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## The Viability of Selected Potassium Solubilizing Rhizobacteria in a Mixture of K-Feldspar and Organic Matter as Carrier Material

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Abstract. The potassium solubilizing biofertilizer based on selected potassium solubilizing bacteria (KSB) can facilitate availability of potassium in primary silicate minerals containing potassium to be absorbed by the plants. The key to successful application of biofertilizer in showing positive effects on inoculated plants is the selection of a carrier material that can guarantee viability of inoculants during shelf life before being applied in the field. The purpose of this study was to determine viability of three selected KSB isolates which were formulated in a mixture of K-feldspar (KF), rice straw compost (RSC), rice husk biochar (RHB), and Aleksandrov liquid medium (ALM) as a carrier material for 24 weeks of shelf life. The experiment used a completely randomized design with nine treatments of carrier material composition and three replications. The results showed formula of potassium solubilizing biofertilizer with a composition of 1% mixed culture KSB + 15% KF + 30% RSC + 45% RHB + 9% ALM was the best formula with high inoculant viability during 4 weeks of shelf life. It was evidenced by the increase in respiration and total KSB population by 17.2% and 213.2%, respectively, compared to formula that produced the lowest respiration and total KSB population.

#### 1. Introduction

The availability of potassium in the soil is very limited because it is largely integrated in the structure of primary silicate minerals such as K-feldspar [1, 2, 3]. Potassium solubilizing bacteria (KSB) formulated in potassium solubilizing biofertilizer can facilitate the availability of potassium in the soil to be absorbed by plants from K-feldspar that already exists in situ or to be applied ex situ into the soil as potassium ameliorant. Potassium solubilizing bacteria produces organic acids to explore sources of insoluble potassium to become soluble in soil solution to use in its metabolism and at the same time can be absorbed by plants [4, 5, 6]. Potassium dissolution occurs when potassium ions as balancing cations in mineral structures are replaced by ionized hydrogen ions from organic acids through the hydrolysis process [7].

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Twenty five KSB isolates could be isolated from the rhizosphere of maize growing on Inceptisols in Jatinangor using Aleksandrov agar medium with K-feldspar as sole source of potassium [8]. The selection result of these KSB isolates obtained three selected isolates which had high activity in dissolving K-feldspar, i.e. BPK-DHJ3-3150, BPK-DHJ1-4125, and BPK-DHJ2-5250 [9]. The three selected KSB isolates have been identified as *Burkholderia cenocepacia*, *Streptomyces pseudovenezuelae*, and *Klebsiella* sp. based on molecular characterization of 16 rRNAs and their activity produced organic acids (coumaric acid, citric acid, virulat acid, syringic acid, malic acid, and oxalic acid) in Aleksandrov liquid medium. These isolates were used in current study as a basic to formulated potassium solubilizing biofertilizer as a potassium availability facilitator to the plants which would be expected to have high viability over a shelf life.

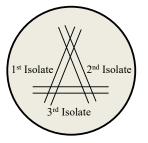
Potassium solubilizing biofertilizer formula will have a positive effect on inoculated plants if it uses selected KSB isolates that have obtained from the selection of their activity at dissolving potassium. In addition, the selection of a carrier material that can guarantee the viability of microbial inoculants to provide a conducive living environment in order to obtain a minimum amount of total microbial population in accordance with the requirements of the biofertilizer, a relatively long shelf life, and its ability to compete with other microbes when it is applied in the field will be the key to successful application of biofertilizers [10, 11, 12, 13, 14, 15, 16]. Carrier materials are an important part of the biofertilizer formulation and must be able to support the intended microbes in the required amounts in biological fertilizers [17]. Potassium solubilizing biofertilizer formulas which be tested in this study were based on selected KSB isolates formulated in a solid carrier material.

