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The Utilization of Modified Cassava Flour (Mocaf) Industrial Waste and Peat as Carrier of Nitrogen-Fixing Bacteria (NFB) and Phosphate Solubilizing Bacteria (PSB) Inoculant

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Biofertilizer is organic fertilizer with addition of specific microorganisms. Carrier material play an important role in maintaining the viability of microorganisms during storage period. Solid waste of Modified Cassava Flour (Mocaf) has great potency as carrier material of good biofertilizers, because of its nutrient content. Peat has also been used as a biofertilizer carrier for a long time. The aim of this study was to determine the potential of mocaf solid waste in its combinations with peat as the carrier, added by addesive materials (starch and clay), in supporting the growth of NFB and PSB during the storage period and carriers quality. It was factorial experimental using completely randomized design (CRD) as the based design, consist of two factors: carrier formulation (C: C1, C2 and C3); and incubation time (T: T1, T2,… T5). All materials were mixed and sterilized, than inoculated by NFB (*Rhizobium* sp., *Azotobacter* sp.) and PSB (*Bacillus* sp. dan *Pseudomanas* sp.), 10^8 CFU g^{-1} carrier, than incubated for 60 days at room temperature. Bacteria colony were analyzed every 15 days and the quality of carrier (pH, moisture, N-total and C-Organic) were analyzed at the beginning and the end of incubation. The results showed the increasing growth of NFB and PSB until day 60 incubation time in all formulas carriers, reach of 10^8-10^9 CFU g^{-1}. It showed that carriers could support the growth and viability of NFB and PSB. All formula of carriers had fulfilled the quality standard for biofertilizer as assigned by Minister of Agriculture Republic of Indonesia No. 70 year 2011.

Key words: biofertilizer, carrier, mocaf, NFB, peat, PSB

Pupuk hayati adalah pupuk organik dengan penambahan mikroorganisme spesifik. Bahan dasar dalam bahan pembawa inokulum mikroorganisme, memegang peranan penting dalam mendukung kehidupan mikroorganisme selama periode penyimpanan. Limbah padat indusri *Modified Cassava Flour* (Mocaf) memiliki potensi tinggi sebagai bahan pembawa inokulum mikroorganisme pupuk hayati yang baik, karena kandungan nutrisinya. Gambut juga sejak lama telah digunakan sebagai bahan dasar bahan pembawa inokulum mikroorganisme. Tujuan penelitian ini adalah mengetahui potensi limbah padat industri mocaf dalam kombinasinya dengan gambut sebagai bahan pembawa inokulum bakteri dengan penambahan bahan perekat tepung gandum dan lempung, dalam mendukung pertumbuhan bakteri penambat N (BPN) dan bakteri pelarut fosfat (BPF), selama periode penyimpanan dan kualitas bahan pembawa. Rancangan percobaan adalah faktorial dengan rancangan dasar Rancangan Acak Lengkap, terdiri dari dua faktor yaitu formula bahan pembawa (C: C1,C2 dan C3); dan lama penyimpanan (T: T0, T2,…T5). Semua bahan dicampur kemudian disterilisasi, selanjutnya diinokulasi BPN (*Rhizobium* sp., *Azotobacter* sp.) dan BPF (*Bacillus* sp. dan *Pseudomanas* sp.) sejumlah 10^8 CFU g^{-1} bahan pembawa, kemudian diinkubasi selama 60 hari pada suhu kamar. Pengamatan dilakukan terhadap koloni bakteri setiap 15 hari sekali dan kualitas bahan pembawa (pH (H_2O), kadar Air, N-total dan kandungan C- Organik) pada awal dan akhir percobaan. Hasil penelitian menunjukkan terjadinya peningkatan pertumbuhan bakteri hingga periode penyimpanan hari ke 60, dengan koloni bakteri sejumlah 10^8-10^9 CFU g^{-1}. Artinya bahwa bahan pembawa inokulum bakteri ini dapat mendukung pertumbuhan dan kehidupan BPN dan BPF. Selanjutnya diketahui bahwa ke tiga formula bahan pembawa telah memenuhi standar baku mutu kualitas pupuk hayati yang ditetapkan oleh Menteri Pertanian Republik Indonesia No. 70 th 2011.

