All other language	Simplify the review process
Databases	ScienceDirect (Elsevier) and Scopus	Article that are not indexed by Scopus	To provide quality assessment for used journal

RESULTS AND DISCUSSION

Using the PRISMA method, 149 articles were retrieved from the databases (ScienceDirect was 24 articles, and Scopus was 127 articles). After removing four duplicates from the database, 145 articles were examined based on their title and abstract content; 73 were eliminated because they did not consider biofertilizer carriers as the core focus, although several articles present potential carriers that can enhance microbial inoculants indirectly. A total of 72 papers were evaluated: 62 fulfilled the exclusion criteria, resulting in 10 articles being selected for systematic review (Table 1, Figure 1). The articles that were not chosen included 13 articles published before 2012 and 49 non-research articles, including 16 review articles,

two encyclopedias, 25 book chapters, and six conference abstracts.

Biofertilizer carrier material greatly affects viability and effectiveness of microbial inoculants on the soil nutrient cycle to support plant growth. A suitable biofertilizer carrier can maintain microbial activity during storage and application. This review article discusses the potential of carrier materials that can improve the viability and effectiveness of microbes, especially nitrogen-fixing microbial inoculants. There are various types of carrier materials, including tea leave biochar, guar gum, halophyte root, organic matrix, lantana charcoal, sludge ash, banana peels, alginate, maltodextrin, and coal fly ash. There are several processes for the formulation of biofertilizer carrier materials, such as pyrolysis (charcoal), combustion (ashing), powdering, encapsulation, and entrapping

in the matrix. Selected material prioritizes the environment. The biofertilizer carrier formulation principle of being non-toxic to microbes and the of each material is shown in Table 2.

Table 2. Various Solid Carrier Material and Formulation Technology

Author, Year	Carrier Material	Formulation
Araujo, 2019	Pine bark biochar	Slow pyrolysis in a semi-continuous electrically heated reactor produced the biochar in a pilot plant. The carrier materials were ground, sieved through a 70-m sieve, placed in autoclavable plastic pots (20-ml volume), and autoclaved for 20 minutes at 120°C. The inoculum was mixed with a cell protector made up of 1% of locust bean gum and 1% of trehalose (weight: volume). The inoculants were stored at 4-6°C immediately after preparation and without incubation.
Azeem, 2021	Tea leaves biochar	Biochar is derived from the pyrolysis process of tea leaves (<i>Camelia sinensis</i>). Tea leaves biochar was produced at 350°C (B1) and 600°C (B2) for 2 hours (h) at a heating rate of 10°C/min in a pyrolysis tank. The 50 g of biochar carrier was inoculated with 20 mL <i>Bacillus cereus</i> culture. The formula was incubated for 24 before being shade-dried and packed into polyethylene plastic.
Baena- Aristizábal, 2019	Guar gum	Spray drying 200 mL samples using a Buchi B-290 lab scale spray drier at a constant spray flow rate of 819 normal liters per hour (Nl/h), 100% aspirator speed, input temperature of 130°C, and 10% pump rate resulted in microcapsules. In a single-cyclone separator, dry powders were collected.
Hassan, 2018	Halophyte root powder	Buffel grass (<i>Cenchrus ciliaris</i>) was chosen because it has greater levels of sugar, protein, and organic matter. The Buffel grass, a naturally growing halophyte, was harvested when it was 13-15 cm tall. The roots were cleaned with sanitized water, shade-dried for 5-7 days, then processed into powder using a grinder (Anex KC106). The root powder was inoculated by <i>Pseudomonas moraviensis, Bacillus cereus</i> , and <i>Stenotrophomonas maltophilia</i> .
Kumar, 2015	Organic Matrix Entrapped (cow dung, rice bran, neem leaf, and clay soil)	Individually oven-dried agricultural waste (cow dung, rice bran, dried neem (<i>Azadirachta indica</i>), leaf powder, and clay soil) was crushed using a grinder and mixer. Appropriate numbers of <i>A. chroococcum</i> and <i>B. subtilis</i> were added to a matrix containing neem leaves, rice bran, cow dung, and clay soil in a 1:1:1:1 ratio, in combination with 15% saresh (plant gum of <i>Acacia</i> sp.). The components were combined to form an aqua paste in order to bond the matrix. The entire mixture was dried at room temperature to produce (Organic Matrix-Entrapped Biofertilizer) OMEB granules with a diameter of 3 to 4 mm.
Mankar, 2021	Lantana charcoal	Lantana camara stems and branches that had been sun-dried were processed to create 30 kg of lantana biomass (LC). The dry materials were pyrolyzed for two hours in an anaerobic condition at 300°C in a kiln. LC was crushed into a 100-mesh size using a crusher. They were sterilized by autoclaving at 121°C for 15 minutes after having their pH adjusted to neutrality using calcium carbonate solution. In an aseptic environment, a log phase culture of bacteria (CFU: 108–109/ml) was combined with the carrier materials. Formulations were placed in sterile polyethylene bags, leaving one-third empty space, sealed right away, and kept at 4°C.