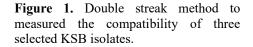
The carrier material composition in the formulation of potassium solubilizing biofertilizer on this study consisted of organic materials (rice straw compost and rice husk biochar), K-feldspar, and Aleksandrov liquid medium mixed with a certain composition in accordance with the tested treatment. Rice straw and rice husk are abundant available as agricultural waste with production of grain:straw at a ratio 1:4 while grain:husks at ratio 1:0.24 [18, 19]. Carbon in organic matter serves as a source of energy to the bacterial activity during the shelf life [5, 20]. Biochar is composed of fine pores with high inner surface area. It becomes a conducive habitat to bacterial inoculants [21, 22, 23, 24, 25]. Potassium insoluble in K-feldspar and Aleksandrov liquid medium can guarantee the continuation of the potassium solubilizing activity of selected KSB isolates during the shelf life. The selected KSB isolates with high viability during shelf life in the carrier material are expected to be effective at facilitating the availability of potassium to be absorbed by plants when applied in the field.

#### 2. Methodology

#### 2.1. Compatibility study of selected KSB isolates

Compatibility study of selected KSB isolates were carried out by the double streak method (figure 1) [26, 27]. Each of the three selected KSB isolates was grown etched in a straight line cut each other on the surface of nutrient agar in a petri dish and incubated in an incubator (33 °C) for three days. Observations have been made of clear zones presence at the confluence of the three KSB isolates that were etched. If a clear zone was formed, it would be indicating that there was antagonistic properties among the KSB isolates. If no clear zone was formed, the KSB isolates were compatible one another.





2.2. Viability study of selected KSB isolates in solid carrier material

This study used the experiment method that carried out based on a single factor experiment in a completely randomized design with nine treatments and three replications. The treatments tested were shown in the table 1 below.

No.	Treatment			Composition (%	)					
INO.	Treatment	KSB <sup>a</sup>	КF <sup>ь</sup>	RSC <sup>c</sup>	RHB <sup>d</sup>	ALM <sup>e</sup>				
1.	$F_1$	0.5	15	30	45	9.5				
2.	$F_2$	0.5	30	30	30	9.5				
3.	F <sub>3</sub>	0.5	45	30	15	9.5				
4.	$F_4$	1	15	30	45	9				
5.	$F_5$	1	30	30	30	9				
6.	$F_6$	1	45	30	15	9				
7.	$F_7$	1.5	15	30	45	8.5				
8.	$F_8$	1.5	30	30	30	8.5				
9.	F9	1.5	45	30	15	8.5				

Table 1. Treatment of potassium solubilizing biofertilizer formulations.

<sup>a</sup>KSB, KSB was an acronym for mixed culture potassium solubilizing bacteria.

<sup>b</sup>KF, K-feldspar.

°RSC, rice straw compost.

<sup>d</sup>RHB, rice husk biochar.

eALM, Aleksandrov liquid medium.

Potassium solubilizing biofertilizer was formulated by inoculating the mixed culture of KSB isolate (Burkholderia cenocepacia, Streptomyces pseudovenezuelae, and Klebsiella sp.) into a solid carrier material consisted a mixture of K-feldspar (24.43% K<sub>2</sub>O, 0.29% moisture content, pass 100 mesh sieve), rice straw compost (17.03% organic-C, C:N ratio 12, 1.21% K<sub>2</sub>O, 7.71 pH, 8.58% moisture content, pass 4 mesh sieve), biochar rice husk (22.14% organic-C, C:N ratio 35, 0.56% K<sub>2</sub>O, 6.56 pH, 3.72% moisture content, pass 4 mesh sieve), and Aleksandrov liquid medium. The stages of potassium solubilizing biofertilizer formulation were as follows: (i) A total of 100 g carrier material of the potassium solubilizing biofertilizer formula with the composition in accordance to the treatment was put in a package of aluminium foil as an experimental unit and then sealed. The water content of the carrier material was regulated to 45%. The experimental unit was sterilized using an autoclave at 121 °C and a pressure of 1 atm for 15 minutes. Preparation of the carrier material was done at CV. Bintang Asri Arthauly, Bandung; (ii) Each selected KSB isolates on Aleksandrov slant agar medium was taken one full loop with ose and then inoculated into 100 mL nutrient broth medium and shaken on a shaker at a speed of 120 rpm for 11 hours at room temperature (26 °C); (iii) Each isolate was inoculated 33.3% by injecting it into the carrier material as mixed culture of KSB isolate in accordance to the composition of the treatment dose. Initial population density of Burkholderia cenocepacia, Streptomyces pseudovenezuelae, and Klebsiella sp. were 2.18 x 10<sup>10</sup> cfu.mL<sup>-1</sup>, 3.84 x 10<sup>10</sup> cfu.mL<sup>-1</sup>, and 2.96 x 10<sup>10</sup> cfu.mL<sup>-1</sup>, respectively; and (iv) The experimental units were placed randomly at room temperature (26 °C) in the Soil Biology Laboratory, Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran.