Kata kunci: bahan pembawa, BPF, BPN, gambut, mocaf, pupuk hayati

Fertilizer demand for agriculture increases 5-10% per year. To support the increasing needs of fertilizer so many studies about utilization of agricultural biomass and industrial waste were done. Large number of small and medium enterprises (SMEs) produce fertilizer derived from agricultural waste and livestock, so the development of organic fertilizer and biofertilizer also increase (Simanungkalit *et al.* 2006). According to Sivasakthivelan and Saranraj (2013), there were many research about formulation of biofertilizer, because formulation of biofertilizer plays a vital role in helping

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to solve many problems in agricultural field in making an organism effective in the field. Carrier material may have allowed better survival of organism. Many materials has been investigated for use as carrier of bacteria, they are charcoal, farmyard manure (FYM), compost, coconut chell powder, vermiculite clay, teak leaf powder, powdered peanut shell, corn cobs, coffee waste, black ash, paddy husk, etc.

Mocaf industrial activities often cause environmental of pollution caused by its solid, liquid and gas waste. The accumulation of bad odor due to the fermentation process indicated that the mocaf waste is the right nutrient for growth of microorganisms (Subagio 2007). Mocaf solid waste has high carbohydrate content reaching 63%-68% and moisture contents level is 20% (Ogbo 2010; Atika and Apsari 2011). The high carbohydrate (Subagio 2007) and moisture contents (Alexander 1976; Foth 1995; Makan *et al.* 2013) in the waste can support microbial activities. By certain treatment the waste could become useful product and not pollute the environment (Chardialani 2008). The waste could be processed into more useful products in agricultural product.

Solid waste of agricultural industry which has high nutrient, potential used as based material of microorganisms carrier, by combined with other material. Peat has been used as the carrier material for seed inoculation for a long time (Simanungkalit *et al.* 2006; Raharjo *et al.* 2007; Forsmann and Kjaergaard 2014). Peat characteristics are moisture and humid which generate good environmental conditions in the growth of microorganisms. Peat also has sufficient permeability for air and water exchange (Somasegaran and Hoben 1994; Allaire *et al.* 1994). Peat is generally as most dependable carrier because of its high content of organic matter and water holding capacity (Sivasakthivelan and Saranraj 2013), so peat is well utilized as a carrier material for seed inoculation.

Rosariastuti *et al.* (2013) explained the mocaf waste characteristics similar with peat and the nutrient content mainly nitrogen and phosphate as a source of nutrients for microorganisms, and it is better than peat. There is no research about the use of solid waste of mocaf industry as carrier of bacteria. Therefore, the potency of mocaf solid waste combined with peat as carrier of functional bacterial is insteresting to be investigated. The purpose of this study was to determine the potency of mocaf solid waste combined with peat as carrier in supporting the growth of nitrogen fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) during the incubation.

MATERIALS AND METHODS

Materials and Equipment. Materials used include: mocaf solid waste obtained from the SME community in Wonogiri - Indonesia, Peat from Histosols soil of Rawapening, Ambarawa - Indonesia, and a bacterial inoculum collection of Microbiology Department, Faculty of Agriculture, Gadjah Mada University in Yogyakarta - Indonesia. Specific media (NFB symbiotic in YMA media, NFB non-symbiotic in Ashby media, PSB in Pikovskaya media and total bacterial in Natrium Agar media) and other chemicals for laboratory analysis.

Research Design and Data Analysis. This research was a laboratory experimental research. The design experimental was completely randomized design (CRD) consist of two factors, first was formulas of the carrier (C) = : C1: 75% Peat +25% mocaf +70g starch kg^{-1}carrier, C2: 75% Peat+25% mocaf+(35g starch + 35g clay) kg^{-1}carrier, and C3: 50% Peat+50% mocaf+70g clays kg^{-1}carrier, second was Incubation time (T): T1: Day - 0, T2: Day – 15, T3: Day – 30, T4: Day - 45, T5: Day - 60) with 2 replications. Each carrier was added by bacterial inoculum : *Rhizobium* sp., *Azotobacter* sp. (NFB), *Bacillus* sp. and *Pseudomanas* sp. (PSB), as much as 10^8 g^{-1} of carrier material.

Data analized by statistical analysis using Anova, followed by Duncan's Multiple Range Test (DMRT) to compare the mean of treatment combinations.

Variables Observations. Variables observations including the viability of bacterial growth (colony number), and quality of carrier: pH, moisture (water content), total nitrogen, total phospate, and organic carbon.

