

1 **Performance of selected superior bacterial isolates from biogas sludge on the growth of**
2 **upland rice in ultisols**

3
4 **Desempenho dos isolados bacterianos superiores seleccionados das lamas de biogás no**
5 **crescimento do arroz upland em ultisóis**

6
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16

17 **ABSTRACT**

18 The study to obtain the influence of selected superior bacterial isolates (SBI), biogas sludge,
19 and their interactions on growth, biomass, and nutrient uptake in upland rice in ultisols. This
20 study used a Randomized Block Design within two factors and seven replications from October
21 2020 until April 2021. The first factor used selected SBI (B0= untreated, B1= nitrogen-fixing
22 bacteria isolate (N3), B2= phosphate solubilizing bacteria isolate (P7), B3= isolates
23 combination (N3+P7). The second factor was dosage of biogas sludge (S0= untreated, S1=
24 157.5; S2= 315; S3= 630 mL polybag⁻¹). The parameters were determined by ANOVA and
25 followed by DMRT at $P < 0.05$. The results showed that the P7 isolate significantly increased

26 total-N uptake and the highest crop growth rate (CGR) of upland rice by 20.77% and 2.81-
27 folds, respectively. Biogas sludge dosage from 315 to 630 mL polybag⁻¹ significantly increased
28 plant height, uptake of total-N and available-P, total fresh and dry weight, and CGR of upland
29 rice. The interaction of N3 with biogas sludge dosage of 630 mL polybag⁻¹ significantly
30 increased the CGR of upland rice. The application of N3 and P7 isolates and their combination
31 within biogas sludge of 630 ml polybag⁻¹ has the potential to archive the CGR of upland rice in
32 acidic soils.

33

34 **Keywords:** acidic soil, crop growth rate, dosage, sludge potential, upland rice.

35

36 **RESUMEN**

37 O estudo para obter a influência de isolados bacterianos superiores selecionados (IBS), lamas
38 de biogás e suas interações no crescimento, biomassa e absorção de nutrientes no arroz upland
39 em ultisóis. Este estudo utilizou um projeto de bloco aleatorizado dentro de dois fatores e sete
40 replicações de outubro de 2020 até abril de 2021. O primeiro factor utilizado foi o IBS
41 seleccionado (B0= não tratado, B1= isolado de bactérias fixadoras de azoto (N3), B2= isolado
42 de bactérias solubilizadoras de fosfato (P7), B3= combinação de isolados (N3+P7). O segundo
43 factor foi a dosagem das lamas de biogás (S0= não tratadas, S1= 157.5; S2= 315; S3= 630 mL
44 o polybag⁻¹). Os parâmetros foram determinados pela ANOVA e seguidos pela DMRT Em P<
45 0,05. Os resultados mostraram que o isolado P7 aumentou significativamente a captação total
46 de N e a maior taxa de crescimento das culturas (TCC) de arroz das terras altas em 20,77% e
47 2,81 dobras, respectivamente. Dosagem das lamas de biogás de 315 a 630 mL o polibag⁻¹
48 aumentou significativamente a altura da planta, a absorção do total-N e do Disponível-P, o peso
49 total fresco e seco e o TCC do arroz das terras altas. Interação de N3 com a dosagem de lamas
50 de biogás de 630 mL o polibag⁻¹ aumentou significativamente o TCC do arroz upland. A

51 aplicação de isolados N3 e P7 e a sua combinação nas lamas de biogás de 630 mL o polibag⁻¹
52 tem o potencial de arquivar o TCC do arroz upland em solos ácidos.

53

54 **Palavras-chave:** arroz de montanha, dosagem, potencial de lamas, solo ácido, taxa de
55 crescimento das culturas.

56

57 **Introduction**

58 Biogas sludge is the waste by-product installation from an anaerobic processing system (Food
59 and Agriculture Organization, 1997) and has a high nutrient content that can be used as organic
60 fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). It has been reported that
61 the characteristics of biogas sludge from palm oil waste such as total-N of 490 mg L⁻¹; NH₃-N
62 was 65 mg L⁻¹; total-P by 110 mg L⁻¹; K of 1.9 mg L⁻¹; Ca by 23 mg L⁻¹; Mg of 256 mg L⁻¹; As
63 <0.01 mg L⁻¹; Zn was 0.61 mg L⁻¹; and Cr by 0.04 mg L⁻¹ (Lubis *et al.*, 2014), C/N 8; 0.14%
64 N, 1.12% C (Tepsour *et al.*, 2019), NH₃-N ranged by 91 to 112 mg L⁻¹ (Choorit & Wisarnwan,
65 2007), pH ranged by 6.8 to 8.3; and the highest bacterial populations was 7.21×10⁷ cells per
66 mL and the lowest was 3.15×10⁷ cells per mL (Alvionita *et al.*, 2019). Mustamu & Triyanto
67 (2020) also reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing
68 which have the potential to availability of nitrogen and phosphate in the soil.

69 The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing has
70 greater potential in increasing soil fertility and plant growth. Zhang *et al.* (2013) reported that
71 phosphate solubilizing bacteria play an important role in increasing soil fertility, plant yield,
72 and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described that the *Bacillus*
73 genera such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B. mycoides*,
74 *B. coagulans*, *B. chitinolyticus*, *B. subtilis* have been reported as phosphate solubilizing.
75 Ambrosini *et al.* (2016) reported that *Bacillus cereus* showed the highest nitrogenase activity

76 among 42 different strains of *Bacillus spp.* Lim *et al.* (2018) also reported the dominant bacteria
77 found in the biogas sludge from anaerobic processing using the pyrosequencing and clone
78 library methods, i.e. *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

79 The bacteria from biogas sludge has never been reported in Indonesia on the application of
80 bacterial isolates from biogas sludge in improving upland rice growth on acidic soils. Thus, it
81 is necessary to test the potential of beneficial bacterial isolates from biogas sludge in increasing
82 the availability of nitrogen, phosphate, and the response to the growth of upland rice due to the
83 biogas sludge and selected isolate in ultisols. The study was aimed to obtain the influence of
84 selected superior bacterial isolates, biogas sludge, and their interaction on the growth of upland
85 rice in ultisols.

86 **Material and Methods**

87 **Study area**

88 The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in
89 the Analytical Laboratory of PT. Socfin Indonesia, Medan. The bacterial isolates were applied
90 to upland rice in Padang Bulan Village, Medan Selayang Subdistrict, Medan City, North
91 Sumatra, Indonesia from October 2020 to April 2021.

92 **Preparation of medium and upland rice seeds**

93 The planting medium used a soil type of ultisols from the Simalingkar area, Medan Tuntungan
94 Subdistrict, Medan City with a soil depth of 0 to 20 cm. 100 g of soil samples were taken and
95 analyzed for chemical characteristics such as pH, organic-C, available-P, total-N, CEC, and
96 base saturation (K, Ca, Na, Mg) (Table 1). The soil was sterilized by burning at 100⁰C for 2 h.
97 After being incubated for a day, the soil was put into a polybag with a size of 10 kg. A basic
98 fertilizer of NPK Mutiara at a dose of 300 kg ha⁻¹ was given by stirring evenly with the soil. In
99 concurrently, the seeds of upland rice used was inbred variety of Inpago-8 then soaked in water

100 for 24 h and followed by a propineb fungicide (70%) for two hours. Upland rice was planted
 101 after one day of basic fertilization with two seeds per polybag at a depth of 2 cm.

102 **TABLE 1.** The chemical characteristics of sterile ultisols.
 103

Chemical characteristics	Value	Category*
Soil pH (H ₂ O)	4.80	Acid
Organic-C (%)	0.44	Very low
Total-N (%)	0.04	Very low
Available-P (mg kg ⁻¹)	870.25	Very high
CEC (me/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (me/100 g)	0.60	High
Ca (me/100 g)	0.34	Very low
Mg (me/100 g)	0.32	Very low
Na (me/100 g)	0.09	Very low
Al (%)	0.02	Very low

104 Source: *Criteria for pH H₂O= 4.5-5.5 (acid); organik-C <1% (very low); total-N <0,1% (very
 105 low); available-P >60 mg kg⁻¹ (very high); CEC= 25-40 me/100 g (high); base saturation <20%
 106 (very low); exchangeable-K= 0.60-1.00 me/100 g (high); exchangeable-Ca <2 me/100 g (very
 107 low); exchangeable-Mg <0.4 me/100 g (very low); exchangeable-Na <0.1 me/100 g (very low);
 108 exchangeable-Al <5% (very low) (Soil Research Institute, 2009).
 109

110 **Preparation of superior bacterial isolates suspension and biogas sludge**

111 The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has
 112 been confirmed by Mustamu *et al.* (2021) and nitrogen-fixing bacteria or N3 (data
 113 unpublished). The isolates were grown on NB medium, and incubated for 48 h. The microbial
 114 mass in the solution was measured using spectrophotometer with a density of 10⁸ cells per mL.
 115 10 mL was taken from solution containing nitrogen-fixing bacteria (N3) and phosphate
 116 solubilizing (P7).

117 The biogas sludge was taken from an identical location in the first phase of the study (palm oil
 118 mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province,
 119 Indonesia). Bacterial isolate and biogas sludge were applied to the soil surface at the plants

120 were one week after planting (WAP). Biogas sludge samples were taken 500 mL then analyzed
 121 the chemical and biological characteristics in the laboratory (Table 2).

122 **TABLE 2.** The chemical biological characteristics of biogas sludge.
 123

Characteristics of biogas sludge	Value
Chemical	
pH	7.41
Chemical oxygen demand/COD (mg L ⁻¹)	4547.8
Biological oxygen demand/BOD (mg L ⁻¹)	1127.5
Total-N (%)	0.051
Total-P (%)	0.0097
Available-P (%)	0.013
Total-K (%)	0.18
Organic-C (%)	0.14
Ca (%)	0.04
Mg (%)	0.04
Na (ppm)	44.41
Cu (%)	0.0001
Biological	
Total nitrogen-fixing bacteria (CFU mL ⁻¹)	29.4×10 ⁵
Total phosphate solubilizing bacteria (CFU mL ⁻¹)	7.0×10 ⁴

124

125 **Treatments Application**

126 This study used a Randomized Block Design within two factors and seven replications. The
 127 first factor with the type of superior bacterial isolates at the similarly likewise dose, namely 10
 128 mL polybag⁻¹ (B0= un-treated; B1= nitrogen-fixing bacterial isolate (N3); B2= phosphate
 129 solubilizing bacteria isolate (P7); B3= combination isolates N3+P7). The second factor was
 130 dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag⁻¹). Each
 131 replication was dismantled at 4, 8, and 12 Weeks After Isolate and Biogas Sludge Application
 132 (WAIBSA) for determination Crop Growth Rate (CGR).

133 **Parameters and Data Analysis**

134 Variable observations were conducted by measuring the growth of upland rice (plant
 135 height, total fresh and dry weight), total-N and available-P content in the shoots, total uptake-
 136 N, and available-P. CGR were conducted on plants at 4-8, 8-12, and 12-16 WAIBSA. Each

137 polybag from each treatment and replication was dismantled at the plants were 4, 8, 12, and 16
 138 WAIBSA, then measured the plant height, the total fresh weight was conducted by weighing
 139 the roots and shoots. The total dry weight was measured by oven at 60⁰C for 48 hours and
 140 weighed by the analytical scales. A sample of the 2nd leaf from the shoots was taken by 200 g
 141 and analyzed for the total-N using the Kjeldahl method and available-P by the dry ashing
 142 method through UV-Vis Spectrophotometer. The total-N and available-P absorption were
 143 measured using equation (1). The CGR was calculated by the dry weight per unit area using
 144 equation (2) (Shon *et al.*, 1997):

$$145 \quad \text{Uptake nutrient} = \text{nutrient content in the shoots} \times \text{total dry weight} \quad (1)$$

$$146 \quad \text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W_2 - W_1}{t_2 - t_1} \quad (2)$$

147 Note:

148 CGR = crop growth rate

149 W1 = dry weight per unit area at t1

150 W2 = dry weight per unit area at t2

151 t1 = first sampling

152 t2 = second sampling

153 The parameters of the second phase of the study were analyzed by ANOVA and if the treatment
 154 had a significant effect, then continued by DMRT at $P < 0.05$ with SPSS v.20 software.

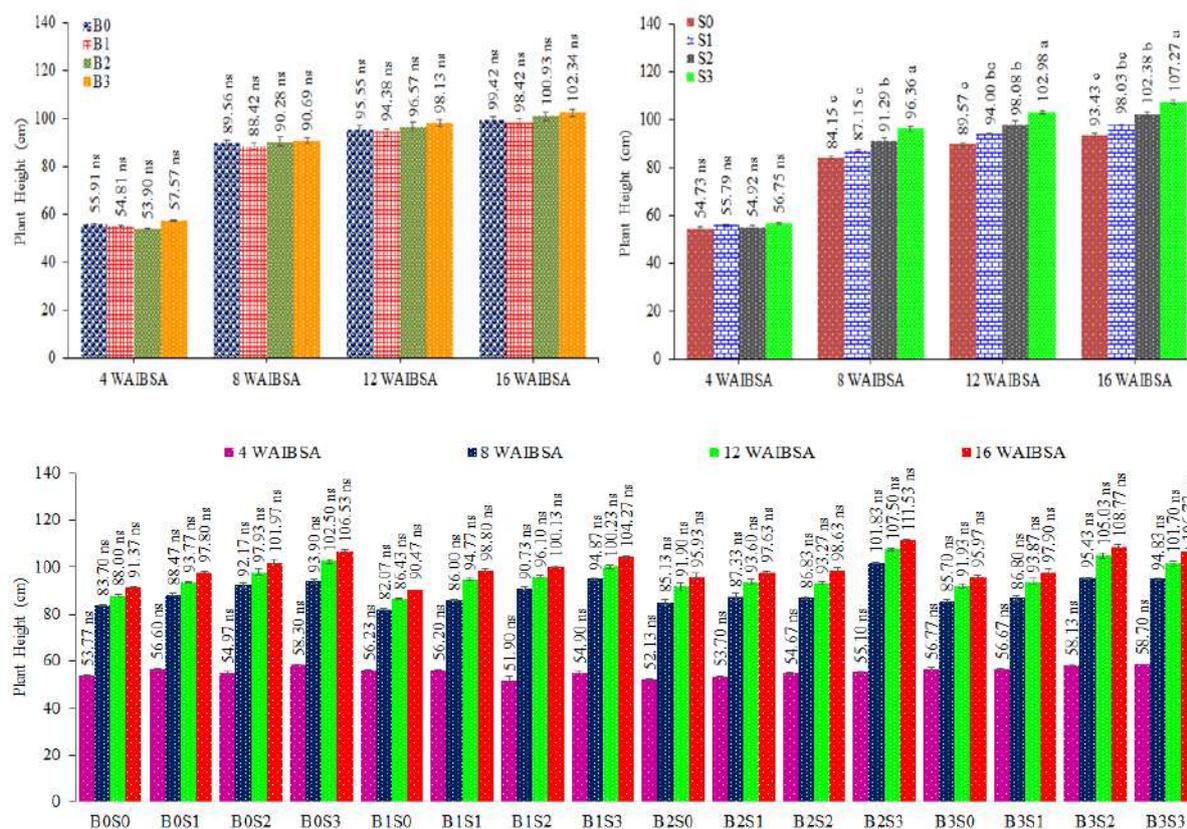
155

156 **Results and discussion**

157 **Plant height of upland rice (cm)**

158 The effect of biogas sludge application was significant on the plant height of upland rice at 8,
 159 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant
 160 effect on the plant height of upland rice at 4, 8, 12, and 16 WAIBSA (Figure 1). A significant

161 increase in plant height of upland rice along with increased doses of biogas sludge to 630 mL
 162 polybag⁻¹ at 8, 12, and 16 WAIBSA with the highest increase of 14.81% compared to the control
 163 at 16 WAIBSA. Although the effect was insignificant, it was seen that the isolates combination
 164 of B3 and the interaction of B2S3 showed the highest increase plant height of upland rice by
 165 2.94% and 22.06%, respectively, compared to the control.