Observations of measured treatment responses in the carrier material were as follows: (i) Respiration on 1 (T<sub>1</sub>), 2 (T<sub>2</sub>), 3 (T<sub>3</sub>), 4 (T<sub>4</sub>), 8 (T<sub>8</sub>), 12 (T<sub>12</sub>), 16 (T<sub>16</sub>), 20 (T<sub>20</sub>), and 24 (T<sub>24</sub>) weeks of shelf life were determined using the titration method [28]; (ii) pH on 1, 2, 3, 4, 8, 12, 16, 20, and 24 weeks of shelf life were determined using the electrometric method [29]; and (iii) Total KSB population on 1, 4, 12, and 24 weeks of shelf life were determined using the electrometric at 5% significance level was carried out to determine the effect of treatments to the measured responses and if there was a significance effect continued with Duncan's test at 5% significance level to determine differences between treatments. Correlation analysis was performed to determine the relationship between responses [32]. The statistical analyzes were carried out using SPSS software version 15.0.

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The best formula of potassium solubilizing biofertilizer was chosen based on: (i) Interpretation of Duncan's test results between the shelf life of each treatment response. Determine the shelf life that produces the highest respiration and total KSB population values. Determine the shelf life that produces the lowest pH value; and (ii) At the selected shelf life based on i item, determine the response that had the strongest correlation coefficient to all responses and then choose the treatment that was significantly different from all treatments on that response.

#### 3. Results and discussion

#### 3.1. Compatibility of selected KSB isolates

The confluence of the three KSB isolates etched on the nutrient agar surface did not form clear zone so that the isolates were compatible one another (figure 2). The purpose of compatibility study is to find out microbes that are grown together in a growth medium do not have antagonistic properties that can inhibit the growth of these microbes [26, 27].



**Figure 2.** Compatibility of three selected KSB isolates. *Burkholderia cenocepacia* (No. 17), *Streptomyces pseudovenezuelae* (No. 2), and *Klebsiella* sp. (No. 16).

The three selected KSB isolates could be used in the formulation of mixed culture potassium solubilizing biofertilizer based on the results of the compatibility study. Inoculants in the mixed culture biofertilizer formula can be formed from a combination of several single microbial cultures which are synergistic in increasing the growth of each microbe [11].

#### 3.2. Viability of KSB isolates in solid carrier material

Respiration of all potassium solubilizing biofertilizer formulas tended to increase until 4 weeks of shelf life. After that, it tended to decrease until the end of shelf life. The respiration average at 4<sup>th</sup> week of a shelf life was higher and significantly different than the other shelf life (table 2).

T	Respiration (mg CO <sub>2</sub> -C.kg <sup>-1</sup> .day <sup>-1</sup> )								
Treatment	T1	T <sub>2</sub>	T <sub>3</sub>	T_4	T <sub>8</sub>	T <sub>12</sub>	T <sub>16</sub>	T <sub>20</sub>	T <sub>24</sub>
$F_1$	49.2	51.6 b	63.2	72.6 al	o 44.6	26.8	2.5	4.2	3.6
$F_2$	48.0	51.6 b	64.0	71.4 al	<b>46.4</b>	22.8	2.4	3.6	3.0
F <sub>3</sub>	46.4	43.5 a	60.0	68.6 a	42.8	26.4	3.0	3.4	1.8
$F_4$	49.6	66.4 c	69.6	80.4 c	48.8	24.0	2.5	4.4	3.6
F <sub>5</sub>	49.2	54.6 b	63.6	76.8 b	e 46.8	23.4	2.4	4.0	3.6
$F_6$	48.6	49.2 b	59.6	72.0 al	46.8	23.2	2.4	3.6	2.4
$\mathbf{F}_7$	49.2	66.0 c	74.0	74.0 b	e 46.0	25.2	3.6	6.0	3.2
F <sub>8</sub>	46.8	52.4 b	66.4	72.0 al	45.0	24.6	4.2	3.6	2.4
F9	47.6	52.4 b	61.6	70.4 al	45.0	25.2	2.4	2.4	3.0
$\overline{x}$	48.3 C	54.2 D	64.7 E	73.1 F	45.8 C	24.6 B	2.8 A	3.9 A	3.0 A