Table 2. Various Solid Carrier Material and Formulation Technology (Continued)

Author,	Carrier	Formulation	
Year	Material	romulation	
Paliya, 2019	Sludge ash amended	The fine sludge ash and lignite powder were sterilized in an autoclave for 3 hours at 121°C and 15 atm. Rhizobium broth inoculums were combined with sterilized sludge ash and lignite powder at 25-30% to create the biofertilizer. The formulations were kept at room temperature in low-density polyethylene bags.	
Raj, 2021	Banana peels powder	Banana peels were dried at 50°C for 8 hours before being ground into powder. The carrier materials underwent a 15 minutes sterilization process at 121°C and 15 atm. The carrier materials were additionally enhanced with MS nutritional medium. The bio formulation was made in a 1:50:4 ratio of CMC, carrier material, and bacterial suspension culture. The bio-formulation was made with 1 x 108 CFU/ml inoculum.	
Rohman, 2020	Alginate/starch blends	Cassava starch was gelatinized in dispersion at 95°C for 15 minutes. The starch content varies from 1-5% (w/v). The control treatment was only alginate without added starch. As much as 2% (w/v) of alginate was added to each starch dispersion and stirred to produce a bead matrix solution of an alginate/starch mixture. Beads were formed by putting the solution into 2% (w/v) calcium chloride using a 10 ml hand-held syringe with a 1.0 cm diameter. The beads were kept for 30 minutes to fully develop and then collected and rinsed in distilled water (DW) to produce new, moist, uninoculated beads. Inoculated beads were prepared using the same process as uninoculated beads, with the addition of a step to inoculate a 10 ml inoculum into 40 ml of the matrix solution.	
Stojanovic, 2022	Fermentation broth containing <i>B. subtilis</i> NCIM 2063 culture subjected to a 1-hour heat shock at 54°C. Fermented broth sawere combined with varying volumes of maltodextrin and incufor 10 minutes to generate a homogeneous solution (according to Stojanovic, Maltodextrin Box-Benkhen experimental design). Each solution was spray-dri		

Effect of Various Carrier Materials on Viability N-fixing Inoculant

The availability of nutrients in carrier material influences microbial cell viability and microbial populations (Głodowska *et al.*, 2016). Mankar *et al.* (2021) use charcoal derived from the biomass of the weed *L. camara*, which has a high carbon content and a pH that tends to be neutral. According to Yadav & Chandra (2014), the carrier medium should have a high carbon concentration and a neutral pH to maintain viable microbial cells throughout storage, such as *L. camara* charcoal-based carrier material has a higher water-holding capacity (WHC) compared to other carriers such as

lignite, wood charcoal, vermicompost, and manure). The high WHC permits the carrier to hold a greater volume of inoculum (Aloo *et al.*, 2022).

The temperature of pyrolysis in the process of producing biochar is a crucial factor that will affect the microbial life and cell density (Hale *et al.*, 2015). Pyrolysis at low temperatures has been shown to release various volatile organic chemicals that can disrupt the growth and proliferation of microorganisms (Spokas *et al.*, 2011). According to Azeem *et al.* (2021), tea leaf biochar can improve the viability and stability of microbial cells even after three months of storage. These results were obtained

when tea leaves were pyrolyzed at 600°C compared to tea leaves pyrolyzed at 300°C.

Undervalued organic waste from agricultural production can be utilized as an alternate source for carrying biological fertilizers. As in the research by Araujo et al. (2020), biochar based on pine bark waste was used as a carrier for Bradyrhizobium. Biochar is recognized as effective in retaining moisture due to its sponge-like matrix and capacity to maintain microbial life (Wang et al., 2019). Other research has found that biochar is a promising carrier for N2-fixing bacteria found in root nodules such as Rhizobium, Mesorhizobium, Bradyrhizobium (Pastor-Bueis et al., 2019; Jahan et al., 2020; Zhang et al., 2022). The utilization of organic materials of agricultural waste, such as cow dung, rice bran, powdered neem leaves (A. indica), and clay combined with acacia gum in granulated form is undoubtedly rich in nutrients for microbes, soil and plants (Kumar et al., 2015). The formulation contains adequate nutrients supports to improve microbial activity and cell viability during shelf life and application.