RESULTS

Chemical Analysis of Treatment Formulation. Three formulas carrier chemical composition (final) analysis were that total nitrogen and total phosphate were accordance with the quality standards of macro nutrients (standard N, P_2O5, K_2O minimum 4%); the organic carbon treatment was appropriate quality standard of Organic carbon (standard > 15%), the moisture appropriate with quality standards of water content (standard ≤35); pH only C1 = 5.08 and C2 = 5.05 were appropriate with quality standards pH (standard 5.0-8.0) (Table 1). Based on mocaf in combination with peat potentially used as carrier material of NFB and PSB, and can produce good biofertilizer which has high quality.

Table 1 Results of Chemical Analysis of Carrier in Treatment Formulation

No	Variable	Treatment	Time			
			Early (day 0)		End (day 60)	
			Value	Criteria	Value	Criteria
1	Total Nitrogen (%)	C1	1.01	√	1.16	√
		C2	0.64	√	0.69	√
		C3	0.68	√	0.77	√
2	Total Phospate (%)	C1	8.49	√	8.54	√
		C2	8.45	√	9.11	√
		C3	8.31	√	9.14	√
3	Organic carbon(%)	C1	43.84	√	26.92	√
		C2	36.91	√	19.92	√
		C3	39.28	√	21.53	√
4	Water moisture (%)	C1	34.99	√	26.21	√
		C2	23.46	√	31.75	√
		C3	33.80	√	21.74	√
5	pH	C1	4.79	x	5.08	√
		C2	4.52	x	5.05	√
		C3	4.43	x	4.93	x

Note. (√) means qualified and (x) means not qualified by biofertilizer quality standard according to Minister of Agriculture Indonesia No. 28 year 2009 and Minister of Agriculture of Indonesia No. 70 year 2011.

The Number Symbiotic of Nitrogen-Fixing Bacteria (NFB). The colony numbers of NFB symbiotic (*Rhizobium* sp.) growth in the carrier, at 60 day incubation were 8.311 - 9.471 Log 10 ($2.05x10^8$ - $3x10^9$) CFU g^{-1}. The results of anova, after 60 days incubation showed that the carrier material formulations (P = 0.178) had no significantly effect, whereas the incubation time (P = 0.005) had very significantly affected to the colony number of NFB. During 60 days incubation, there were fluctuation growth of NFB symbiotic *Rhizobium* sp. (Fig 1). At this stage bacterial growth were in exponential phase, which the multiplication of cells was not disordering or because of the availability of nutrients.

The Number of Non-Symbiotic Nitrogen-Fixing Bacteria (NFB). The colony numbers NFB non-symbiotic (*Azotobacter* sp.) lived in the carrier were 6.387 - 6.903 Log 10 ($2.6x10^6$ - $8x10^6$) CFU g^{-1}. Based on the results of the F testlevel of 95%after 60 days incubation showed that the carrier material formulations (P = 0.715) had no significantly effect, whereas the incubation time (P = 0.047) significantly affected to the number of NFB. Based on Figure 2, it could be seen that during 60 days of incubation there were the fluctuation NFB non-symbiotic *Azotobacter* sp. growth (Fig 2). The decrease of the number of *Azotobacter* sp.because of the adaptation process (Lag Phase). In environment bacteria needs to adjust the environmental conditions.

The Number of Phosphate-Solubilizing Bacteria (PSB). Each population of PSB microbial isolate inoculated into the carrier formulations can be associated with the ability of phosphate dissolve and pH changes. The observation of microbial populations of phosphate solubilizing bacteria are presented (Fig 3). Based on anova, incubation showed highly significantly (P = 0.001) influenced on the number of PSB, while the formulas of carrier material and the interaction between incubation time and formulas were not significantly effected (P = 0.484) to the number of PSB. Based on the DMRT showed that there were significantly differences in the changing of the number of PSB. Figure 3 showed that the incubation time, increase of the number of bacterial colonies. It can be seen on the 30th day of incubation, the number of colonies reached 9.616- 10.571 Log 10 ($4.1x10^9$ - $3.7x10^{10}$) CFU g^{-1}. *Bacillus* sp. and *Pseudomonas* sp. isolates could degrade the nutrients present in a carrier material, so the bacterias still in increasing growth until the 60th day of incubation.