**Table 2.** The effect of potassium solubilizing biofertilizer formula on respiration.

The number followed by the same letter notation was not significantly different based on Duncan's test at 5% significance level. Lowercase notation was read vertically based on columns while uppercase notation horizontally based on row. The treatments did not significantly affect responses based on analysis of variance at 5% significance level were not tested further and were not given letter notation.

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The pH of potassium solubilizing biofertilizer formulas in the 24<sup>th</sup> week at the end a shelf life decreased compared to the 1<sup>st</sup> week of a shelf life. The pH average on 4, 20, and 24 weeks of shelf life were lower and significantly different than the pH on 1, 8, and 16 weeks of shelf life (table 3).

Treatment					pН				
Treatment	$T_1$	$T_2$	T <sub>3</sub>	$T_4$	$T_8$	T <sub>12</sub>	T <sub>16</sub>	T <sub>20</sub>	T <sub>24</sub>
$F_1$	7.6	7.5	7.4 ab	7.6 b	7.3 a	7.7	7.7 abc	7.3	7.4
$F_2$	7.6	7.4	7.6 bc	7.5 ab	7.9 b	7.4	7.5 ab	7.5	7.4
F <sub>3</sub>	8.0	7.9	7.8 c	7.7 b	7.9 b	7.4	7.8 bc	7.8	7.5
$F_4$	7.5	7.3	7.4 ab	7.3 a	7.4 a	7.4	7.5 ab	7.2	7.3
$F_5$	7.8	7.5	7.6 bc	7.3 a	7.6 ab	7.5	7.6 ab	7.6	7.4
$F_6$	7.6	7.8	7.7 c	7.4 ab	8.0 b	7.6	7.7 bc	7.5	7.6
$\mathbf{F}_{7}$	7.5	7.3	7.3 а	7.3 a	7.4 a	7.4	7.4 а	7.3	7.3
$F_8$	7.6	7.8	7.4 ab	7.5 ab	7.6 ab	7.6	7.6 ab	7.4	7.5
F9	7.8	7.6	7.6 bc	7.5 ab	7.7 ab	7.7	7.9 c	7.4	7.5
x	7.7 B	7.6 AB	7.5 AB	7.4 A	7.6 B	7.5 AB	7.6 B	7.4 A	7.4 A

Table 3. The effect of potassium solubilizing biofertilizer formula on pH.

The number followed by the same letter notation was not significantly different based on Duncan's test at 5% significance level. Lowercase notation was read vertically based on columns while uppercase notation horizontally based on row. The treatments did not significantly affect responses based on analysis of variance at 5% significance level were not tested further and were not given letter notation.

The total KSB population in all potassium solubilizing biofertilizer formulas tended to increased until the 4<sup>th</sup> week of a shelf life and decreased until the end of a shelf life. The total KSB population average at the 4<sup>th</sup> week of a shelf life was higher and significantly different than the 12<sup>th</sup> and 24<sup>th</sup> week of a shelf life, except with the 1<sup>st</sup> week of a shelf life was not significantly different (table 4).