166 **FIGURE 1.** Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions
 167 on plant height of upland rice at 4, 8, 12, and 16 WAIBSA. Values followed by the different
 168 letter in the graph indicated significantly by DMRT at $P < 0.05$. ns= not significantly. Dosage of
 169 biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag⁻¹). Superior bacterial
 170 isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).
 171

172 Biomass of upland rice (g)

173 The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12,
 174 and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect
 175 on the total fresh weight of upland rice at 4-16 WAIBSA (Table 3).

176 **TABLE 3.** Effect of superior bacterial isolates, biogas sludge, and their interactions on total
 177 fresh weight of upland rice at 4, 8, 12, and 16 WAIBSA.
 178

Treatments	Total fresh weight \pm SE (g)			
	4 WAIBSA	8 WAIBSA	12 WAIBSA	16 WAIBSA
Superior bacterial isolates (B)				
B0	4.15 \pm 0.21ns	169.31 \pm 8.90ns	215.27 \pm 8.42ns	229.82 \pm 8.94ns
B1	3.12 \pm 0.12ns	194.50 \pm 9.35ns	235.08 \pm 10.32 ns	252.02 \pm 10.22ns
B2	4.52 \pm 0.23ns	162.89 \pm 11.15ns	201.85 \pm 9.89ns	230.70 \pm 9.28ns
B3	3.30 \pm 0.25ns	173.91 \pm 12.55ns	220.40 \pm 15.96ns	245.03 \pm 16.32ns
Biogas sludge (S)				
S0	3.72 \pm 0.24ns	144.07 \pm 9.37b	182.67 \pm 7.14b	197.56 \pm 6.58b
S1	3.58 \pm 0.27ns	153.41 \pm 7.93b	190.70 \pm 8.90b	215.65 \pm 7.03b
S2	3.64 \pm 0.27ns	199.68 \pm 10.30a	258.70 \pm 9.63a	280.15 \pm 9.25a
S3	4.15 \pm 0.25ns	203.45 \pm 1.36a	240.52 \pm 2.81a	264.21 \pm 2.42a
Interactions (B \times S)				
B0S0	4.99 \pm 0.33ns	124.08 \pm 5.60ns	185.64 \pm 3.32ns	192.78 \pm 2.96ns
B0S1	3.47 \pm 0.26ns	160.43 \pm 1.16ns	188.60 \pm 5.76ns	207.05 \pm 3.97ns
B0S2	3.42 \pm 0.42ns	185.97 \pm 6.80ns	232.60 \pm 8.75ns	250.84 \pm 7.40ns
B0S3	4.71 \pm 0.42ns	206.76 \pm 5.49ns	254.23 \pm 10.27ns	268.61 \pm 8.85ns
B1S0	2.80 \pm 0.18ns	155.79 \pm 1.12ns	183.96 \pm 5.20ns	202.88 \pm 2.88ns
B1S1	3.74 \pm 0.29ns	174.82 \pm 9.01ns	227.91 \pm 6.38ns	236.60 \pm 6.32ns
B1S2	3.28 \pm 0.40ns	241.17 \pm 5.25ns	283.60 \pm 7.76ns	296.08 \pm 8.05ns
B1S3	2.67 \pm 0.22ns	206.20 \pm 7.23ns	244.85 \pm 6.26ns	272.52 \pm 4.34ns
B2S0	3.19 \pm 0.18ns	190.90 \pm 7.77ns	215.36 \pm 7.67ns	229.11 \pm 6.75ns
B2S1	4.85 \pm 0.38ns	106.74 \pm 13.42ns	143.16 \pm 13.02ns	179.61 \pm 10.36ns
B2S2	5.20 \pm 0.24ns	148.40 \pm 11.59ns	219.65 \pm 5.26ns	248.72 \pm 6.94ns
B2S3	4.82 \pm 0.45ns	205.53 \pm 10.50ns	229.21 \pm 16.57ns	265.34 \pm 9.58ns
B3S0	3.91 \pm 0.30ns	105.53 \pm 3.94ns	145.72 \pm 1.96ns	165.45 \pm 1.11ns
B3S1	2.25 \pm 0.09ns	171.63 \pm 4.90ns	203.14 \pm 7.07ns	239.34 \pm 12.07ns
B3S2	2.66 \pm 0.14ns	223.17 \pm 7.84ns	298.95 \pm 1.51ns	324.94 \pm 3.03ns
B3S3	4.37 \pm 0.07ns	195.31 \pm 6.77ns	233.79 \pm 8.40ns	250.38 \pm 8.16ns
CV (%)	56.09	29.68	26.31	20.78

179 Note: values followed by the different letter in the column indicated significantly by DMRT at
 180 $P < 0.05 \pm SE$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 181 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 182 isolate P7, B3= combination isolates N3+P7).
 183

184 A significant increase in total fresh weight of upland rice along with the increase in the dosage
 185 of biogas sludge to 315 mL polybag⁻¹ at 16 WAIBSA with the highest increase by 41.81%
 186 compared to the control. Although the effect was insignificant, it was seen that the B1 and the
 187 interaction of B3S2 showed the highest increase in the total fresh weight of upland rice were
 188 9.66% and 68.55%, respectively compared to the control.

189 The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and
 190 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the
 191 total dry weight of upland rice at 4-16 WAIBSA (Table 4).

192 **TABLE 4.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the total
 193 dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.
 194

Treatments	Total dry weight \pm SE (g)			
	4 WAIBSA	8 WAIBSA	12 WAIBSA	16 WAIBSA
Superior bacterial isolates (B)				
B0	1.38 \pm 0.06ns	48.01 \pm 1.29ns	73.60 \pm 3.99ns	82.52 \pm 4.18ns
B1	1.13 \pm 0.05ns	54.09 \pm 2.41ns	76.83 \pm 2.66ns	99.72 \pm 4.15ns
B2	1.49 \pm 0.06ns	47.30 \pm 3.30ns	73.20 \pm 2.28ns	98.25 \pm 3.90ns
B3	1.15 \pm 0.07ns	52.32 \pm 3.39ns	77.18 \pm 4.90ns	98.47 \pm 4.56ns
Biogas sludge (S)				
S0	1.26 \pm 0.06ns	45.51 \pm 2.63ns	62.88 \pm 2.19b	76.78 \pm 1.63c
S1	1.23 \pm 0.08ns	44.47 \pm 1.71ns	68.52 \pm 2.00ab	87.65 \pm 2.84bc
S2	1.26 \pm 0.08ns	55.36 \pm 3.43ns	85.69 \pm 1.08a	98.95 \pm 1.86b
S3	1.40 \pm 0.06ns	56.38 \pm 1.05ns	83.73 \pm 3.44a	115.59 \pm 2.11a
Interactions (B \times S)				
B0S0	1.58 \pm 0.08ns	41.73 \pm 2.78ns	58.08 \pm 1.54ns	67.23 \pm 0.96ns
B0S1	1.12 \pm 0.08ns	45.87 \pm 0.83ns	62.74 \pm 1.83ns	71.08 \pm 1.91ns
B0S2	1.20 \pm 0.12ns	52.25 \pm 2.07ns	81.39 \pm 5.48ns	88.28 \pm 5.02ns
B0S3	1.60 \pm 0.12ns	52.18 \pm 0.29ns	92.20 \pm 3.05ns	103.49 \pm 2.43ns
B1S0	0.97 \pm 0.04ns	46.64 \pm 1.39ns	69.53 \pm 4.90ns	80.30 \pm 4.51ns
B1S1	1.40 \pm 0.07ns	48.13 \pm 2.78ns	78.91 \pm 0.53ns	96.23 \pm 1.50ns
B1S2	1.12 \pm 0.10ns	67.79 \pm 1.44ns	91.05 \pm 2.25ns	101.80 \pm 2.40ns
B1S3	1.02 \pm 0.08ns	53.81 \pm 3.76ns	67.84 \pm 1.77ns	120.54 \pm 2.15ns
B2S0	1.17 \pm 0.05ns	59.32 \pm 2.33ns	70.92 \pm 4.20ns	81.43 \pm 3.82ns
B2S1	1.54 \pm 0.10ns	34.47 \pm 2.16ns	61.69 \pm 1.97ns	89.84 \pm 1.41ns
B2S2	1.73 \pm 0.05ns	37.37 \pm 3.74ns	83.10 \pm 1.19ns	105.46 \pm 1.37ns
B2S3	1.53 \pm 0.10ns	58.05 \pm 1.76ns	77.07 \pm 4.27ns	116.28 \pm 1.30ns
B3S0	1.30 \pm 0.07ns	34.35 \pm 7.04ns	52.98 \pm 0.73ns	78.16 \pm 0.48ns
B3S1	0.85 \pm 0.03ns	49.40 \pm 0.08ns	70.72 \pm 1.29ns	93.44 \pm 2.19ns
B3S2	0.99 \pm 0.05ns	64.05 \pm 4.68ns	87.22 \pm 2.90ns	100.26 \pm 1.93ns
B3S3	1.44 \pm 0.02ns	61.48 \pm 2.47ns	97.80 \pm 0.77ns	122.04 \pm 0.20ns
CV (%)	43.80	31.22	26.54	18.38

195 Note: values followed by the different letter in the column indicated significantly by DMRT at
 196 $P < 0.05 \pm$ SE. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 197 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 198 isolate P7, B3= combination isolates N3+P7).
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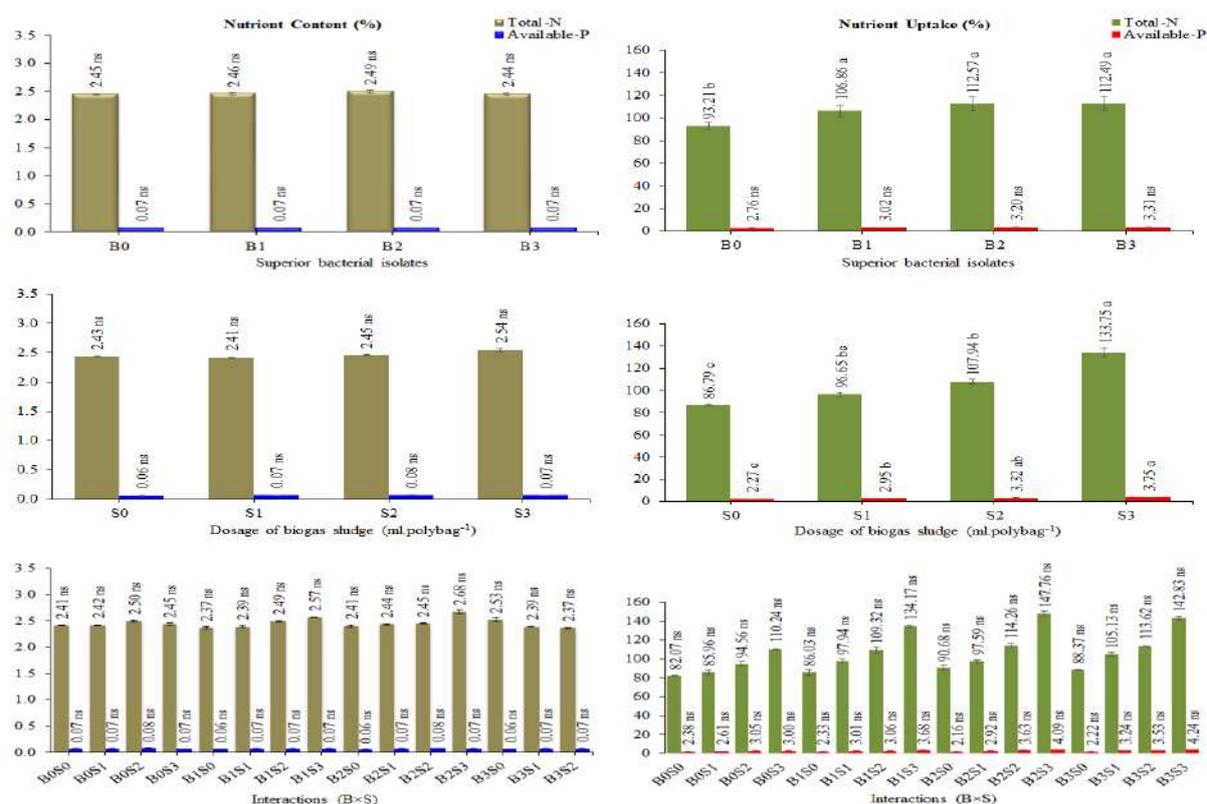
200 A significant increase in total dry weight of upland rice along with the increase in the dosage
 201 of biogas sludge to 630 mL polybag⁻¹ at 16 WAIBSA with the highest increase of 50.55%

202 compared to the control. Although the effect was insignificant, it was seen that the B1 and the
 203 interaction of B3S3 showed the highest increase in the total dry weight of upland rice were
 204 20.84% and 81.53%, respectively compared to the control.

205

206 Content and uptake of total-N and available-P nutrient of upland rice

207 The effect of biogas sludge, superior bacterial isolates, and their interactions had an
 208 insignificant effect on the nutrient content of total-N and available-P in upland rice. The effect
 209 of biogas sludge significantly increased in the nutrient uptake of total-N and available-P.
 210 Superior bacterial isolates significantly increased in the nutrient uptake of total-N. The
 211 interaction of biogas sludge with superior bacterial isolates had an insignificant effect on the
 212 nutrient uptake of total-N and available-P of upland rice (Figure 2).



213 **FIGURE 2.** The effect of superior bacterial isolates, dosage of biogas sludge, and their
 214 interactions in the content and uptake of total-N and available-P nutrient of upland rice. Values
 215 followed by the different letter in graph indicated significantly by DMRT at $P < 0.05$. ns= not
 216 significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL
 217 polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3=
 218 combination isolates N3+P7).

219 A significant increase in the nutrient uptake of total-N and available-P in upland rice along with
220 the increase in the dose of biogas sludge to 630 mL polybag⁻¹ with the highest increase of
221 54.11% and 65.20%, respectively compared to the control. The superior bacterial isolates (B1-
222 B3) also significantly increased the nutrient uptake of total-N for upland rice with the highest
223 increase in the B2 by 20.77% compared to the control. Although the effect was insignificant, it
224 was seen that the B3 showed the highest increase in nutrient uptake of available-P in upland
225 rice was 19.93% compared to the control. Likewise, the interaction of B2S3 and B3S3 showed
226 the highest increase in nutrient uptake of total-N and available-P in upland rice by 80.04% and
227 79.41%, respectively, compared to the control.

228 The biogas sludge doses of 315 and 630 mL polybag⁻¹ (S2 and S3) explained that the nutrient
229 content of available-P and total-N in the plant tissue of upland rice were higher by 33.33% and
230 4.53%, respectively compared to the control. The B2 isolate showed the highest nutrient content
231 of total-N in the plant tissue of upland rice by 1.63% compared to the control, but all isolates
232 (B1-B3) showed a similar level of available-P in the plant tissue of upland rice with the control.
233 The interactions of B2S3 and B2S2 also showed the highest nutrient content of total-N and
234 available-P compared to other interactions.