The Number of Total Bacterial. The next observation was analyzing the total bacterial viability consisting of a consortium bacteria such as *Rhizobium* sp., *Azotobacter* sp., *Bacillus* sp. and *Pseudomonas* sp. growth in all carrier formulations. The colony numbers of total bacterial lived in the carrier were 9.613 - 10.571 Log 10 ($4.09x10^9$ - $1.6x10^{10}$) CFU g^{-1}. The results of anova, after 60 days incubation showed that the

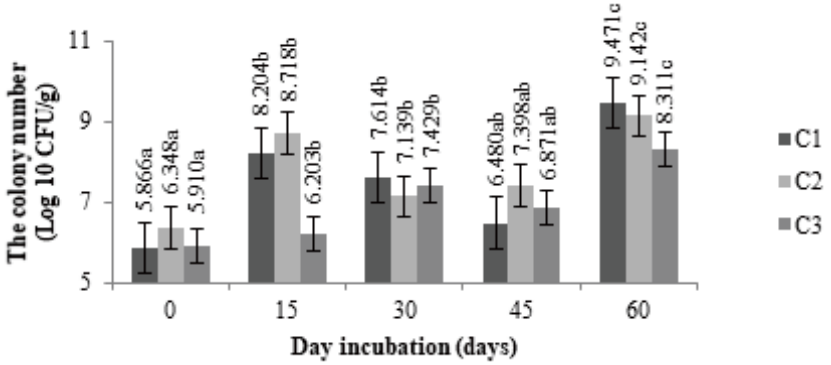

Fig 1 Histogram number colony of *Rhizobium* sp. during 60-days incubation.

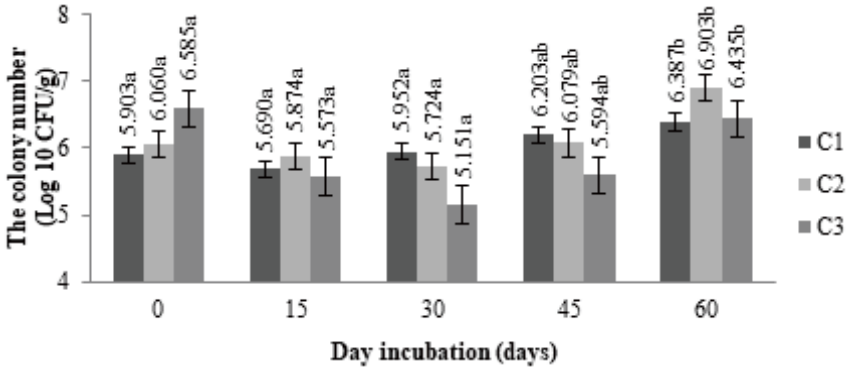

Fig 2 Histogram the number colony of *Azotobacter* sp. during 60-days incubation.

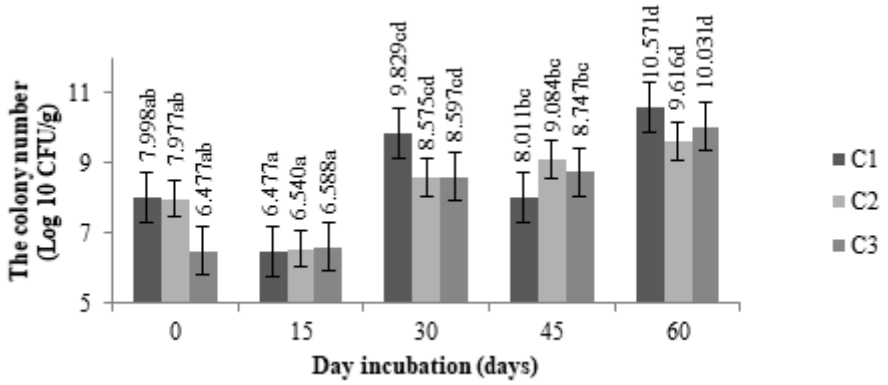

Fig 3 Histogram number colony of PSB during 60-days incubation.

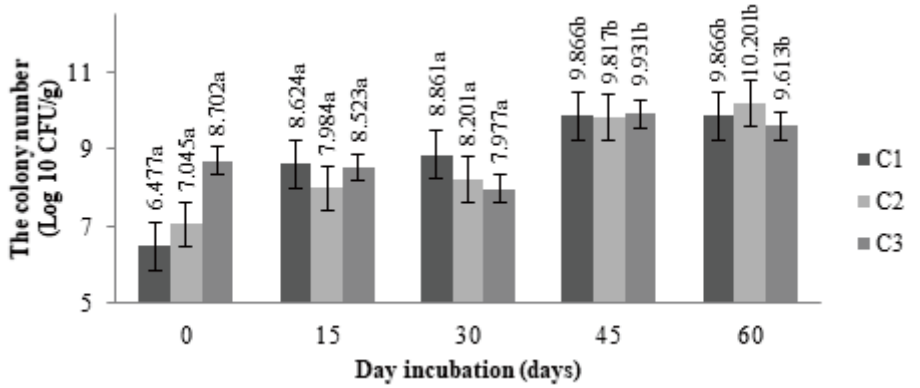

Fig 4 Histogram number of total colony bacterial during 60-days incubation.