T		Total KSB Pop	ulation (	x 10 <sup>5</sup> cfu.g <sup>-1</sup> )	
Treatment	T1	T <sub>4</sub>		T <sub>12</sub>	T <sub>24</sub>
$F_1$	4.8	6.9 a	ı	4.3	3.3
$F_2$	4.9	5.1 a	ı	4.1	2.4
F <sub>3</sub>	4.3	4.9 a	ı	3.6	3.4
$F_4$	7.7	11.9 b	,	6.2	2.8
F <sub>5</sub>	6.8	7.7 a	ıb	7.1	3.3
$F_6$	4.5	3.8 a	ı	3.9	3.2
F <sub>7</sub>	6.1	11.8 b	,	5.5	3.8
$F_8$	5.7	7.5 a	ıb	5.1	3.1
F9	5.1	4.8 a	ı	4.5	2.6
$\overline{x}$	5.5 BC	7.2 C		4.9 B	3.1 A

Table 4. The effect of potassium solubilizing biofertilizer formula on total KSB population.

The number followed by the same letter notation was not significantly different based on Duncan's test at 5% significance level. Lowercase notation was read vertically based on columns while uppercase notation horizontally based on row. The treatments did not significantly affect responses based on analysis of variance at 5% significance level were not tested further and were not given letter notation.

All potassium solubilizing biofertilizer formula treatments showed significant differences in respiration, pH, and total KSB population at the 4<sup>th</sup> week of a shelf life (tables 2-4). Respiration showed a strong negatively correlation with pH (r = -0.82) and a strong positively correlation with the total KSB population (r = 0.74) [33] at the 4<sup>th</sup> week of a shelf life (table 5).

Table 5. Correlations between respiration,		d total K	SB population	in potassium	solubilizing
biofertilizer formula at the 4 <sup>th</sup> week of a shell	f life.				

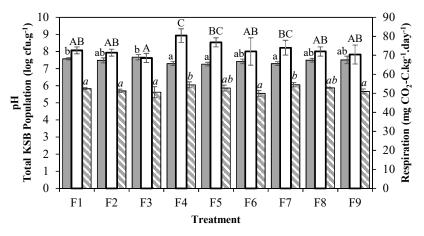
Response	Correlation Coefficient $(r)$						
Response	Respiration	pН	Total KSB Population				
Respiration	1	-0.823ª	0.743 <sup>b</sup>				
pH	-0.823ª	1	-0.637				
Total KSB Population	0.743 <sup>b</sup>	-0.637	1				
	· · 10/ · · · · · · 1	1					

<sup>a</sup>Correlation had a significant effect at 1% significance level.

<sup>b</sup>Correlation had a significant effect at 5% significance level.

The respiration response that had due to the influence of various potassium solubilizing biofertilizer formula at the 4<sup>th</sup> week of a shelf life was chosen to represent the selection of the best potassium solubilizing biofertilizer formula. The F<sub>4</sub> potassium solubilizing biofertilizer formula with a composition of 1% mixed culture KSB + 15% KF + 30% RSC + 45% RHB + 9% ALM was the best potassium solubilizing biofertilizer formula. It showed the highest respiration and was significantly different from other formulas, except with the F<sub>5</sub> and F<sub>7</sub> formulas were not significantly different (table 2, figure 3). The F<sub>4</sub> formula produced the highest respiration and total KSB population with an increase of 17.2% and 213.2% compared to the F<sub>3</sub> formula (0.5% mixed culture KSB + 45% KF + 30% RSC + 15% RHB + 9.5% ALM) and F<sub>6</sub> formula (1% mixed culture KSB + 45% KF + 30% RSC + 15% RHB + 9% ALM), respectively. In addition, the F<sub>4</sub> formula showed the lowest pH at the 4<sup>th</sup> week of a shelf life.

■pH ■Total KSB Population ■Respiration



**Figure 3**. The effect of potassium solubilizing biofertilizer formula on respiration, pH, and total KSB population at the 4<sup>th</sup> week of a shelf life. Error bar was mean value  $\pm$  standard deviation. Mean value right at the top of bar followed by the same letter notation was not significantly different based on Duncan's test at 5% significance level. Uppercase notation used for respiration, lowercase pH, and italics lowercase for total KSB population.