Thermal neutralization has been considered an effective method for reducing sludge generated by wastewater treatment plants (Zhang et al., 2018), and sludge ash was produced during this process. The pyrolysis method is commonly used for the thermal neutralization of sludge waste by direct combustion of the sludge in the casting burner. The burning process eliminates hazardous pollutants and odors while also re-energizing the sludge, which gives it the potential to be used as a carrier for biofertilizers. Sludge ash amended is identified to contain a high concentration of carbon, nitrogen, phosphorus, and micronutrients that can encourage rhizobium development (Paliya et al., 2019). The microbial density of sludge ash amended is higher compared to lignite amendment.

Other organic resources, such as halophyte roots and banana peels, are used and ground into powder and used as the carriers (Hassan *et al.*, 2018; Raj *et al.*, 2021). The organic matter, protein, and glucose content of root powder provides a source of microbial C/N in the carrier and when applied to the soil. The carrier's high C/N content provides an energy source for microorganisms to grow and proliferate. Banana peel powder is known to

naturally contain a variety of macro and micronutrients. Hussein *et al.* (2019) reported that dry banana peel contains potassium (78 g/kg), iron (0.6 g/kg), protein (52 g/kg), and tryptophan (0.517 g/kg). The tryptophan amino acid concentration in banana peel is the primary precursor for IAA-producing bacteria (Bhutani *et al.*, 2018). Other investigations have found that giving tryptophan to bacteria like *Rhizobium* and *Azospirillum* increased IAA synthesis (Sarkar & Laha, 2013; Molina *et al.*, 2018).

Alginate is a natural polymer obtained from seaweed and brown algae (Kothale *et al.*, 2020). Alginate creates a hydrogel with a stable polymer structure, which can trap microbial organisms. The viability and shelf life of microbial cells can be preserved during storage and handling via this protective method. Furthermore, the starch added with alginate of cassava is practicable because it is cheap, easy to obtain, biodegradable, and comes from a renewable source (Rohman *et al.*, 2021). The addition of cassava starch increases the strength of the alginate grains and keeps bacteria alive during the encapsulation process and storage.

The encapsulation by spray drying method in Baena-Aristizábal et al. (2019) and Stojanović et al. (2022) research is commonly utilized as a microencapsulation technology in the chemical and food sectors. Bacterial cells in the carrier material emulsion are sprayed in a hot chamber, inducing evaporation and production of microcapsules. The selection of coating material for the encapsulation drying process is a crucial step. Coating materials such as guar gum, a natural polysaccharide produced from the endosperm of Cyamopsis tetragonolobus, are hydrophilic, non-toxic, and biodegradable. Maltodextrin acts as a thickening and preservative agent (Muhamad et al., 2018), extending the shelf life of bacteria in the package. Microencapsulation of B. subtilis with maltodextrin using the spray drying method obtains the formulation stable for up to 1 year of storage (Stojanović et al., 2022). The spray drying method has the potential to improve the microbial resistance and longevity biofertilizer products.

A comparison of shelf life and viable cell microbes in each formulation of biofertilizer is shown in Table 3.

Table 3. Shelf life and viable microbial cells on multiple carriers

Author, Year	Carrier Material	Microbial	Shelf Life (viable cells after storage)	
Araujo, 2019	Pine bark biochar	Bradyrhizobium	12 months stored at 4-6°C (around 10°CFU/g)	
Azeem, 2021	Tea leaves biochar	B. cereus	More than 3 months (around 10° CFU/g)	
Baena- Aristizábal, 2019	Guar gum	Rhizobium leguminosarum bv. trifolii	N/A (around 10 ⁷ CFU dry/g)	
Hassan, 2018	Halophyte root powder	B. cereus, P. moraviensis, S. maltophilia	4 months (around 10 ¹⁰ CFU/g each isolate)	
Kumar, 2015	Organic Matrix Entrapped (cow dung, rice bran, neem leaf, and clay soil)	Azotobacter chroococcum & Bacillus subtilis	N/A	
Mankar, 2021	Lantana charcoal	A. chroococcum	6 months at 4°C (around 10 ⁷ CFU/g)	
Paliya, 2019	Sludge ash amended	Rhizobium	5 months at room temperature (around 10 ⁵ CFU/g)	
Raj, 2021	Banana peels powder	B. asahi & B. cereus	N/A	
Rohman, 2020	Alginate & cassava starch	Rhodopseudomonas palustris	N/A (around 10° CFU/g)	
Stojanovic, 2022	Maltodextrin	B. subtilis	12 months at room temperature (around 10° CFU/g)	

Effect of Various Carrier Materials on the Effectiveness N-Fixing Inoculant

The effectiveness of nitrogen-fixing symbiont microbes cannot be separated from their ability to colonize and nodule. In some types of carriers, there is an increase in nodulation ability and the number of nodules in plants. Pine bark biochar has been reported to increase *Bradyrhizobium* nodulation in pigeon pea roots but has not shown a significant increase in yield (Araujo *et al.*, 2020). Increasing nitrogen fixation of mung bean plants by *Bacillus cereus* was also shown when using the tea leaf biochar biofertilizer (Azeem *et al.*, 2021). Biochar is positively correlated with pH, EC, and nutrient availability in the soil which determines the level of root nodulation of legumes.