235

236 **Crop growth rate of upland rice**

237 The effect of superior bacterial isolates, biogas sludge, and their interactions significantly
238 increased the crop growth rate of upland rice at 12 to 16 WAIBSA, but it had an insignificant
239 effect at 4-8 and 8-12 WAIBSA (Table 5).

240 The biogas sludge dose of 630 mL polybag⁻¹ (S3) significantly increased the highest crop
241 growth rate for upland rice at 12 to 16 WAIBSA by 129.44% compared to the control. The
242 ability of B1-B3 isolates significantly increased the crop growth rate of upland rice with the
243 highest increase in the B2 by 181.45% compared to the controls at 12 to 16 WAIBSA. The

244 interaction of the B1S3 significantly increased the crop growth rate of upland rice by 5.76-folds
 245 greater compared to the control.

246 **TABLE 5.** The effect of superior bacterial isolates, biogas sludge, and their interactions on the
 247 crop growth rate of upland rice.

248

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
4-8 WAIBSA					
B0	1.434ns	1.598ns	1.823ns	1.806ns	1.665ns
B1	1.631ns	1.669ns	2.381ns	1.885ns	1.892ns
B2	2.077ns	1.176ns	1.273ns	2.019ns	1.636ns
B3	1.180ns	1.734ns	2.252ns	2.144ns	1.828ns
Average	1.580ns	1.544ns	1.932ns	1.964ns	CV= 32.28%
8-12 WAIBSA					
B0	0.584ns	0.602ns	1.041ns	1.430ns	0.914ns
B1	0.818ns	1.099ns	0.831ns	0.501ns	0.812ns
B2	0.414ns	0.972ns	1.633ns	0.679ns	0.925ns
B3	0.665ns	0.761ns	0.828ns	1.297ns	0.888ns
Average	0.620ns	0.859ns	1.083ns	0.977ns	CV= 56.17%
12-16 WAIBSA					
B0	0.327fgh	0.298gh	0.246h	0.403b-h	0.318b
B1	0.385c-h	0.619a-h	0.384d-h	1.882a	0.817a
B2	0.375e-h	1.005a-h	0.798a-h	1.400a-h	0.895a
B3	0.899a-h	0.811a-h	0.466a-h	0.866a-h	0.761a
Average	0.496b	0.683b	0.474b	1.138a	CV= 51.07%

249 Note: values followed by the different letter in the column indicated significantly by DMRT at
 250 $P < 0.05$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315;
 251 S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate
 252 P7, B3= combination isolates N3+P7).

253

254 **The effect of selected superior bacterial isolates**

255 The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake
 256 of total-N and crop growth rate of upland rice on ultisols at 12 to 16 WAIBSA, but it had an
 257 insignificant effect on plant height, total fresh weight, total dry weight, nutrient content (total-
 258 N and available-P) in leaf tissue, nutrient uptake of available-P, and crop growth rate of upland
 259 rice at 4 to 8 and 8 to 12 WAIBSA. The superior bacterial isolates (N3, P7, and N3+P7) could
 260 increase the nutrient uptake of total-N in upland rice by 14.64%, 20.77%, and 20.68%,
 261 respectively compared to control (Figure 2). Similar results are also shown in Table 5, that the

262 crop growth rate of upland rice at 12 to 16 WAIBSA due to selected superior bacterial isolates
263 (N3, P7, N3+P7) has increased by 2.57; 2.81; and 2.39-folds, respectively compared to the
264 control. The finding results indicated that the ability of a single isolate by P7 bacteria was
265 greater in increasing total-N and crop growth rate of upland rice compared to a single isolate
266 by N3 bacteria and a combination of N3+P7 bacterial isolates. It was due to the presence of
267 several organic acids and hormones produced by P7 that can increase the nutrient uptake of
268 total-N and crop growth rate of upland rice. This result was supported by Mustamu *et al.* (2021)
269 that the phosphate solubilizing bacteria isolate (P7) from the biogas sludge contains organic
270 acids produced such as lactic, oxalic, acetic, citric acids, and it had the highest ability to
271 solubilize phosphate from calcium triphosphate and rock phosphate was 4.62 and 2.66-folds,
272 respectively compared to the control. Meena *et al.* (2016) reported that the availability of
273 nitrogen and phosphorus slightly increased in the application of bio fertilization with *Bacillus*
274 *cereus*, it was due to the production of organic acids and other chemicals such as citric, tartaric,
275 and oxalic acids which can stimulate plant growth and nutrients availability. Youssef & Eissa
276 (2017) reported that the increase in vegetative growth and total biomass was due to increased
277 photosynthesis, translocation, and accumulation of nutrients. Khan *et al.* (2020) reported that
278 *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and
279 organic acids. Ferrara *et al.* (2012) reported that the hormone gibberellin, IAA, and other
280 hormones can increase plant growth under stressful conditions. Kang *et al.* (2014) said that
281 PGPB has several mechanisms to increase plant growth with nitrogen-fixation, phosphate
282 solubilizing, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of
283 palm oil solid waste from anaerobic digester include: *Ruminococcus sp.*, *Thiomargarita sp.*,
284 *Clostridium sp.*, *Anaerobacter sp.*, *Bacillus sp.*, *Sporobacterium sp.*, *Saccharofermentans sp.*,
285 *Oscillibacter sp.*, *Sporobacter sp.*, and *Enterobacter sp.* Liaquat *et al.* (2017) also reported that

286 an abundance of *Bacillus*, *Clostridium*, and *Enterobacter spp* in anaerobic digester of
287 wastewater in producing biogas.

288

289 **The effect of biogas sludge**

290 The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16
291 WAIBSA), total dry weight (12 and 16 WAIBSA), nutrient uptake (total-N and available-P),
292 and the crop growth rate of upland rice at 8 to 12 WAIBSA, but it had an insignificant effect
293 on nutrient content (total-N and available-P) in leaf tissue, and crop growth rate of upland rice
294 (4-8 and 8-12 WAIBSA). An increase in plant height, total dry weight, nutrient uptake of total-
295 N and available-P, and also crop growth rate of upland rice on ultisols along with the increase
296 in the dose of biogas sludge to 630 mL polybag⁻¹ at the end of this study (16 WAIBSA).
297 However, in contrast to the total fresh weight, an increase along with the increase in the biogas
298 sludge dose to 315 mL polybag⁻¹ then decreased at the dose of 630 mL polybag⁻¹. It was caused
299 the biogas sludge had chemical characteristics such as pH (7.41), total-N (0.051%), available-
300 P (0.013%), organic-C (0.14%), total-K (0.18%), and biological characteristics such as total
301 nitrogen-fixing bacteria (29.4×10^5 CFU mL⁻¹) and total phosphate solubilizing bacteria
302 (7.0×10^4 CFU mL⁻¹) (Table 2). C-organic content and the total population of nitrogen-fixing
303 and phosphate solubilizing bacteria from the biogas sludge could be increased the nutrient
304 uptake of total-N and available-P in upland rice along with increasing the dose of biogas sludge
305 to 630 mL polybag⁻¹ (Figure 2). Therefore, the nutrients absorbed are used for plant metabolic
306 processes and stimulate the growth of plant height, biomass, and crop growth rate of upland
307 rice. A similar result was reported by Mustamu & Triyanto (2020) that the macro and
308 micronutrients from the biogas sludge and also had the population of nitrogen-fixing and
309 phosphate solubilizing bacteria by 480×10^4 and 42×10^4 CFU mL⁻¹, respectively. Ndubuisi-
310 Nnaji *et al.* (2020) reported that total phosphate solubilizing bacteria (1.6 to 2.5 CFU mL⁻¹) was

311 significantly higher compared to nitrogen-fixing bacteria ($0.5\text{--}1.4\text{ CFU mL}^{-1}$) and a significant
312 increase in nutrient concentration in the order of $\text{N}>\text{K}>\text{P}>\text{Ca}>\text{Mg}>\text{S}$ in all anaerobic digester
313 bioreactors. Möller & Müller (2012) reported that an increase in concentrations of $\text{NH}_4^+\text{-N}$
314 ranged from 45 to 80% after anaerobic waste.

315

316 **The interaction effect of selected superior bacterial isolates and biogas sludge**

317 The interaction of biogas sludge and superior bacterial isolates only significantly increased the
318 crop growth rate of upland rice on ultisols at 8 to 12 WAIBSA, but it had an insignificant effect
319 on other parameters in this study. The interaction of NFB *Bacillus paramycoides* with biogas
320 sludge at the dose of $630\text{ mL polybag}^{-1}$ (B1S3) showed the highest crop growth rate of upland
321 rice compared to other interactions and 5.76-folds greater compared to the control. It was caused
322 by the application of biogas sludge could be increased soil organic matter and the total
323 population of beneficial bacteria. Likewise, the characteristics of the biogas sludge had the
324 organic-C was 0.14%, total nitrogen-fixing bacteria was $29.4\times 10^5\text{ CFU mL}^{-1}$, and total
325 phosphate solubilizing bacteria was $7.0\times 10^4\text{ CFU mL}^{-1}$ (Table 2) could improve soil quality
326 and support the crop growth rate. This result is supported by Urrea *et al.* (2019) that the
327 application of sewage sludge in the long-term significantly increases the organic matter in the
328 soil, which causes a decrease in soil pH due to the nitrification of ammonium in sewage sludge
329 and the production of organic acids along with the decomposition of organic matter. Bhardwaj
330 *et al.* (2014); Carvajal-Muñoz *et al.* (2012) reported that the application of biofertilizer had
331 advantages in the plant such as availability of nutrients that are balanced for plant health,
332 stimulating nutrient mobilization that can increase soil biological activity, availability of
333 microbial food to encourage the growth of beneficial microorganisms, increasing the soil
334 organic matter content thereby increasing the cation exchange capacity. Siswanti & Lestari
335 (2019) reported that the interaction of biogas sludge+biofertilizer ($36\text{ mL}+10\text{ L ha}^{-1}$)

336 significantly increased the plant height, number of leaves, and capcaisin content in chili
337 compared to a single treatment of biogas sludge and biofertilizer.

338

339 **Conclusions**

340 The isolates of N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient uptake
341 of total-N and crop growth rate of upland rice on ultisols with the highest increase found in the
342 P7 isolate of 20.77% and 2.81-folds, respectively. The dose of biogas sludge significantly
343 increased plant height, total dry weight, nutrient uptake of total-N and available-P, and also
344 crop growth rate of upland rice on ultisols with the highest increase at a dose of 630 mL polybag⁻¹
345 ¹ by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively. Likewise, the dose of
346 biogas sludge significantly increased the total fresh weight of upland rice with the highest
347 increase at the dose of 315 mL polybag⁻¹ by 41.81%. The interaction of isolates N3, P7, N3+P7
348 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice
349 on ultisols with the highest increase found in the B1S3 by 5.76-folds.

350

351 **Conflict of interest statement**

352 The authors declare that there is no conflict of interest regarding the publication of this article.

353

354 **Author's contributions**

355 Novilda Elizabeth Mustamu (NEM), Zulkifli Nasution (ZN), Irvan (I), and Mariani Sembiring
356 (MS). All authors formulated the overarching research goals and aims, provided the study
357 materials, developed or designed the methodology. NEM analyze and interpretation study data.
358 NEM and MS wrote the initial draft, managed and coordinated the research activity in field,
359 data collection. ZN and I verified the overall reproducibility of results and other research
360 outputs. All authors conducted the critical review/commentary/revision of the manuscript.

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Dear Elizabeth Mustamu:

Your recent submission to Agronomia Colombiana, entitled "Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols" (id. 97583)., has at first glance some format issues that must be addressed before it can undergo a preliminary revision by the Editor in Chief.

- Please provide us with an institutional email for the corresponding author, Mrs.. Mustamu.
- Please confirm that Ivan is the sole name of the third author of the manuscript
- Provide us with email addresses and ORCID numbers for all authors different from Mustamu
- The parallel title, resumen, and palabras clave must be in Spanish (they were left in Portuguese).

Please make all the corrections in the enclosed file, 97583.docx, that already contains some format and style improvements introduced by us, and send us the corrected manuscript in response to this email. Thank you.

Sincerely,

Stanislav Magnitskiy

Editor-in-Chief

1 **Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice**

2
3 **Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la**
4 **nutrición del arroz de secano**

5
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15
16 **ABSTRACT**

17 The **investigation evaluated** the influence of selected superior bacterial isolates (SBI), biogas
18 sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice in ultisols.
19 This study used a Randomized Block Design within two factors and seven replications from
20 October 2020 until April 2021. The first factor used selected SBI (B0= untreated, B1= nitrogen-
21 fixing bacteria isolate (N3), B2= phosphate solubilizing bacteria isolate (P7), B3= isolates
22 combination (N3+P7). The second factor was dosage of biogas sludge (S0= untreated, S1=
23 157.5; S2= 315; S3= 630 mL polybag⁻¹). The parameters were determined by ANOVA and
24 followed by **Duncan's multiple range test (DMRT)** at $P < 0.05$. The results showed that the P7
25 isolate significantly increased total-N uptake and the highest crop growth rate (CGR) of upland

26 rice by 20.77% and 2.81-times, respectively. Biogas sludge dosage from 315 to 630 mL
27 polybag⁻¹ significantly increased plant height, uptake of total-N and available-P, total fresh and
28 dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630
29 mL polybag⁻¹ significantly increased the CGR of upland rice. The application of N3 and P7
30 isolates and their combination within biogas sludge of 630 mL polybag⁻¹ has the potential to
31 archive the CGR of upland rice in acidic soils.

32

33 **Keywords:** acidic soil, crop growth rate, dosage, sludge potential, upland rice.

34

35 RESUMEN

36 La investigación evaluó la influencia de aislados bacterianos superiores seleccionados (ABS),
37 lodos de biogás y sus interacciones en el crecimiento, la biomasa y la absorción de nutrientes
38 en el arroz de tierras altas en ultisoles. Este estudio utilizó un Diseño de Bloques Aleatorizados
39 dentro de dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer
40 factor utilizado seleccionó ABS (B0= sin tratar, B1 = aislado de bacterias fijadoras de nitrógeno
41 (N3), B2= aislado de bacterias solubilizantes de fosfato (P7), B3= combinación de aislados
42 (N3+P7). El segundo factor fue la dosificación del lodo de biogás (S0= sin tratar, S1= 157,5;
43 S2= 315; S3= 630 mL de polybag⁻¹). Los parámetros fueron determinados por ANOVA y
44 seguidos de la prueba de rangos múltiples de Duncan (DMRT) en P< 0,05. Los resultados
45 mostraron que el aislado P7 aumentó significativamente la captación total de N y la mayor tasa
46 de crecimiento del cultivo (TCC) de arroz de tierras altas en 20.77% y 2.81-veces,
47 respectivamente. La dosificación de fangos de biogás de 315 a 630 mL de polybag⁻¹ aumentó
48 significativamente la altura de la planta, la absorción de N total y P disponible, el peso fresco y
49 seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosificación de lodo
50 de biogás de 630 mL de polybag⁻¹ aumentó significativamente el TCC del arroz de tierras altas.