Potassium solubilizing biofertilizer formula with composition of 1% mixed culture KSB + 15% KF + 30% RSC + 45% RHB + 9% ALM with 4 weeks of shelf life was the best formula obtained from this study. The result was in line with previous study that showed a carrier material with a mixture of 40% K-feldspar + 60% rice straw compost + 2% KSB (*Bacillus cereus*) produced the highest KSB activity, however on a longer shelf life (12 weeks) [34]. The biochemical characteristics of the best formula obtained from this study were a pH of 7.3 (table 3) and a total KSB population of 1,2 x 10<sup>6</sup> cfu.g<sup>-1</sup> (table 4). The biochemical requirements of biofertilizer in solid form at the shelf life are pH of 6.0-7.5 and a total microbial population of 1 x 10<sup>6</sup> cfu.g<sup>-1</sup> [35]. In addition, the technical requirements

for biological fertilizers consisting of two or more microbial genera in mixed culture must have a minimum microbial population of  $1 \times 10^6$  cfu.g<sup>-1</sup> at the end of the shelf life [36].

The best potassium solubilizing biofertilizer formula with 4 weeks of shelf life was a conducive carrier material for the viability of mixed culture KSB isolates before being applied in the field. K-feldspar as a source of insoluble potassium still ensured continuity of the potassium dissolving activity by mixed culture of KSB isolates during the shelf life. The carbon content in rice straw compost was an energy source that used by mixed culture KSB isolates for 4 weeks of shelf life with high viability. Potassium solubilizing bacteria is a heterotrophic bacterium that obtains carbon sources to produce energy from organic matter [20]. The organic carbon content serves as a source of energy to the bacterial activity [5]. Rice husk biochar was a place of life to the mixed culture KSB isolates during the shelf life. It has fine porous with an inner surface area can reach 200-400 mm<sup>2</sup>.g<sup>-1</sup> [24]. The biochar morphology is composed by pores which can be a conducive habitat to the bacterial inoculants [21, 22, 23, 25].

The metabolic activity of mixed culture KSB isolates in the carrier material during the shelf life was determined through respiration measurement. Respiration shows the amount of CO<sub>2</sub> produced by microbial activity. It has a good correlation with other responses related to microbial activity such as total microbial population, organic matter content, pH, transformation of nitrogen, phosphorus and potassium nutrients. Maximum respiration will occur when the microbial population has reached its maximum number [28]. In contrast to this study, previous studies have not used respiration as an indicator of KSB activity in carrier materials during the shelf life. The results of this study showed respiration of mixed culture KSB isolates in the formula of potassium solubilizing biofertilizer was strong negatively correlated to pH (r = -0.82) and strong positively correlated to the total KSB population (r = 0.74) [33] at the 4<sup>th</sup> week of a shelf life (table 5). An increase in respiration would be followed by a decrease in pH and an increase in the total KSB population.

The mixed cultures of KSB isolates could dissolve potassium in K-feldspar which was insoluble to be dissolved actively with energy derived from rice straw compost and rice husk biochar. Potassium dissolving activity by KSB is facilitated by organic acids which are produced by themselves and can reduce the pH of the medium [37, 38, 16, 39, 40]. Previous study showed the KSB activity to disolved K-feldspar in Aleksandrov liquid medium had a strong negatively correlation with pH (r = -0.9) [9]. Selected KSB isolates which used in present study produced organic acids mainly coumaric acid in dissolving potassium contained in K-feldspar mineral. Potassium solubilizing bacteria explore sources of insoluble potassium so that it become dissolved to meet the needs of potassium [5, 6]. The function of K<sup>+</sup> ions in bacterial cells playe a role in the process of regulating homeostatic turgor pressure and maintain the cytoplasmic pH around neutral [41, 42]. Adequacy of K<sup>+</sup> ions in mixed culture of KSB isolate cells made the metabolic process work well so that it stimulated cell division and increased in the total KSB population.