The use of amended sludge ash as a carrier also significantly affected nodulation in lentil seeds (Paliya *et al.*, 2019). The content of macro and micronutrients (Mo, Fe, and Cu) in sludge ash plays an important role in the growth of nitrogen-fixing bacteria. The Fe-Mo cofactor is the active site in the nitrogenase enzyme and also a minor component of the nitrate reductase (Paliya *et al.*, 2019). Fe-Mo

cofactor plays a vital role in the nitrogen fixation activity, which leads to nodulation and growth of legume plants (Swain & Abhijita, 2013). The rate of biological nitrogen fixation can increase space and sites of Rhizobium infection in roots, which leads to an increase in the number of nodules. Sludge ash with a slightly alkaline pH is also able to neutralize soil acidity and promote plant growth.

Some carriers also increase microbial activity by producing enzymes and phytohormones that support plant growth. Tea leaf biochar is known to increase microbial biomass and enzyme activity (Azeem *et al.*, 2021). Biochar facilitates a suitable habitat for bacteria growth because it is rich in nutrients (C, N). The organic matrix entrapped biofertilizer (OMEB) can increase the microbial activity of *A. chroococcum* and *B. subtilis*, which is indicated by an increase in dehydrogenase and phosphatase enzymes secreted by microbes (Kumar *et al.*, 2015).

The use of banana peel powder carrier material can increase the activity of *B. asahi* and *B. cereus* in IAA phytohormones secretion in rice (Raj *et al.*, 2021). It is caused by the tryptophan amino

acid in the banana peel powder influencing microbes' synthesis of auxin. IAA is a phytohormone that controls plant growth in addition to mediates plant responses to biotic and abiotic stressors (Emenecker & Strader, 2020). A similar thing was discovered when the halophyte root powder was used as a carrier. Root powder treatment boosted IAA and GA hormone synthesis in wheat plants (Hassan *et al.*, 2018). The microbial consortia in the carrier had been reported to accelerate nutrient availability and acquisition in wheat plants. The microbial activity affects increasing plant growth

and yields such as stem length, root length, plant dry weight, chlorophyll content, nutrient content (protein), and crop yields.

Prospect in Indonesia

Almost all carrier materials in the articles reviewed can be utilized in Indonesia (Table 4). However, it is important to consider the cost, the availability of the material, as well as the availability of tools that can support the process of formulating the biological fertilizer carrier material.

Table 4. Potential use of carrier material in Indonesia

Carrier Material	Potential use in Indonesia	Justification
Pine bark biochar	Yes	Can be developed in Indonesia but only in certain areas that have an area of pine wood forest
Tea leaves biochar	Yes	Can be developed in Indonesia by utilizing the non- resale value of the tea leaf as a beverage product
Guar gum	Maybe	Guar gum in Indonesia is easy to obtain, but we must consider the production capital of guar gum-based biofertilizers
Halophyte root powder	Yes	Buffle grass (C. ciliaris) is widely found in Indonesia
Organic Matrix Entrapped (cow dung, rice bran, neem leaf, and clay soil)	Yes	Due to the extent of the agricultural sector in Indonesia, it is feasible to produce formulation of an organic matrix based on agricultural waste
Lantana charcoal	Maybe	<i>L. camara</i> is commonly used as herbal medicine in Indonesia, allowing it to be used as a carrier in the future
Sludge ash amended	Maybe	Waste sludge can be utilized but the availability of tools for processing sewage sludge into ash have to be reviewed
Banana peels powder	Yes	In the areas of banana production and banana processing industry, it is feasible to make banana peels powder because banana peel waste can be easy to get for free
Alginate & cassava starch Yes Alginate and cassava starch in Indonesia available at affordable prices		Alginate and cassava starch in Indonesia are easily available at affordable prices
Maltodextrin	Maybe	Maltodextrin in Indonesia is generally used by pharmaceutical companies but it is sold in relatively high prices

CONCLUSION

Many innovations in carrier materials can be developed to maintain the viability and effectiveness of microbes in carriers. Various forms of solid carriers, such as biochar, powder, granule, hydrogel, and capsules, have been evaluated. Each carrier

material has advantages in providing nutrients and a beneficial environment for microbial life. Overall, some carrier materials are feasible and practicable to be developed in Indonesia. However, thorough consideration of production costs, availability of raw materials, and tools that support the formulation process is necessary.

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