51 La aplicación de aislamientos de N3 y P7 y su combinación dentro de lodos de biogás de 630
52 mL de polybag⁻¹ tiene el potencial de archivar el TCC de arroz de tierras altas en suelos ácidos.
53 **Palavras-chave:** arroz de tierras altas, dosis, potencial de lodo, suelo ácido, tasa de crecimiento
54 de los cultivos.

55

56 **Introduction**

57 Biogas sludge is the waste by-product installation from an anaerobic processing system (Food
58 and Agriculture Organization, 1997) and has a high nutrient content that can be used as organic
59 fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). It has been reported that
60 the characteristics of biogas sludge from palm oil waste such as total-N of 490 mg L⁻¹; total-P
61 by 110 mg L⁻¹; and K was 1.9 mg L⁻¹ (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour
62 *et al.*, 2019), NH₃-N ranged by 91 to 112 mg L⁻¹ (Choorit & Wisarnwan, 2007), pH ranged by
63 6.8 to 8.3; and the highest bacterial populations was 7.21×10⁷ cells per mL and the lowest was
64 3.15×10⁷ cells per mL (Alvionita *et al.*, 2019). Mustamu & Triyanto (2020) also reported that
65 the biogas sludge has nitrogen-fixing and phosphate solubilizing which have the potential to
66 availability of nitrogen and phosphate in the soil.

67 The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing has
68 greater potential in increasing soil fertility and plant growth. Zhang *et al.* (2013) reported that
69 phosphate solubilizing bacteria play an important role in increasing soil fertility, plant yield,
70 and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described that the *Bacillus*
71 genera such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B. mycoides*,
72 *B. coagulans*, *B. chitinolyticus*, *B. subtilis* have been reported as phosphate solubilizing.
73 Ambrosini *et al.* (2016) reported that *Bacillus cereus* showed the highest nitrogenase activity
74 among 42 different strains of *Bacillus spp.* Lim *et al.* (2018) also reported the dominant bacteria

75 found in the biogas sludge from anaerobic processing using the pyrosequencing and clone
76 library methods, i.e. *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

77 The bacteria from biogas sludge has never been reported in Indonesia on the application of
78 bacterial isolates from biogas sludge in improving upland rice growth on acidic soils. Thus, it
79 is necessary to test the potential of beneficial bacterial isolates from biogas sludge in increasing
80 the availability of nitrogen, phosphate, and the response to the growth of upland rice due to the
81 biogas sludge and selected isolate in ultisols. The study was aimed to **evaluated** the influence
82 of selected superior bacterial isolates, biogas sludge, and their interaction on the **nutrition** of
83 upland rice in ultisols.

84 **Materials and methods**

85 **Study area**

86 The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in
87 the Analytical Laboratory of PT. Socfin Indonesia, Medan. The bacterial isolates were applied
88 to upland rice in Padang Bulan Village (**3°37.760'N; 98°38.898'E; altitude 18 m above sea**
89 **level**), Medan Selayang Subdistrict, Medan City, North Sumatra, Indonesia from October 2020
90 to April 2021. **Furthermore the average humidity was 82%, temperature was 27.4°C and the**
91 **average rainfall was recorded 228.5 mm by month.**

92 **Preparation of medium and upland rice seeds**

93 **The medium used ultisols order from Simalingkar area, Medan Tuntungan Subdistrict, Medan**
94 **City with a depth of 0 to 20 cm.** 100 g of soil samples were taken and analyzed for chemical
95 characteristics such as **pH using HCl 25% method with spectrophotometer, organic-C by**
96 **Walkley-Black method with spectrophotometer, available-P by Bray-II method with**
97 **spectrophotometer, total-N using Kjeldahl method with spectrophotometer, Cation Exchange**
98 **Capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH 7 method with**

99 **atomic absorption spectrophotometry** (Tab. 1). The soil was sterilized by burning at 100⁰C for
 100 2 **hours**. After being incubated for 1 day, the soil was put into a polybag with a size of 10 kg. A
 101 basic fertilizer of NPK Mutiara at a dose of 300 kg ha⁻¹ was given by stirring evenly with the
 102 soil. Concurrently, the seeds of upland rice used was inbred variety of Inpago-8 then soaked in
 103 water for 24 **hours** and followed by a propineb fungicide (70%) for 2 hours. Upland rice was
 104 planted after one day of basic fertilization with two seeds per polybag at a depth of 2 cm.

105 **TABLE 1.** The chemical characteristics of sterile ultisols.
 106

Chemical characteristics	Value	Category*
Soil pH (H ₂ O)	4.80	Acid
Organic-C (%)	0.44	Very low
Total-N (%)	0.04	Very low
Available-P (mg kg ⁻¹)	870.25	Very high
CEC (me/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (me/100 g)	0.60	High
Ca (me/100 g)	0.34	Very low
Mg (me/100 g)	0.32	Very low
Na (me/100 g)	0.09	Very low
Al (%)	0.02	Very low

107 Source: *Criteria for pH H₂O= 4.5-5.5 (acid); organik-C <1% (very low); total-N <0,1% (very
 108 low); available-P >60 mg kg⁻¹ (very high); CEC= 25-40 me/100 g (high); base saturation <20%
 109 (very low); exchangeable-K= 0.60-1.00 me/100 g (high); exchangeable-Ca <2 me/100 g (very
 110 low); exchangeable-Mg <0.4 me/100 g (very low); exchangeable-Na <0.1 me/100 g (very low);
 111 exchangeable-Al <5% (very low) (Soil Research Institute, 2009).
 112

113 **Preparation of superior bacterial isolates suspension and biogas sludge**

114 The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has
 115 been confirmed by Mustamu *et al.* (2021a) and nitrogen-fixing bacteria or N3 (Mustamu *et al.*,
 116 2021b). The isolates were grown on NB medium, and incubated for 48 h. The **bacteria growth**
 117 in the solution was measured using spectrophotometer with a density of 10⁸ cells per mL. 10
 118 mL was taken from solution containing nitrogen-fixing bacteria (N3) and phosphate
 119 solubilizing (P7).

120 The biogas sludge was taken from the digester tank at the palm oil mill of PT. Nubika Jaya,
 121 Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. Bacterial isolate and
 122 biogas sludge were applied to the soil surface at the plants were one week after planting (WAP).
 123 Biogas sludge samples were taken 500 mL then analyzed the chemical and biological
 124 characteristics in the laboratory (Tab. 2).

125 **TABLE 2.** The chemical biological characteristics of biogas sludge.
 126

Characteristics of biogas sludge	Method	Value
Chemical		
pH	Electrometry	7.41
Chemical oxygen demand/COD (mg L ⁻¹)	Spectrophotometry	4547.8
Biological oxygen demand/BOD (mg L ⁻¹)	Titrimetry	1127.5
Total-N (%)	Spectrophotometry	0.051
Total-P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total-K (%)	Graphite furnace-AAS	0.18
Organic-C (%)	Atomic absorption spectrophotometry (AAS)	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (ppm)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Biological		
Total nitrogen-fixing bacteria (CFU mL ⁻¹)	Plate count	29.4×10 ⁵
Total phosphate solubilizing bacteria (CFU mL ⁻¹)	Plate count	7.0×10 ⁴

127

128 **Treatments application**

129 This study used a Randomized Block Design within two factors and seven replications. The
 130 first factor was the type of superior bacterial isolates (B0= un-treated; B1= nitrogen-fixing
 131 bacterial isolate (N3); B2= phosphate solubilizing bacteria isolate (P7); B3= combination
 132 isolates N3+P7) at the similar dose, namely 10 mL polybag⁻¹. The second factor was dosage of
 133 biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag⁻¹). Determination of
 134 biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m³ ha⁻¹ equal
 135 to 126,000 L ha⁻¹ (Sutarta *et al.*, 2003) then converted to soil weight per polybag (equation 1).

136 Each replication was **disassembled** at 4, 8, and 12 Weeks After Isolate and Biogas Sludge
 137 Application (WAIBSA) for determination Crop Growth Rate (CGR).

$$138 \text{ Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer per ha}}{\text{soil weight per ha}} \times \text{soil weight per polybag} \quad (1)$$

$$139 = \frac{126,000 \text{ L ha}^{-1}}{2,000,000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 630 \text{ ml}$$

140 **Parameters and data analysis**

141 Variable observations were conducted by measuring the growth of upland rice (plant
 142 height, total fresh and dry weight), total-N and available-P content in the shoots, total uptake-
 143 N, and available-P. CGR were conducted on plants at 4-8, 8-12, and 12-16 WAIBSA. Each
 144 polybag from each treatment and replication was **disassembled** at the plants were 4, 8, 12, and
 145 16 WAIBSA, then measured the plant height, the total fresh weight was conducted by weighing
 146 the roots and shoots. The total dry weight was measured by oven at 60°C for 48 hours and
 147 weighed by the analytical scales. A sample of the **second** leaf from the shoots was taken by 200
 148 g and analyzed for the total-N using the Kjeldahl method and available-P by the dry ashing
 149 method through UV-Vis Spectrophotometer. The total-N and available-P absorption were
 150 measured using equation (2). The CGR was calculated by the dry weight per unit area using
 151 equation (3) (Shon *et al.*, 1997):

$$152 \text{ Uptake nutrient} = \text{nutrient content in the shoots} \times \text{total dry weight} \quad (2)$$

$$153 \text{ CGR} = \frac{\Delta W}{\Delta t} = \frac{W_2 - W_1}{t_2 - t_1} \quad (3)$$

154 Note:

155 CGR = crop growth rate

156 W1 = dry weight per unit area at t1

157 W2 = dry weight per unit area at t2

158 t1 = first sampling

159 t2 = second sampling

160 The parameters of the second phase of the study were analyzed by ANOVA and if the treatment
 161 had a significant effect, then continued by **Duncan's multiple range test (DMRT)** at $P < 0.05$ with
 162 SPSS v.20 software.

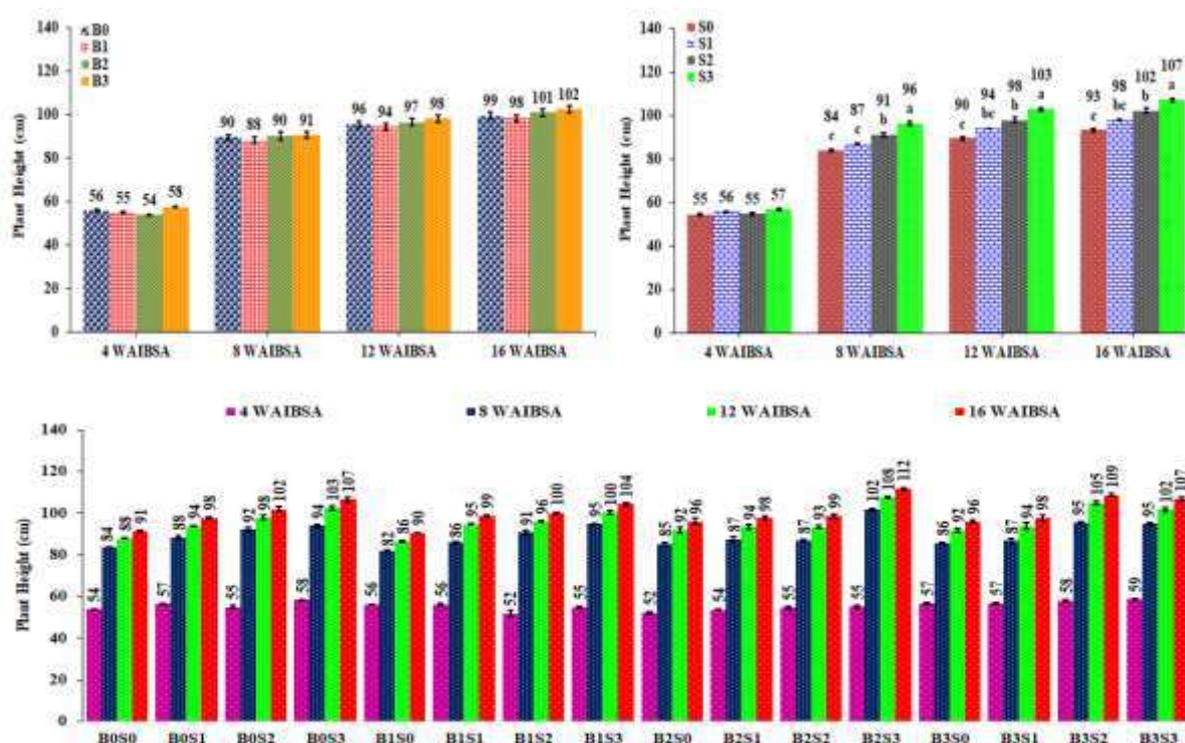
163

164 Results

165 Effect of bacterial isolates and biogas sludge on upland rice growth

166 Plant height of upland rice (cm)

167 The effect of biogas sludge application was significant on the plant height of upland rice at 8,
 168 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant
 169 effect on the plant height of upland rice at 4, 8, 12, and 16 WAIBSA (Fig. 1). A significant
 170 increase in plant height of upland rice along with increased doses of biogas sludge to 630 mL
 171 polybag⁻¹ at 8, 12, and 16 WAIBSA with the highest increase of 14.81% compared to the control
 172 at 16 WAIBSA. Although the effect was insignificant, it was seen that the isolates combination
 173 of B3 and the interaction of B2S3 showed the highest increase plant height of upland rice by
 174 2.94% and 22.06%, respectively, compared to the control.



175 **FIGURE 1.** Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions
 176 on plant height of upland rice at 4, 8, 12, and 16 WAIBSA. Values followed by the different
 177 letter in the graph indicated significantly by DMRT at $P<0.05$. ns= not significantly. Dosage of
 178 biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag⁻¹). Superior bacterial
 179 isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3= combination isolates N3+P7).
 180

181 Biomass of upland rice (g)

182 The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12,
 183 and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect
 184 on the total fresh weight of upland rice at 4-16 WAIBSA (Tab. 3).