In this study, the highest viability of the mixed culture of KSB isolates in the carrier was achieved at the 4<sup>th</sup> week of a shelf life then decreased drastically. This may due to the ineffective sterilization of the carrier material so that the intended microbes were unable to compete with indigenous microbes in the carrier material to obtain nutrition and optimal environmental factors or the accumulation of microbial metabolites results in becoming toxic substances for the intended microbial inoculants. However, the 4 weeks of shelf life was a relatively short when compared to previous studies regarding the use of solid carriers to provide biofertilizer inoculant viability. Bacteria inoculant in the carrier material consisted of rice straw compost mixture with 40% K-feldspar could increased the dissolving activity of potassium in the carrier during 12 weeks of shelf life (3 months) with lower organic C at the end of shelf life when compared to other carrier material compositions because it has been used as a energy source by inoculant and lost as CO<sub>2</sub> [34]. The shelf life of biofertilizer based on solid carrier is generally around six months. Long shelf life with high microbial cell viability is a challenge in the formulation of biofertilizers based on solid carriers. Several strategies to increase the extended shelf life include the selection of the appropriate sterilization method such as gamma irradiation to ensure that the microbes growing in the carrier material are the intended microbe and enrichment of solid carriers with additional nutrients or microbial cell protectors such as sucrose, maltose, trehalose, molasses, glucose and glycerol [15]. Further study will be recommended to improve the best

potassium solubilizing biofertilizer formula that has been obtained so that it has an extended shelf life of more than 4 weeks.

#### 4. Conclusion

Respiration resulted from various tested potassium solubilizing biofertilizer formulas showed a strong positively correlation with the total KSB population and a strong negatively correlation with pH at the 4<sup>th</sup> week of a shelf life. The formula of potassium solubilizing biofertilizer with a composition of 1% mixed culture KSB + 15% KF + 30% RSC + 45% RHB + 9% ALM with 4 weeks of shelf life had the potential to be a potassium solubilizing biofertilizer with high viability which was indicated by the high value of respiration and total KSB population and low pH compared to other formulas that could facilitate potassium availability to plants effectively when applied in the field.

#### 5. References

- [1] Brady N C 1984 *The Nature and Properties of Soils* (New York: Macmillan Publishing Company) chapter 10 pp 327–362
- [2] Foth H D 1990 Fundamentals of Soil Science (New York: John Wiley & Sons) chapter 12 pp 186–209
- [3] [DIKTI] Directorate General of Higher Education 1991 *Soil Fertility* (Jakarta: Directorate General of Higher Education Republic of Indonesia) chapter 8 pp 151–172 (in Indonesian)
- [4] Meena V S, Maurya B R and Verma J P 2014 Microbiological Research 169 337–47
- [5] Alexander M 1977 Introduction to Soil Microbiology (New York: John Wiley & Sons) chapter 23 pp 380–401
- [6] Bennett P C, Rogers J R and Choi W J 2001 Geomicrobiology Journal 18:3-19
- [7] Hardjowigeno S 1993 Soil Classification and Pedogenesis (Jakarta: Akademika Pressindo) chapter 9 pp 175–199 (in Indonesian)
- [8] Herdiyantoro D, Simarmata T, Setiawati M R, Nurlaeny N, Joy B, Hamdani J S, Handayani I 2018 Pros. Sem. Nas. Masy. Biodiv. Indon. 4 178–83
- [9] Herdiyantoro D, Setiawati M R, Simarmata T, Nurlaeny N, Joy B, Hamdani J S, Handayani I 2018 IOP Conf. Ser.: Earth Environ. Sci. 205 012011
- [10] Rao N S S 1994 Soil Microorganism and Plant Growth (Jakarta: UI-Press) chapter 9 pp 143– 214 (in Indonesian)
- [11] Bashan Y 1998 Biotechnol. Adv. 16:729-70
- [12] Nelson L M 2004 Crop Management 1–7
- [13] Ferreira E M and Castro I V 2005 Silva Lusitana 13 159-67
- [14] Simanungkalit R D M, Husen E and Saraswati R 2012 Biofertilizer Quality Standards and Their Monitoring System Organic Fertilizer and Biofertilizer ed R D M Simanungkalit et al. (Bogor: Center for Agricultural Research and Development, Agricultural Research and Development Agency, Ministry of Agriculture Republic of Indonesia) chapter 12 pp 245–64
- [15] Brar S K, Sarma S J and Chaabouni E 2012 Shelf-life of biofertilizers: An accord between formulations and genetics. J. Biofertil. Biopestici. 3 1–2
- [16] Parmar P and Sindhu S S 2013 Journal of Microbiology Research 3 25-31
- [17] Yardin M R, Kennedy I R and Thies J E. 2000. Radiation Physics and Chemistry 57 565-68
- [18] Kim S and Dale B E 2004 *Biomass and Bioenergy* **26** 361–75
- [19] Nur S M 2014. Characteristics of Rice Waste as a Bioenergy Raw Material (Bogor: Sandesign Press) chapter 6 p 10 (in Indonesian)
- [20] Zakaria A A B 2009 Growth Optimization of Potassium Solubilizing Bacteria Isolated from Biofertilizer (Pahang: Universiti Malaysia Pahang) chapter 2 p 13
- [21] Santi L P and Goenadi D H 2010 Menara Perkebunan 78 52–60
- [22] Lehmann J, Rillig M C, Thies J, Masiello C A, Hockaday W C and Crowley D 2011 Soil Biology & Biochemistry 43 1812–36
- [23] Maftu'ah E and Nursyamsi D 2015 Pros. Sem. Nas. Masy. Biodiv. Indon. 1 776-81
- [24] Tamtomo F, Rahayu S and Suyanto A 2015 Jurnal Agrosains 12 1–7