formulas of carrier material (P = 0.757) had no significantly effected, whereas the incubation time (P = 0.005) had very significantly effected to the total bacterial. The observation of total bacterial is presented in Figure 4. Incubation time had highly significantly effected (P = 0.005) to the total number of bacterial colonies, whereas the formulas of carrier material and the interaction between incubation time and formulas of carrier material are not significantly affected (P = 0.757) to the total number of bacterial colonies.

DISCUSSION

There were many research about formulation of biofertilizer for example charcoal + soil mixture is the best carrier for BPF; FYM + soil, FYM + charcoal, soil + FYM + charcoal, were good for Azospirillum carrier, etc. (Sivasakthivelan and Saranraj 2013). In Indonesia, characteristics of carrier materials in biofertilizer should refer to the government quality standards of the Minister of Agriculture of Republic of Indonesia No. 28 year 2009 and No. 70 year 2011 about organic fertilizer, biofertilizer and soil improvement. The growth rate was determined by the composition of the media and environmental factors, while *Rhizobium* sp. population decline due to nutrient reduced, resulting the competition among the NFB to acquire nutrients (Mansur *et al.* 2003). Moreover, mocaf waste would be energy source of *Rhizobium* sp. that could increasing the population. This was same with the statement of Ahmad *et al.* (2011), that the high content of protein, starch and total sugars in the waste water of mocaf flour industry made the waste became source of carbon and nitrogen for microbial growth. One of the factors of population decline of *Rhizobium* sp. was pH. pH between 4.5 to 5.1 on all treatments cause declining bacterial populations. According to Martani and Margino (2005), most *Rhizobium* sp. bacteria have good growth at neutral pH and optimum at a pH of 5.5 to 7.0. According to Kawuri Research (1997), *Rhizobium* sp. has the ability to adapt within the low pH (4.0 to 5.7) by realizing response called Acid Tolerance Response (ATR). In this research, low pH causes the bacteria undergo to adaptation process (Lag Phase), followed by the increasing growth of *Rhizobium* sp. which have ability to live in the low pH environment.

According to Wyss *et al.* (1961), *Azotobacter* needs a break from the vegetative cells to survive in adverse environmental factors. After the environment become in optimum conditions, including certain pH value, carbon source and new vegetative cells, *Azotobacter* sp. would grow and return to the exponential phase (log phase). The increase of bacteria population growth in each carrier formulation cause by the availability of energy source of bacteria in the form of simple sugars such as sucrose and glucose. According to Stella and Sivasak (2009) the addition of nutrients such as glucose and sucrose led the increasing of the viability of microbes in biofertilizer. Viability of *Azotobacter* sp. increased due to the sources energy derived from mocaf waste. Chun and Vidaver (2001) found *Bacillus* sp. reached to a lag phase quickly relatively and the process to reached rapid exponential phase was also quickly relatively. Simultaneously on the 30th days incubation the number of colonies on all formulations of carrier material were increasing.

NA media is not selective media, but could be used as an indicator of microbial growth. NA media has rich of protein, nitrogen, vitamins and from beef extract and peptone (Simanungkalit 2001). The result of Total bacterial viability test during incubation time could be used as a marker of the carrier material quality.

Good carrier materials are expected to maintain the viability and the effectiveness of microbial inoculant during storage (Rao 1982). In accordance with the biofertilizers, quality standards of biofertilizers Regulated by the Minister of Agriculture of Republic of Indonesia No. 28 year 2009 and No. 70 year 2011 about Organic Fertilizer, Biofertilizer and Soil improvement, biological fertilizers consortium must have at least 10^7 CFU g^{-1}, pH value between 5.0 to 8.0 with a moisture of <30% in a state of solid fertilizer.

The Carrier formulation in this research potential to support the growth and sustain the life of functional NFB and PSB bacterial. So they were potential use as carrier material in making biofertilizers. Functional bacterial in specific media accordance with quality standards. The pH value during incubation period was within the proper criteria and the viability of total bacteria supported the development of carrier material formulations. These carrier formulation are new, because they have never investigated before.

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