185 **TABLE 3.** Effect of superior bacterial isolates, biogas sludge, and their interactions on total
 186 fresh weight of upland rice at 4, 8, 12, and 16 WAIBSA.
 187

Treatments	Total fresh weight \pm SE (g)			
	4 WAIBSA	8 WAIBSA	12 WAIBSA	16 WAIBSA
Superior bacterial isolates (B)				
B0	4.15 \pm 0.21	169.31 \pm 8.90	215.27 \pm 8.42	229.82 \pm 8.94
B1	3.12 \pm 0.12	194.50 \pm 9.35	235.08 \pm 10.32	252.02 \pm 10.22
B2	4.52 \pm 0.23	162.89 \pm 11.15	201.85 \pm 9.89	230.70 \pm 9.28
B3	3.30 \pm 0.25	173.91 \pm 12.55	220.40 \pm 15.96	245.03 \pm 16.32
Biogas sludge (S)				
S0	3.72 \pm 0.24	144.07 \pm 9.37 b	182.67 \pm 7.14 b	197.56 \pm 6.58 b
S1	3.58 \pm 0.27	153.41 \pm 7.93 b	190.70 \pm 8.90 b	215.65 \pm 7.03 b
S2	3.64 \pm 0.27	199.68 \pm 10.30 a	258.70 \pm 9.63 a	280.15 \pm 9.25 a
S3	4.15 \pm 0.25	203.45 \pm 1.36 a	240.52 \pm 2.81 a	264.21 \pm 2.42 a
Interactions (B \times S)				
B0S0	4.99 \pm 0.33	124.08 \pm 5.60	185.64 \pm 3.32	192.78 \pm 2.96
B0S1	3.47 \pm 0.26	160.43 \pm 1.16	188.60 \pm 5.76	207.05 \pm 3.97
B0S2	3.42 \pm 0.42	185.97 \pm 6.80	232.60 \pm 8.75	250.84 \pm 7.40
B0S3	4.71 \pm 0.42	206.76 \pm 5.49	254.23 \pm 10.27	268.61 \pm 8.85
B1S0	2.80 \pm 0.18	155.79 \pm 1.12	183.96 \pm 5.20	202.88 \pm 2.88
B1S1	3.74 \pm 0.29	174.82 \pm 9.01	227.91 \pm 6.38	236.60 \pm 6.32
B1S2	3.28 \pm 0.40	241.17 \pm 5.25	283.60 \pm 7.76	296.08 \pm 8.05
B1S3	2.67 \pm 0.22	206.20 \pm 7.23	244.85 \pm 6.26	272.52 \pm 4.34
B2S0	3.19 \pm 0.18	190.90 \pm 7.77	215.36 \pm 7.67	229.11 \pm 6.75
B2S1	4.85 \pm 0.38	106.74 \pm 13.42	143.16 \pm 13.02	179.61 \pm 10.36
B2S2	5.20 \pm 0.24	148.40 \pm 11.59	219.65 \pm 5.26	248.72 \pm 6.94
B2S3	4.82 \pm 0.45	205.53 \pm 10.50	229.21 \pm 16.57	265.34 \pm 9.58
B3S0	3.91 \pm 0.30	105.53 \pm 3.94	145.72 \pm 1.96	165.45 \pm 1.11
B3S1	2.25 \pm 0.09	171.63 \pm 4.90	203.14 \pm 7.07	239.34 \pm 12.07
B3S2	2.66 \pm 0.14	223.17 \pm 7.84	298.95 \pm 1.51	324.94 \pm 3.03
B3S3	4.37 \pm 0.07	195.31 \pm 6.77	233.79 \pm 8.40	250.38 \pm 8.16
CV (%)	56.09	29.68	26.31	20.78

188 Note: values followed by the different letter in the column indicated significantly by DMRT at
 189 $P < 0.05 \pm SE$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 190 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 191 isolate P7, B3= combination isolates N3+P7).

192

193 A significant increase in total fresh weight of upland rice along with the increase in the dosage
 194 of biogas sludge to 315 mL polybag⁻¹ at 16 WAIBSA with the highest increase by 41.81%
 195 compared to the control. Although the effect was insignificant, it was seen that the B1 and the
 196 interaction of B3S2 showed the highest increase in the total fresh weight of upland rice were
 197 9.66% and 68.55%, respectively compared to the control.

198 The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and
 199 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect on the
 200 total dry weight of upland rice at 4-16 WAIBSA (Tab. 4).

201 **TABLE 4.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the total
 202 dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.

203

Treatments	Total dry weight \pm SE (g)			
	4 WAIBSA	8 WAIBSA	12 WAIBSA	16 WAIBSA
Superior bacterial isolates (B)				
B0	1.38 \pm 0.06	48.01 \pm 1.29	73.60 \pm 3.99	82.52 \pm 4.18
B1	1.13 \pm 0.05	54.09 \pm 2.41	76.83 \pm 2.66	99.72 \pm 4.15
B2	1.49 \pm 0.06	47.30 \pm 3.30	73.20 \pm 2.28	98.25 \pm 3.90
B3	1.15 \pm 0.07	52.32 \pm 3.39	77.18 \pm 4.90	98.47 \pm 4.56
Biogas sludge (S)				
S0	1.26 \pm 0.06	45.51 \pm 2.63	62.88 \pm 2.19 b	76.78 \pm 1.63 c
S1	1.23 \pm 0.08	44.47 \pm 1.71	68.52 \pm 2.00 ab	87.65 \pm 2.84 bc
S2	1.26 \pm 0.08	55.36 \pm 3.43	85.69 \pm 1.08 a	98.95 \pm 1.86 b
S3	1.40 \pm 0.06	56.38 \pm 1.05	83.73 \pm 3.44 a	115.59 \pm 2.11 a
Interactions (B \times S)				
B0S0	1.58 \pm 0.08	41.73 \pm 2.78	58.08 \pm 1.54	67.23 \pm 0.96
B0S1	1.12 \pm 0.08	45.87 \pm 0.83	62.74 \pm 1.83	71.08 \pm 1.91
B0S2	1.20 \pm 0.12	52.25 \pm 2.07	81.39 \pm 5.48	88.28 \pm 5.02
B0S3	1.60 \pm 0.12	52.18 \pm 0.29	92.20 \pm 3.05	103.49 \pm 2.43
B1S0	0.97 \pm 0.04	46.64 \pm 1.39	69.53 \pm 4.90	80.30 \pm 4.51
B1S1	1.40 \pm 0.07	48.13 \pm 2.78	78.91 \pm 0.53	96.23 \pm 1.50
B1S2	1.12 \pm 0.10	67.79 \pm 1.44	91.05 \pm 2.25	101.80 \pm 2.40
B1S3	1.02 \pm 0.08	53.81 \pm 3.76	67.84 \pm 1.77	120.54 \pm 2.15
B2S0	1.17 \pm 0.05	59.32 \pm 2.33	70.92 \pm 4.20	81.43 \pm 3.82
B2S1	1.54 \pm 0.10	34.47 \pm 2.16	61.69 \pm 1.97	89.84 \pm 1.41

B2S2	1.73±0.05	37.37±3.74	83.10±1.19	105.46±1.37
B2S3	1.53±0.10	58.05±1.76	77.07±4.27	116.28±1.30
B3S0	1.30±0.07	34.35±7.04	52.98±0.73	78.16±0.48
B3S1	0.85±0.03	49.40±0.08	70.72±1.29	93.44±2.19
B3S2	0.99±0.05	64.05±4.68	87.22±2.90	100.26±1.93
B3S3	1.44±0.02	61.48±2.47	97.80±0.77	122.04±0.20
CV (%)	43.80	31.22	26.54	18.38

204 Note: values followed by the different letter in the column indicated significantly by DMRT at
 205 $P < 0.05 \pm SE$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 206 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 207 isolate P7, B3= combination isolates N3+P7).

208

209 A significant increase in total dry weight of upland rice along with the increase in the dosage
 210 of biogas sludge to 630 mL polybag⁻¹ at 16 WAIBSA with the highest increase of 50.55%
 211 compared to the control. Although the effect was insignificant, it was seen that the B1 and the
 212 interaction of B3S3 showed the highest increase in the total dry weight of upland rice were
 213 20.84% and 81.53%, respectively compared to the control.

214

215 **Crop growth rate of upland rice**

216 The effect of superior bacterial isolates, biogas sludge, and their interactions significantly
 217 increased the crop growth rate of upland rice at 12 to 16 WAIBSA, but it had an insignificant
 218 effect at 4-8 and 8-12 WAIBSA (Tab. 5).

219 **TABLE 5.** The effect of superior bacterial isolates, biogas sludge, and their interactions on the
 220 crop growth rate of upland rice.

221

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
4-8 WAIBSA					
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV= 32.28%
8-12 WAIBSA					
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888

Average	0.620	0.859	1.083	0.977	CV= 56.17%
12-16 WAIBSA					
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV= 51.07%

222 Note: values followed by the different letter in the column indicated significantly by DMRT at
 223 $P < 0.05$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315;
 224 S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate
 225 P7, B3= combination isolates N3+P7).
 226

227 The biogas sludge dose of 630 mL polybag⁻¹ (S3) significantly increased the highest crop
 228 growth rate for upland rice at 12 to 16 WAIBSA by 129.44% compared to the control. The
 229 ability of B1-B3 isolates significantly increased the crop growth rate of upland rice with the
 230 highest increase in the B2 by 181.45% compared to the controls at 12 to 16 WAIBSA. The
 231 interaction of the B1S3 significantly increased the crop growth rate of upland rice by 5.76-times
 232 greater compared to the control.
 233

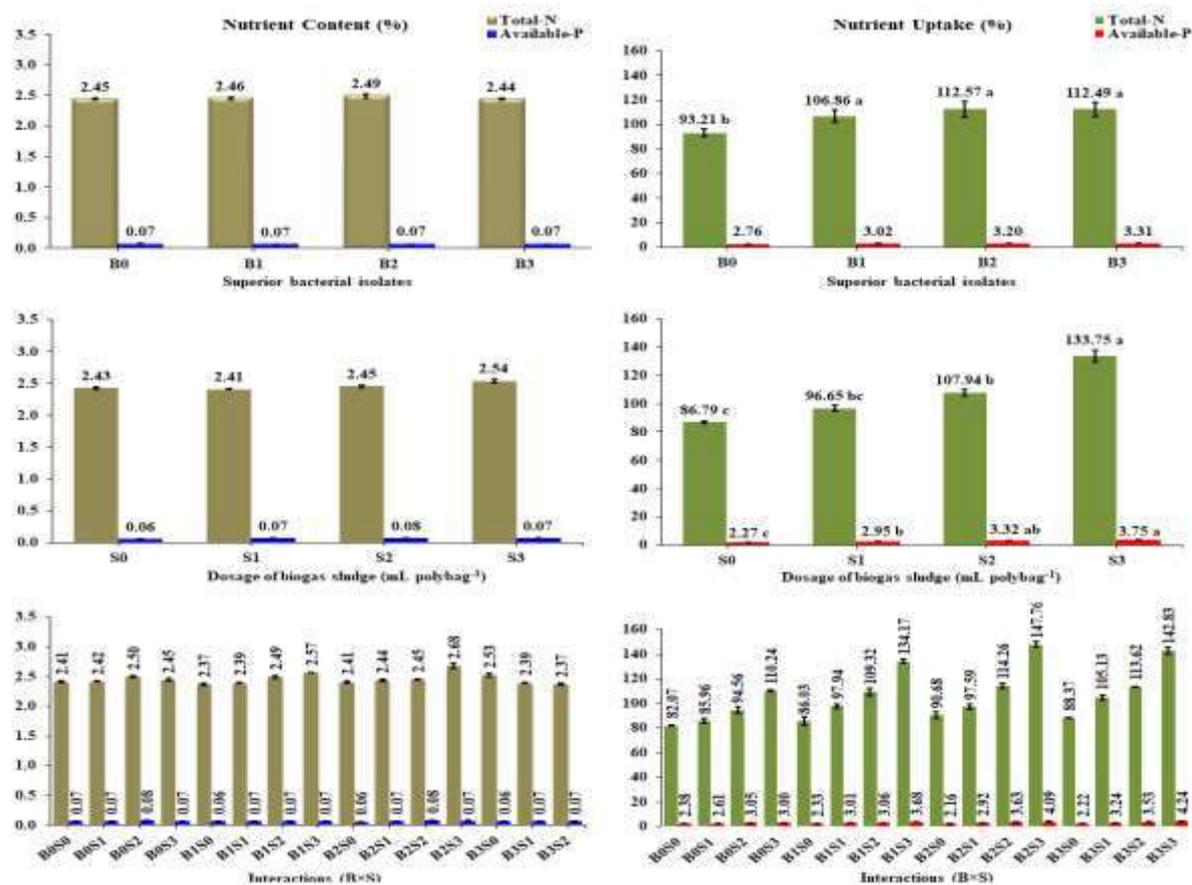
234 **Effect of bacterial isolates and biogas sludge on upland rice nutrition**

235 **Content of total-N and available-P nutrient of upland rice**

236 The effect of biogas sludge, superior bacterial isolates, and their interactions had an
 237 insignificant effect on the nutrient content of total-N and available-P in upland rice (Fig. 2).
 238 The biogas sludge doses of 315 and 630 mL polybag⁻¹ (S2 and S3) explained that the nutrient
 239 content of available-P and total-N in the plant tissue of upland rice were higher by 33.33% and
 240 4.53%, respectively compared to the control. The B2 isolate showed the highest nutrient content
 241 of total-N in the plant tissue of upland rice by 1.63% compared to the control, but all isolates
 242 (B1-B3) showed a similar level of available-P in the plant tissue of upland rice with the control.
 243

244 **Uptake of total-N and available-P nutrient of upland rice**

245 The effect of biogas sludge significantly increased in the nutrient uptake of total-N and
 246 available-P. Superior bacterial isolates significantly increased in the nutrient uptake of total-N.
 247 The interaction of biogas sludge with superior bacterial isolates had an insignificant effect on
 248 the nutrient uptake of total-N and available-P of upland rice (Fig. 2).



249 **FIGURE 2.** The effect of superior bacterial isolates, dosage of biogas sludge, and their
 250 interactions in the content and uptake of total-N and available-P nutrient of upland rice. Values
 251 followed by the different letter in graph indicated significantly by DMRT at $P < 0.05$. ns= not
 252 significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL
 253 polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3=
 254 combination isolates N3+P7).
 255

256 A significant increase in the nutrient uptake of total-N and available-P in upland rice along with
 257 the increase in the dose of biogas sludge to 630 mL polybag⁻¹ with the highest increase of
 258 54.11% and 65.20%, respectively compared to the control. The superior bacterial isolates (B1-
 259 B3) also significantly increased the nutrient uptake of total-N for upland rice with the highest
 260 increase in the B2 by 20.77% compared to the control. Although the effect was insignificant, it

261 was seen that the B3 showed the highest increase in nutrient uptake of available-P in upland
262 rice was 19.93% compared to the control.

263

264 **Discussion**

265 **The effect of selected superior bacterial isolates**

266 The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake
267 of total-N and crop growth rate of upland rice on ultisols at 12 to 16 WAIBSA, but it had an
268 insignificant effect on plant height, total fresh weight, total dry weight, nutrient content (total-
269 N and available-P) in leaf tissue, nutrient uptake of available-P, and crop growth rate of upland
270 rice at 4 to 8 and 8 to 12 WAIBSA. The superior bacterial isolates (N3, P7, and N3+P7) could
271 increase the nutrient uptake of total-N in upland rice by 14.64%, 20.77%, and 20.68%,
272 respectively compared to control (Fig. 2). Similar results are also shown in Table 5, that the
273 crop growth rate of upland rice at 12 to 16 WAIBSA due to selected superior bacterial isolates
274 (N3, P7, N3+P7) has increased by 2.57; 2.81; and 2.39-times, respectively compared to the
275 control. The finding results indicated that the ability of a single isolate by P7 bacteria was
276 greater in increasing total-N and crop growth rate of upland rice compared to a single isolate
277 by N3 bacteria and a combination of N3+P7 bacterial isolates. It was due to the presence of
278 several organic acids and hormones produced by P7 that can increase the nutrient uptake of
279 total-N and crop growth rate of upland rice. This result was supported by Mustamu *et al.* (2021)
280 that the phosphate solubilizing bacteria isolate (P7) from the biogas sludge contains organic
281 acids produced such as lactic, oxalic, acetic, citric acids, and it had the highest ability to
282 solubilize phosphate from calcium triphosphate and rock phosphate was 4.62 and 2.66-times,
283 respectively compared to the control. Meena *et al.* (2016) reported that the availability of
284 nitrogen and phosphorus slightly increased in the application of bio fertilization with *Bacillus*
285 *cereus*, it was due to the production of organic acids and other chemicals such as citric, tartaric,

286 and oxalic acids which can stimulate plant growth and nutrients availability. Youssef and Eissa
287 (2017) reported that the increase in vegetative growth and total biomass was due to increased
288 photosynthesis, translocation, and accumulation of nutrients. Khan *et al.* (2020) reported that
289 *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and
290 organic acids. Ferrara *et al.* (2012) reported that the hormone gibberellin, IAA, and other
291 hormones can increase plant growth under stressful conditions. Kang *et al.* (2014) said that
292 PGPB has several mechanisms to increase plant growth with nitrogen-fixation, phosphate
293 solubilizing, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of
294 palm oil solid waste from anaerobic digester include: *Ruminococcus sp.*, *Thiomargarita sp.*,
295 *Clostridium sp.*, *Anaerobacter sp.*, *Bacillus sp.*, *Sporobacterium sp.*, *Saccharofermentans sp.*,
296 *Oscillibacter sp.*, *Sporobacter sp.*, and *Enterobacter sp.* Liaquat *et al.* (2017) also reported that
297 an abundance of *Bacillus*, *Clostridium*, and *Enterobacter spp* in anaerobic digester of
298 wastewater in producing biogas.