- [25] Shareef T M E and Zhao B W 2017 Journal of Agricultural Chemistry and Environment 6 38–61
- [26] Hidayati U, Anas I, Munif A, Siswanto and Santosa D A 2014 Jurnal Penelitian Karet 32 129–38
- [27] Prasad A A and Babu S 2017 An. Acad. Bras. Cienc. 89 1027-40
- [28] Anas I 1989 Soil Biology in Practice (Bogor: Directorate General of Higher Education Republic of Indonesia, Inter-University Center, Institut Pertanian Bogor) chapter 6 pp 76–95 (in Indonesian)
- [29] Sulaeman, Suparto and Eviati 2005 Technical Guidelines of Chemical Analysis for Soil, Plant, Water and Fertilizer ed B H Prasetyo et al. (Bogor: Soil Research Institute, Agricultural Research and Development Agency, Ministry of Agriculture Republic of Indonesia). chapter 4 pp 102–3 (in Indonesian)
- [30] Hadioetomo R S 1990 Microbiology in Practice: Basic Techniques and Procedures of Laboratory (Jakarta: Gramedia) chapter 12 pp 76–81 (in Indonesian)
- [31] Hu X F, Chen J and Guo J F 2006 World J. Micro. Biotech. 22 983–90
- [32] Mattjik A A and Sumertajaya I M 2002 Experiments Design (Bogor: IPB Press) chapter 5 pp 63–99 (in Indonesian)
- [33] Taylor R 1990 JDMS 1 35-39
- [34] Badr M A 2006 J. App. Sci. Res. 2 1191–98
- [35] Ghosh T K, Singh R P, Duhan J S and Yadav D S 2001 Fertiliser Marketing News 32 1–9
- [36] [Kepmentan] Decree of the Minister of Agriculture Republic of Indonesia 2019 Kepmentan No. 261/KPTS/SR. 310/M/4/2019 (Jakarta: Ministry of Agriculture Republic of Indonesia) pp 1– 18 (in Indonesian)
- [37] Sheng X F and He L Y 2006 Canadian J. Microbiol. 52 66-72
- [38] Meena O P, Maurya B R and Meena V S 2013 Agriculture for Sustainable Development 1 53-6
- [39] Styriakova I, Styriak I, Galko I, Hradil D and Bezdicka P 2003 Ceramic Silicaty 47 20-26
- [40] Girgis M G Z, Khalil H M A and Sharaf M S 2008 Australian Journal of Basic and Applied Sciences 2 68–81
- [41] Varnam A H and Evans M G 2000 Environmental Microbiology (Boca Raton: CRC Press) chapter 1 pp 7–28
- [42] Moat A G, Foster J W and Spector M P 2002 Microbial Physiology (New York: Wiley-Liss, Inc.) chapter 9 pp 368–393

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