299

300 **The effect of biogas sludge**

301 The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16
302 WAIBSA), total dry weight (12 and 16 WAIBSA), nutrient uptake (total-N and available-P),
303 and the crop growth rate of upland rice at 8 to 12 WAIBSA, but it had an insignificant effect
304 on nutrient content (total-N and available-P) in leaf tissue, and crop growth rate of upland rice
305 (4-8 and 8-12 WAIBSA). An increase in plant height, total dry weight, nutrient uptake of total-
306 N and available-P, and also crop growth rate of upland rice on ultisols along with the increase
307 in the dose of biogas sludge to 630 mL polybag⁻¹ at the end of this study (16 WAIBSA).
308 However, in contrast to the total fresh weight, an increase along with the increase in the biogas
309 sludge dose to 315 mL polybag⁻¹ then decreased at the dose of 630 mL polybag⁻¹. It was caused
310 the biogas sludge had chemical characteristics such as pH (7.41), total-N (0.051%), available-

311 P (0.013%), organic-C (0.14%), total-K (0.18%), and biological characteristics such as total
312 nitrogen-fixing bacteria (29.4×10^5 CFU mL⁻¹) and total phosphate solubilizing bacteria
313 (7.0×10^4 CFU mL⁻¹) (Table 2). C-organic content and the total population of nitrogen-fixing
314 and phosphate solubilizing bacteria from the biogas sludge could be increased the nutrient
315 uptake of total-N and available-P in upland rice along with increasing the dose of biogas sludge
316 to 630 mL polybag⁻¹ (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic
317 processes and stimulate the growth of plant height, biomass, and crop growth rate of upland
318 rice. A similar result was reported by Mustamu and Triyanto (2020) that the macro and
319 micronutrients from the biogas sludge and also had the population of nitrogen-fixing and
320 phosphate solubilizing bacteria by 480×10^4 and 42×10^4 CFU mL⁻¹, respectively. Ndubuisi-
321 Nnaji *et al.* (2020) reported that total phosphate solubilizing bacteria (1.6 to 2.5 CFU mL⁻¹) was
322 significantly higher compared to nitrogen-fixing bacteria (0.5–1.4 CFU mL⁻¹) and a significant
323 increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester
324 bioreactors. Möller and Müller (2012) reported that an increase in concentrations of NH₄⁺-N
325 ranged from 45 to 80% after anaerobic waste.

326

327 **The interaction effect of selected superior bacterial isolates and biogas sludge**

328 The interaction of biogas sludge and superior bacterial isolates only significantly increased the
329 crop growth rate of upland rice on ultisols at 12-16 WAIBSA, but it had an insignificant effect
330 on other parameters in this study. The interaction of NFB *Bacillus paramycoides* with biogas
331 sludge at the dose of 630 mL polybag⁻¹ (B1S3) showed the highest crop growth rate of upland
332 rice compared to other interactions and 5.76-times greater compared to the control. It was
333 caused by the application of biogas sludge could be increased soil organic matter and the total
334 population of beneficial bacteria. Likewise, the characteristics of the biogas sludge had the
335 organic-C was 0.14%, total nitrogen-fixing bacteria was 29.4×10^5 CFU mL⁻¹, and total

336 phosphate solubilizing bacteria was 7.0×10^4 CFU mL⁻¹ (Tab. 2) could improve soil quality and
337 support the crop growth rate. This result is supported by Urra *et al.* (2019) that the application
338 of sewage sludge in the long-term significantly increases the organic matter in the soil, which
339 causes a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the
340 production of organic acids along with the decomposition of organic matter. Bhardwaj *et al.*
341 (2014); Carvajal-Muñoz *et al.* (2012) reported that the application of biofertilizer had
342 advantages in the plant such as availability of nutrients that are balanced for plant health,
343 stimulating nutrient mobilization that can increase soil biological activity, availability of
344 microbial food to encourage the growth of beneficial microorganisms, increasing the soil
345 organic matter content thereby increasing the cation exchange capacity. Siswanti and Lestari
346 (2019) reported that the interaction of biogas sludge+biofertilizer (36 mL+10 L ha⁻¹)
347 significantly increased the plant height, number of leaves, and capcaisin content in chili
348 compared to a single treatment of biogas sludge and biofertilizer.

349 **Conclusions**

350 The isolates of N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient uptake
351 of total-N and crop growth rate of upland rice on ultisols with the highest increase found in the
352 P7 isolate of 20.77% and 2.81-times, respectively. The dose of biogas sludge significantly
353 increased plant height, total dry weight, nutrient uptake of total-N and available-P, and also
354 crop growth rate of upland rice on ultisols with the highest increase at a dose of 630 mL polybag⁻¹
355 by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively. Likewise, the dose of
356 biogas sludge significantly increased the total fresh weight of upland rice with the highest
357 increase at the dose of 315 mL polybag⁻¹ by 41.81%. The interaction of isolates N3, P7, N3+P7
358 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice
359 on ultisols with the highest increase found in the B1S3 by 5.76-times.

360 **Conflict of interest statement**

361 The authors declare that there is no conflict of interest regarding the publication of this article.

362 **Author's contributions**

363 All authors formulated the overarching research goals and aims, provided the study materials,
364 developed or designed the methodology. NEM analyze and interpretation study data. NEM and
365 MS wrote the initial draft, managed and coordinated the research activity in field, data
366 collection. ZN and I verified the overall reproducibility of results and other research outputs.
367 All authors conducted the critical review/commentary/revision of the manuscript.

368

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Novilda Elisabeth Mustamu <nemustamu@gmail.com>

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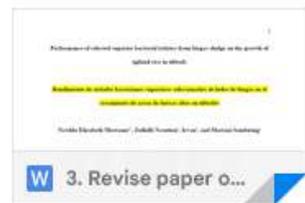
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Dear Novilda:

We are pleased to inform you that at least two reviewers, which have evaluated your manuscript entitled "Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols" (id. 97583), are recommending its publication.

We enclose the last version of your manuscript read by reviewers (SA_revCA_97583.docx), the evaluation forms (Eva files), and the reviewed versions of your manuscript (with some comments and suggestions from reviewers, Doc Eva files; N. B. reviewer #2 did not make comments to the manuscript).

Please reply to all questions from reviewers or follow their suggestions and recommendations (usually this is done in a separate file (i.e. "Answers to reviewers"), and indicate precisely the places where you are making the changes in the corrected manuscript. This is essential in order to continue with the editorial process. In the case you don't agree with some of the reviewers' suggestions or recommendations, you must justify (usually in the same separated Word file) why you will not follow them. According to the journal policy, the Editor-in-Chief has the last word about manuscript publication, and that decision is usually made after the author's reply to suggestions and corrections from reviewers.

We consider that **20 calendar days** is a reasonable time to receive from you the manuscript with the changes and suggestions discussed or incorporated. Therefore we hope to receive the manuscript and the responses to the reviewers before **October 23rd, 2021**. When reaching that date, if we have not received the manuscript from you, we will be reminding you about the deadline. If you require an extension of the deadline, let us know.

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Sincerely,

Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols

Rendimiento de aislados bacterianos superiores seleccionados de lodos de biogás en el crecimiento de arroz de tierras altas en ultisoles

ABSTRACT

The study searched to obtain the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice in ultisols. This study was conducted from October 2020 to April 2021 in a Randomized Block Design within two factors and seven replicates. The first factor was selected SBI (B0= untreated, B1= nitrogen-fixing bacteria isolate (N3), B2= phosphate solubilizing bacteria isolate (P7), B3= isolates combination (N3+P7)). The second factor was dosage of biogas sludge (S0= untreated, S1= 157.5; S2= 315; S3= 630 mL polybag⁻¹). The parameters were determined by ANOVA and followed by DMRT at $P < 0.05$. The results showed that the P7 isolate significantly increased total-N uptake and the highest crop growth rate (CGR) of upland rice by 20.77% and 2.81-folds, respectively. Biogas sludge dosage from 315 to 630 ml polybag⁻¹ significantly increased plant height, uptake of total-N and available-P, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 ml polybag⁻¹ significantly increased the CGR of upland rice. The application of N3 and P7 isolates and their combination within biogas sludge of 630 mL polybag⁻¹ has the potential to archive the CGR of upland rice in acidic soils.

Keywords: acidic soil, crop growth rate, dosage, sludge potential, upland rice.

RESUMEN

El estudio se realizó para obtener la influencia de aislados bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones en el crecimiento, la biomasa y la

Commented [MOU1]: The investigation/study evaluated

Commented [MOU2]: the first time it is cited in text do not use abbreviations, Duncan's multiple range test

Commented [MOU3]: nowhere is it explained what the "folds" are or how they were measured

28 absorción de nutrientes en el arroz de tierras altas en ultisoles. Este estudio utilizó un Diseño
29 de Bloques Aleatorizados dentro de dos factores y siete repeticiones desde octubre de 2020
30 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0= sin tratar, B1 = aislado de
31 bacterias fijadoras de nitrógeno (N3), B2= aislado de bacterias solubilizantes de fosfato (P7),
32 B3= combinación de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de
33 biogás (S0= sin tratar, S1= 157.5; S2= 315; S3= 630 mL de polybag⁻¹). Los parámetros fueron
34 determinados por ANOVA y seguidos por DMRT en $P < 0.05$. Los resultados mostraron que
35 el aislado P7 aumentó significativamente la captación total de N y la mayor tasa de
36 crecimiento del cultivo (TCC) de arroz de tierras altas en 20.77% y 2.81-pliegues,
37 respectivamente. La dosificación de fangos de biogás de 315 a 630 mL de polybag⁻¹ aumentó
38 significativamente la altura de la planta, la absorción de N total y P disponible, el peso fresco
39 y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosificación de
40 lodo de biogás de 630 mL de polybag⁻¹ aumentó significativamente el TCC del arroz de
41 tierras altas. La aplicación de aislamientos de N3 y P7 y su combinación dentro de lodos de
42 biogás de 630 mL de polybag⁻¹ tiene el potencial de archivar el TCC de arroz de tierras altas
43 en suelos ácidos.

44 **Palabras clave:** arroz de tierras altas, dosis, potencial de lodo, suelo ácido, tasa de
45 crecimiento de los cultivos.

46

47 **Introduction**

48 Biogas sludge is the waste by-product installation from an anaerobic processing system (Food
49 and Agriculture Organization, 1997) and has a high nutrient content that can be used as
50 organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). The biogas
51 sludge from palm oil waste contained total-N of 490 mg L⁻¹; NH₃-N was 65 mg L⁻¹; total-P by
52 110 mg L⁻¹; K of 1.9 mg L⁻¹; Ca by 23 mg L⁻¹; Mg of 256 mg L⁻¹; As <0.01 mg L⁻¹; Zn was
53 0.61 mg L⁻¹; and Cr by 0.04 mg L⁻¹ (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour

54 *et al.*, 2019), NH₃-N ranged by 91 to 112 mg L⁻¹ (Choorit & Wisarnwan, 2007), pH ranged by
55 6.8 to 8.3; and the highest bacterial populations was 7.21×10⁷ cells per ml and the lowest one
56 was 3.15×10⁷ cells per ml (Alvionita *et al.*, 2019). Mustamu and Triyanto (2020) also
57 reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria which
58 have the potential to increase availability of nitrogen and phosphate in soils.

59 The diversity of beneficial bacteria, such as nitrogen-fixing and phosphate solubilizing, has
60 greater potential for increasing soil fertility and plant growth. Zhang *et al.* (2013) reported that
61 phosphate solubilizing bacteria play an important role in increasing soil fertility, plant yield,
62 and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described that the *Bacillus*
63 genera such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B. mycoides*,
64 *B. coagulans*, *B. chitinolyticus*, and *B. subtilis* have been reported as having phosphate
65 solubilizing activities. Ambrosini *et al.* (2016) reported that *B. cereus* showed the highest
66 nitrogenase activity among 42 different strains of *Bacillus spp.* Lim *et al.* (2018) also reported
67 the dominant bacteria found in the biogas sludge from anaerobic processing using the
68 pyrosequencing and clone library methods, i.e. *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and
69 *Thermotogae*.

70 Application of bacterial isolates from biogas sludge for the improvement of upland rice
71 growth on acidic soils has never been reported in Indonesia. Thus, it is necessary to test the
72 potential of beneficial bacterial isolates from biogas sludge in increasing the availability of
73 nitrogen, phosphate, and the growth responses of upland rice due to the biogas sludge and
74 selected isolates in ultisols. The study was aimed at obtaining the influence of selected
75 superior bacterial isolates, biogas sludge, and their interaction on the growth of upland rice in
76 ultisols.

77 **Materials and methods**

78 **Study area**

79 The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in
80 the Analytical Laboratory of PT. Socfin Indonesia, Medan. The bacterial isolates were applied
81 to upland rice in Padang Bulan Village, Medan Selayang Subdistrict, Medan City, North
82 Sumatra, Indonesia from October 2020 to April 2021.

83 **Preparation of medium and upland rice seeds**

84 The planting medium used a soil type of ultisols from the Simalingkar area, Medan
85 Tuntungan Subdistrict, Medan City with a soil depth of 0 to 20 cm. One hundred grams of
86 soil samples were taken and analyzed for chemical characteristics such as pH, organic-C,
87 available-P, total-N, CEC, and base saturation (K, Ca, Na, Mg) (Tab. 1). The soil was
88 sterilized by drying at 100°C for 2 hours. After being incubated for 1 day, the soil was put into
89 a polybag of 10 kg. A basic fertilizer of NPK Mutiara at a dose of 300 kg ha⁻¹ was given by
90 stirring evenly with the soil. Concurrently, the seeds of upland rice were inbred varieties of
91 Inpago-8 then soaked in water for 24 h and followed by a propineb fungicide (70%) for 2 h.
92 Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth
93 of 2 cm.

94 **TABLE 1.** Chemical characteristics of sterile ultisols

95

Chemical characteristics	Value	Category*
Soil pH (H ₂ O)	4.80	Acid
Organic-C (%)	0.44	Very low
Total-N (%)	0.04	Very low
Available-P (mg kg ⁻¹)	870.25	Very high
CEC (me/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (me/100 g)	0.60	High
Ca (me/100 g)	0.34	Very low
Mg (me/100 g)	0.32	Very low
Na (me/100 g)	0.09	Very low
Al (%)	0.02	Very low

Commented [MOU4]: it is important to know environmental conditions such as rainfall, average temperature, humidity, altitude

Commented [MOU5]: why incubated?

Commented [MOU6]: Improve the wording, it is somewhat confusing

96 Source: *Criteria for pH H₂O= 4.5-5.5 (acid); organik-C <1% (very low); total-N <0,1% (very
 97 low); available-P >60 mg kg⁻¹ (very high); CEC= 25-40 me/100 g (high); base saturation
 98 <20% (very low); exchangeable-K= 0.60-1.00 me/100 g (high); exchangeable-Ca <2 me/100
 99 g (very low); exchangeable-Mg <0.4 me/100 g (very low); exchangeable-Na <0.1 me/100 g
 100 (very low); exchangeable-Al <5% (very low) (Soil Research Institute, 2009).
 101

102 Preparation of superior bacterial isolates suspension and biogas sludge

103 The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has
 104 been confirmed by Mustamu *et al.* (2021) and nitrogen-fixing bacteria or N3 (data
 105 unpublished). The isolates were grown on an NB medium, and incubated for 48 h. The
 106 microbial mass in the solution was measured using a spectrophotometer with a density of 10⁸
 107 cells per mL. ~~10⁷ Fe_n mL~~ was taken from the solution containing nitrogen-fixing bacteria
 108 (N3) and phosphate solubilizing bacteria (P7).

109 The biogas sludge was taken from an identical location in the first phase of the study (palm
 110 oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province,
 111 Indonesia). Bacterial isolate and biogas sludge were applied to the soil surface of the plants
 112 one week after planting (WAP). Biogas sludge of 500 mL samples were taken and then the
 113 chemical and biological characteristics were analyzed in the laboratory (Tab. 2).

114 **TABLE 2.** Chemical and biological characteristics of biogas sludge
 115

Characteristics of biogas sludge	Value
Chemical	
pH	7.41
Chemical oxygen demand/COD (mg L ⁻¹)	4547.8
Biological oxygen demand/BOD (mg L ⁻¹)	1127.5
Total-N (%)	0.051
Total-P (%)	0.0097
Available-P (%)	0.013
Total-K (%)	0.18
Organic-C (%)	0.14
Ca (%)	0.04
Mg (%)	0.04
Na (ppm)	44.41
Cu (%)	0.0001
Biological	

Commented [MOU7]: why are they superior bacteria? How were they selected? what species are they?

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Total nitrogen-fixing bacteria (CFU mL ⁻¹)	29.4×10 ⁵
Total phosphate solubilizing bacteria (CFU mL ⁻¹)	7.0×10 ⁴

116

117 **Treatments application**

118 This study used a Randomized Block Design within two factors and seven replicates. The first
 119 factor was the type of superior bacterial isolates at the similarly likewise dose, namely 10 mL
 120 polybag⁻¹ (B0= un-treated; B1= nitrogen-fixing bacterial isolate (N3); B2= phosphate
 121 solubilizing bacteria isolate (P7); B3= combination isolates N3+P7). The second factor was
 122 dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag⁻¹). Each
 123 replicate was dismantled at 4, 8, and 12 Weeks After Isolate and Biogas Sludge Application
 124 (WAIBSA) for determination of the CGR.

Commented [MOU9]: Improve the wording, it is somewhat confusing

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125 **Parameters and data analysis**

126 Variable observations were conducted by measuring the growth of upland rice (plant height,
 127 total fresh and dry weight), total-N and available-P content in the shoots, total uptake-N, and
 128 available-P. CGR was determined on plants at 4-8, 8-12, and 12-16 WAIBSA. Each polybag
 129 from each treatment and replicate was dismantled at the plants at 4, 8, 12, and 16 WAIBSA,
 130 then the plant height was measured, the total fresh weight was determined by weighing the
 131 roots and shoots. The total dry weight was measured by oven at 60°C for 48 h and weighed
 132 with analytical scales. A 200 g sample of the 2nd leaf from the shoots was taken by 200 g and
 133 analyzed for total-N using the Kjeldahl method and available-P by the dry ashing method with
 134 a UV-Vis Spectrophotometer. The total-N and available-P absorption were measured using
 135 equation (1). The CGR was calculated by the dry weight per unit area using equation (2)
 136 (Shon *et al.*, 1997):

Commented [MOU11]: dismantled? is the term appropriate?

Commented [MOU12]: second

$$137 \quad \text{Uptake nutrient} = \text{nutrient content in the shoots} \times \text{total dry weight} \quad (1)$$

$$138 \quad \text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W_2 - W_1}{t_2 - t_1} \quad (2)$$

139 where:

140 CGR = crop growth rate

141 W1 = dry weight per unit area at t1

142 W2 = dry weight per unit area at t2

143 t1 = first sampling

144 t2 = second sampling

145 The parameters of the second phase of the study were analyzed by ANOVA and if the
146 treatment had a significant effect, it was continued by DMRT at $P < 0.05$ with SPSS v.20
147 software.

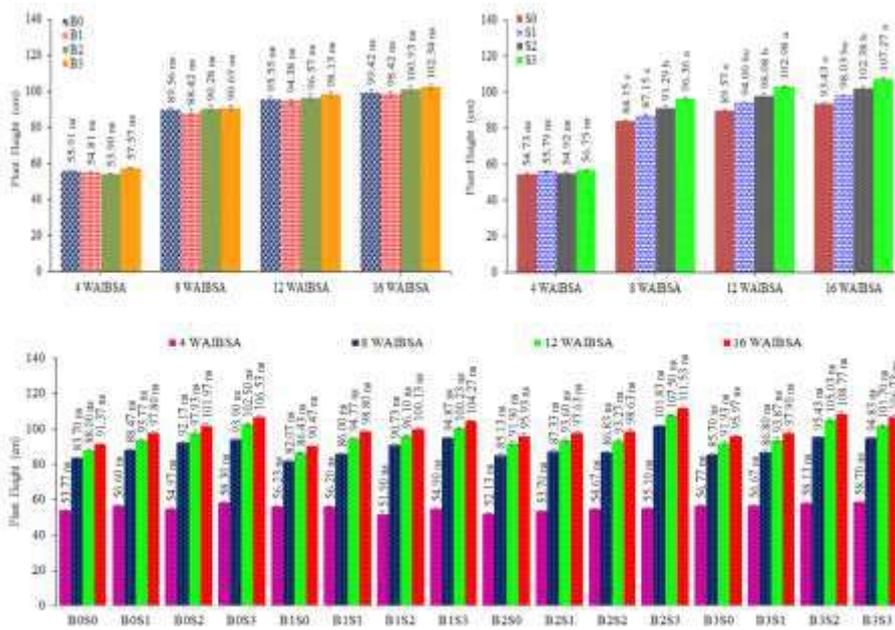
148

149 **Results and discussion**

150 **Plant height of upland rice**

151 The effect of biogas sludge application on the plant height of upland rice was significant at 8,
152 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant
153 effect on the plant height of upland rice at 4, 8, 12, and 16 WAIBSA (Fig. 1). A significant
154 increase in plant height of upland rice along with increased doses of biogas sludge to 630 mL
155 polybag⁻¹ at 8, 12, and 16 WAIBSA with the highest increase of 14.81% compared to the
156 control at 16 WAIBSA. Although the effect was insignificant, the isolates combination of B3
157 and the interaction of B2S3 showed the highest increase plant height of upland rice by 2.94%
158 and 22.06%, respectively, compared to the control.

Commented [MOU13]: I recommend that the results part be restructured in its figures and tables. It could only show figures that show a significant difference, without showing those that do not have a significant difference.



159 **FIGURE 1.** Effect of superior bacterial isolates, dosage of biogas sludge, and their
 160 interactions on plant height of upland rice at 4, 8, 12, and 16 WAIBSA. Values followed by
 161 the different letter in the graph indicated significantly by DMRT at $P < 0.05$. ns= not
 162 significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL
 163 polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3=
 164 combination isolates N3+P7).
 165

166 **Biomass of upland rice (g)**

167 The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8,
 168 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant
 169 effect on the total fresh weight of upland rice at 4-16 WAIBSA (Tab. 3).

170 **TABLE 3.** Effect of superior bacterial isolates, biogas sludge, and their interactions on total
 171 fresh weight of upland rice at 4, 8, 12, and 16 WAIBSA.
 172

Treatments	Total fresh weight \pm SE (g)			
	4 WAIBSA	8 WAIBSA	12 WAIBSA	16 WAIBSA
Superior bacterial isolates (B)				
B0	4.15 \pm 0.21ns	169.31 \pm 8.90ns	215.27 \pm 8.42ns	229.82 \pm 8.94ns
B1	3.12 \pm 0.12ns	194.50 \pm 9.35ns	235.08 \pm 10.32 ns	252.02 \pm 10.22ns
B2	4.52 \pm 0.23ns	162.89 \pm 11.15ns	201.85 \pm 9.89ns	230.70 \pm 9.28ns

B3	3.30±0.25ns	173.91±12.55ns	220.40±15.96ns	245.03±16.32ns
Biogas sludge (S)				
S0	3.72±0.24ns	144.07±9.37b	182.67±7.14b	197.56±6.58b
S1	3.58±0.27ns	153.41±7.93b	190.70±8.90b	215.65±7.03b
S2	3.64±0.27ns	199.68±10.30a	258.70±9.63a	280.15±9.25a
S3	4.15±0.25ns	203.45±1.36a	240.52±2.81a	264.21±2.42a
Interactions (B×S)				
B0S0	4.99±0.33ns	124.08±5.60ns	185.64±3.32ns	192.78±2.96ns
B0S1	3.47±0.26ns	160.43±1.16ns	188.60±5.76ns	207.05±3.97ns
B0S2	3.42±0.42ns	185.97±6.80ns	232.60±8.75ns	250.84±7.40ns
B0S3	4.71±0.42ns	206.76±5.49ns	254.23±10.27ns	268.61±8.85ns
B1S0	2.80±0.18ns	155.79±1.12ns	183.96±5.20ns	202.88±2.88ns
B1S1	3.74±0.29ns	174.82±9.01ns	227.91±6.38ns	236.60±6.32ns
B1S2	3.28±0.40ns	241.17±5.25ns	283.60±7.76ns	296.08±8.05ns
B1S3	2.67±0.22ns	206.20±7.23ns	244.85±6.26ns	272.52±4.34ns
B2S0	3.19±0.18ns	190.90±7.77ns	215.36±7.67ns	229.11±6.75ns
B2S1	4.85±0.38ns	106.74±13.42ns	143.16±13.02ns	179.61±10.36ns
B2S2	5.20±0.24ns	148.40±11.59ns	219.65±5.26ns	248.72±6.94ns
B2S3	4.82±0.45ns	205.53±10.50ns	229.21±16.57ns	265.34±9.58ns
B3S0	3.91±0.30ns	105.53±3.94ns	145.72±1.96ns	165.45±1.11ns
B3S1	2.25±0.09ns	171.63±4.90ns	203.14±7.07ns	239.34±12.07ns
B3S2	2.66±0.14ns	223.17±7.84ns	298.95±1.51ns	324.94±3.03ns
B3S3	4.37±0.07ns	195.31±6.77ns	233.79±8.40ns	250.38±8.16ns
CV (%)	56.09	29.68	26.31	20.78

173 Note: values followed by the different letter in the column indicated significantly by DMRT
 174 at $P < 0.05 \pm SE$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 175 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 176 isolate P7, B3= combination isolates N3+P7).
 177

178 A significant increase in total fresh weight of upland rice along with the increase in the
 179 dosage of biogas sludge to 315 mL polybag⁻¹ at 16 WAIBSA with the highest increase by
 180 41.81% compared to the control. Although the effect was insignificant, the B1 and the
 181 interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with
 182 9.66% and 68.55%, respectively, compared to the control.

183 The effect of biogas sludge significantly increased the total dry weight of upland rice at 12
 184 and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect
 185 on the total dry weight of upland rice at 4-16 WAIBSA (Tab. 4).

186 **TABLE 4.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the
 187 total dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Commented [MOU14]: This is not an interaction assessment, it is the treatments. In the ANOVA of this trial there are 16 treatments and 112 experimental units. The sources of variation evaluated would be (treatments, bacteria, sludge, and bacteria x sludge interaction). And there may be a significant difference in treatments without any interaction between the factors.

Treatments	Total dry weight \pm SE (g)			
	4 WAIBSA	8 WAIBSA	12 WAIBSA	16 WAIBSA
Superior bacterial isolates (B)				
B0	1.38 \pm 0.06ns	48.01 \pm 1.29ns	73.60 \pm 3.99ns	82.52 \pm 4.18ns
B1	1.13 \pm 0.05ns	54.09 \pm 2.41ns	76.83 \pm 2.66ns	99.72 \pm 4.15ns
B2	1.49 \pm 0.06ns	47.30 \pm 3.30ns	73.20 \pm 2.28ns	98.25 \pm 3.90ns
B3	1.15 \pm 0.07ns	52.32 \pm 3.39ns	77.18 \pm 4.90ns	98.47 \pm 4.56ns
Biogas sludge (S)				
S0	1.26 \pm 0.06ns	45.51 \pm 2.63ns	62.88 \pm 2.19b	76.78 \pm 1.63c
S1	1.23 \pm 0.08ns	44.47 \pm 1.71ns	68.52 \pm 2.00ab	87.65 \pm 2.84bc
S2	1.26 \pm 0.08ns	55.36 \pm 3.43ns	85.69 \pm 1.08a	98.95 \pm 1.86b
S3	1.40 \pm 0.06ns	56.38 \pm 1.05ns	83.73 \pm 3.44a	115.59 \pm 2.11a
Interactions (B \times S)				
B0S0	1.58 \pm 0.08ns	41.73 \pm 2.78ns	58.08 \pm 1.54ns	67.23 \pm 0.96ns
B0S1	1.12 \pm 0.08ns	45.87 \pm 0.83ns	62.74 \pm 1.83ns	71.08 \pm 1.91ns
B0S2	1.20 \pm 0.12ns	52.25 \pm 2.07ns	81.39 \pm 5.48ns	88.28 \pm 5.02ns
B0S3	1.60 \pm 0.12ns	52.18 \pm 0.29ns	92.20 \pm 3.05ns	103.49 \pm 2.43ns
B1S0	0.97 \pm 0.04ns	46.64 \pm 1.39ns	69.53 \pm 4.90ns	80.30 \pm 4.51ns
B1S1	1.40 \pm 0.07ns	48.13 \pm 2.78ns	78.91 \pm 0.53ns	96.23 \pm 1.50ns
B1S2	1.12 \pm 0.10ns	67.79 \pm 1.44ns	91.05 \pm 2.25ns	101.80 \pm 2.40ns
B1S3	1.02 \pm 0.08ns	53.81 \pm 3.76ns	67.84 \pm 1.77ns	120.54 \pm 2.15ns
B2S0	1.17 \pm 0.05ns	59.32 \pm 2.33ns	70.92 \pm 4.20ns	81.43 \pm 3.82ns
B2S1	1.54 \pm 0.10ns	34.47 \pm 2.16ns	61.69 \pm 1.97ns	89.84 \pm 1.41ns
B2S2	1.73 \pm 0.05ns	37.37 \pm 3.74ns	83.10 \pm 1.19ns	105.46 \pm 1.37ns
B2S3	1.53 \pm 0.10ns	58.05 \pm 1.76ns	77.07 \pm 4.27ns	116.28 \pm 1.30ns
B3S0	1.30 \pm 0.07ns	34.35 \pm 7.04ns	52.98 \pm 0.73ns	78.16 \pm 0.48ns
B3S1	0.85 \pm 0.03ns	49.40 \pm 0.08ns	70.72 \pm 1.29ns	93.44 \pm 2.19ns
B3S2	0.99 \pm 0.05ns	64.05 \pm 4.68ns	87.22 \pm 2.90ns	100.26 \pm 1.93ns
B3S3	1.44 \pm 0.02ns	61.48 \pm 2.47ns	97.80 \pm 0.77ns	122.04 \pm 0.20ns
CV (%)	43.80	31.22	26.54	18.38

189 Note: values followed by the different letter in the column indicated significantly by DMRT
 190 at $P < 0.05 \pm SE$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 191 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 192 isolate P7, B3= combination isolates N3+P7).
 193

194 A significant increase in total dry weight of upland rice along with the increase in the dosage
 195 of biogas sludge to 630 mL polybag⁻¹ at 16 WAIBSA with the highest increase of 50.55%
 196 compared to the control. Although the effect was insignificant, the B1 and the interaction of
 197 B3S3 showed the highest increase in the total dry weight of upland rice, with 20.84% and
 198 81.53%, respectively, compared to the control.

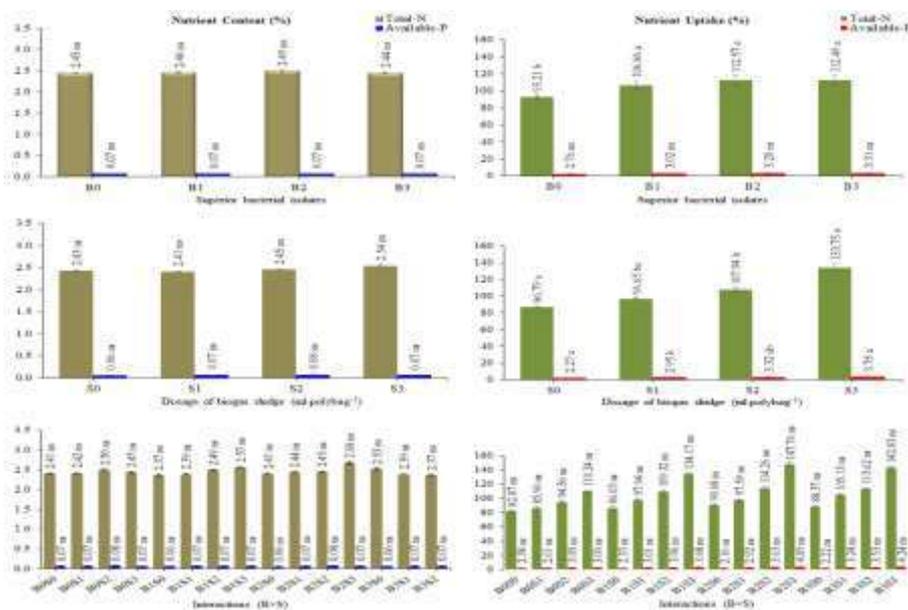
199

200 **Content and uptake of total-N and available-P nutrient of upland rice**

Commented [MOU15]: Idem table 3

Commented [MOU16]: The interaction was insignificant

201 The effect of biogas sludge, superior bacterial isolates, and their interactions had an
 202 insignificant effect on the nutrient content of total-N and available-P in upland rice. The effect
 203 of biogas sludge significantly increased the nutrient uptake of total-N and available-P.
 204 Superior bacterial isolates significantly increased the nutrient uptake of total-N. The
 205 interaction of biogas sludge with superior bacterial isolates had an insignificant effect on the
 206 nutrient uptake of total-N and available-P of upland rice (Fig. 2).



207 **FIGURE 2.** The effect of superior bacterial isolates, dosage of biogas sludge, and their
 208 interactions in the content and uptake of total-N and available-P nutrient of upland rice.
 209 Values followed by the different letter in graph indicated significantly by DMRT at $P < 0.05$.
 210 ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630
 211 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7,
 212 B3= combination isolates N3+P7).

213
 214 A significant increase in the nutrient uptake of total-N and available-P in upland rice along
 215 with the increase in the dose of biogas sludge to 630 mL polybag⁻¹ with the highest increase
 216 of 54.11% and 65.20%, respectively compared to the control. The superior bacterial isolates
 217 (B1-B3) also significantly increased the nutrient uptake of total-N for upland rice with the
 218 highest increase in the B2 by 20.77% compared to the control. Although the effect was

219 insignificant, the B3 showed the highest increase in nutrient uptake of available-P in upland
 220 rice, with 19.93% compared to the control. Likewise, the interaction of B2S3 and B3S3
 221 showed the highest increase in nutrient uptake of total-N and available-P in upland rice by
 222 80.04% and 79.41%, respectively, compared to the control.

Commented [MOU17]: there is no interaction, should not be analyzed, the results are a result of chance

223 The biogas sludge doses of 315 and 630 mL polybag⁻¹ (S2 and S3) explained that the nutrient
 224 content of available-P and total-N in the plant tissue of upland rice were higher by 33.33%
 225 and 4.53%, respectively, compared to the control. The B2 isolate showed the highest nutrient
 226 content of total-N in the plant tissue of upland rice by 1.63% compared to the control, but all
 227 isolates (B1-B3) showed a similar level of available-P in the plant tissue of upland rice with
 228 the control. The interactions of B2S3 and B2S2 also showed the highest nutrient content of
 229 total-N and available-P compared to other interactions.

Commented [MOU18]: there is no significant difference should not be analyzed, the differences are given only by chance

230

231 **Crop growth rate of upland rice**

232 The effect of superior bacterial isolates, biogas sludge, and their interactions significantly
 233 increased the crop growth rate of upland rice at 12 to 16 WAIBSA, but it had an insignificant
 234 effect at 4-8 and 8-12 WAIBSA (Tab. 5).

235 The biogas sludge dose of 630 mL polybag⁻¹ (S3) significantly increased the highest crop
 236 growth rate for upland rice at 12 to 16 WAIBSA by 129.44% compared to the control. The
 237 ability of B1-B3 isolates significantly increased the crop growth rate of upland rice with the
 238 highest increase in the B2 by 181.45% compared to the controls at 12 to 16 WAIBSA. The
 239 interaction of the B1S3 significantly increased the crop growth rate of upland rice by 5.76-
 240 folds greater compared to the control.

Commented [MOU19]: how it was calculated and where it compares with the other treatments

241 **TABLE 5.** The effect of superior bacterial isolates, biogas sludge, and their interactions on
 242 the crop growth rate of upland rice.

Commented [MOU20]: This table can generate confusion, take into account the recommendations of the previous tables

243

Superior bacterial	Biogas sludge (S)	Average
--------------------	-------------------	---------

isolates (B)	S0	S1	S2	S3	
4-8 WAIBSA					
B0	1.434ns	1.598ns	1.823ns	1.806ns	1.665ns
B1	1.631ns	1.669ns	2.381ns	1.885ns	1.892ns
B2	2.077ns	1.176ns	1.273ns	2.019ns	1.636ns
B3	1.180ns	1.734ns	2.252ns	2.144ns	1.828ns
Average	1.580ns	1.544ns	1.932ns	1.964ns	CV= 32.28%
8-12 WAIBSA					
B0	0.584ns	0.602ns	1.041ns	1.430ns	0.914ns
B1	0.818ns	1.099ns	0.831ns	0.501ns	0.812ns
B2	0.414ns	0.972ns	1.633ns	0.679ns	0.925ns
B3	0.665ns	0.761ns	0.828ns	1.297ns	0.888ns
Average	0.620ns	0.859ns	1.083ns	0.977ns	CV= 56.17%
12-16 WAIBSA					
B0	0.327fgh	0.298gh	0.246h	0.403b-h	0.318b
B1	0.385c-h	0.619a-h	0.384d-h	1.882a	0.817a
B2	0.375e-h	1.005a-h	0.798a-h	1.400a-h	0.895a
B3	0.899a-h	0.811a-h	0.466a-h	0.866a-h	0.761a
Average	0.496b	0.683b	0.474b	1.138a	CV= 51.07%

244 Note: values followed by the different letter in the column indicated significantly by DMRT
 245 at $P < 0.05$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 246 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 247 isolate P7, B3= combination isolates N3+P7).
 248

249 **The effect of selected superior bacterial isolates**

250 The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake
 251 of total-N and crop growth rate of upland rice on ultisols at 12 to 16 WAIBSA, but it had an
 252 insignificant effect on plant height, total fresh weight, total dry weight, nutrient content (total-
 253 N and available-P) in leaf tissue, nutrient uptake of available-P, and crop growth rate of
 254 upland rice at 4 to 8 and 8 to 12 WAIBSA. The superior bacterial isolates (N3, P7, and
 255 N3+P7) could increase the nutrient uptake of total-N in upland rice by 14.64%, 20.77%, and
 256 20.68%, respectively compared to control (Fig. 2). Similar results are also shown in Table 5,
 257 that the crop growth rate of upland rice at 12 to 16 WAIBSA due to selected superior bacterial
 258 isolates (N3, P7, N3+P7) has increased by 2.57; 2.81; and 2.39-folds, respectively compared
 259 to the control. The finding results indicated that the ability of a single isolate by P7 bacteria
 260 was greater in increasing total-N and crop growth rate of upland rice compared to a single
 261 isolate by N3 bacteria and a combination of N3+P7 bacterial isolates. It was due to the

Commented [MOU21]: In this analysis, it is necessary to justify the reason why the bacteria achieve a greater absorption of nutrients such as nitrogen and a higher growth rate, but do not achieve a greater height, dry weight and content of N and P

262 presence of several organic acids and hormones produced by P7 that can increase the nutrient
263 uptake of total-N and crop growth rate of upland rice. This result was supported by Mustamu
264 *et al.* (2021) that the phosphate solubilizing bacteria isolate (P7) from the biogas sludge
265 contains organic acids produced such as lactic, oxalic, acetic, citric acids, and it had the
266 highest ability to solubilize phosphate from calcium triphosphate and rock phosphate was
267 4.62 and 2.66-folds, respectively compared to the control. Meena *et al.* (2016) reported that
268 the availability of nitrogen and phosphorus slightly increased in the application of bio
269 fertilization with *Bacillus cereus*, it was due to the production of organic acids and other
270 chemicals such as citric, tartaric, and oxalic acids which can stimulate plant growth and
271 nutrients availability. Youssef and Eissa (2017) reported that the increase in vegetative growth
272 and total biomass was due to increased photosynthesis, translocation, and accumulation of
273 nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the
274 gibberellins, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that
275 gibberellins, IAA, and other hormones can increase plant growth under stressful conditions.
276 Kang *et al.* (2014) said that PGPB has several mechanisms to increase plant growth with
277 nitrogen-fixation, phosphate solubilizing, increasing nutrient availability. Suksong *et al.*
278 (2016) reported that bacteria of palm oil solid waste from anaerobic digester include:
279 *Ruminococcus sp.*, *Thiomargarita sp.*, *Clostridium sp.*, *Anaerobacter sp.*, *Bacillus sp.*,
280 *Sporobacterium sp.*, *Saccharofermentans sp.*, *Oscillibacter sp.*, *Sporobacter sp.*, and
281 *Enterobacter sp.* Liaquat *et al.* (2017) also reported that an abundance of *Bacillus*,
282 *Clostridium*, and *Enterobacter spp* in anaerobic digester of wastewater in producing biogas.

283

284 **The effect of biogas sludge**

285 The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and
286 16 WAIBSA), total dry weight (12 and 16 WAIBSA), nutrient uptake (total-N and available-

287 P), and the crop growth rate of upland rice at 8 to 12 WAIBSA, but it had an insignificant
288 effect on nutrient content (total-N and available-P) in leaf tissue, and crop growth rate of
289 upland rice (4-8 and 8-12 WAIBSA). An increase in plant height, total dry weight, nutrient
290 uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols along
291 with the increase in the dose of biogas sludge to 630 mL polybag⁻¹ at the end of this study (16
292 WAIBSA). However, in contrast to the total fresh weight, an increase along with the increase
293 in the biogas sludge dose to 315 mL polybag⁻¹ then decreased at the dose of 630 mL polybag⁻¹.
294 It was caused the biogas sludge had chemical characteristics such as pH (7.41), total-N
295 (0.051%), available-P (0.013%), organic-C (0.14%), total-K (0.18%), and biological
296 characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU mL⁻¹) and total phosphate
297 solubilizing bacteria (7.0×10^4 CFU mL⁻¹) (Tab. 2). C-organic content and the total population
298 of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could be
299 increased the nutrient uptake of total-N and available-P in upland rice along with increasing
300 the dose of biogas sludge to 630 mL polybag⁻¹ (Fig. 2). Therefore, the nutrients absorbed are
301 used for plant metabolic processes and stimulate the growth of plant height, biomass, and crop
302 growth rate of upland rice. A similar result was reported by Mustamu and Triyanto (2020)
303 that the macro and micronutrients from the biogas sludge and also had the population of
304 nitrogen-fixing and phosphate solubilizing bacteria by 480×10^4 and 42×10^4 CFU mL⁻¹,
305 respectively. Ndubuisi-Nnaji *et al.* (2020) reported that total phosphate solubilizing bacteria
306 (1.6 to 2.5 CFU mL⁻¹) was significantly higher compared to nitrogen-fixing bacteria (0.5 – 1.4
307 CFU mL⁻¹) and a significant increase in nutrient concentration in the order of
308 N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported
309 that an increase in concentrations of NH₄⁺-N ranged from 45 to 80% after anaerobic waste.

310

311 **The interaction effect of selected superior bacterial isolates and biogas sludge**

312 The interaction of biogas sludge and superior bacterial isolates only significantly increased
 313 the crop growth rate of upland rice on ultisols at 8 to 12 WAIBSA, but it had an insignificant
 314 effect on other parameters in this study. The interaction of NFB *Bacillus paramycooides* with
 315 biogas sludge at the dose of 630 mL polybag⁻¹ (B1S3) showed the highest crop growth rate of
 316 upland rice compared to other interactions and 5.76-fold greater compared to the control. It
 317 was caused by the application of biogas sludge that could increase the contents of soil organic
 318 matter and the total population of beneficial bacteria. Likewise, the characteristics of the
 319 biogas sludge had the organic-C was 0.14%, total nitrogen-fixing bacteria was 29.4×10^5 CFU
 320 mL⁻¹, and total phosphate solubilizing bacteria was 7.0×10^4 CFU mL⁻¹ (Tab. 2) could improve
 321 soil quality and support the crop growth rate. This result is supported by Urrea *et al.* (2019)
 322 that the application of sewage sludge in the long-term significantly increases the organic
 323 matter in the soil, which causes a decrease in soil pH due to the nitrification of ammonium in
 324 sewage sludge and the production of organic acids along with the decomposition of organic
 325 matter. Bhardwaj *et al.* (2014); Carvajal-Muñoz *et al.* (2012) reported that the application of
 326 biofertilizer had advantages for plants, such as availability of nutrients that are balanced for
 327 plant health, stimulating nutrient mobilization that can increase soil biological activity,
 328 availability of microbial food to encourage the growth of beneficial microorganisms,
 329 increasing the soil organic matter content thereby increasing the cation exchange capacity.
 330 Siswanti and Lestari (2019) reported that the interaction of biogas sludge+biofertilizer (36
 331 mL+10 L ha⁻¹) significantly increased the plant height, number of leaves, and capcaisin
 332 content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

333

334 Conclusions

335 The isolates of N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient
 336 uptake of total-N and crop growth rate of upland rice on ultisols with the highest increase

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337 found in the P7 isolate of 20.77% and 2.81-folds, respectively. The dose of biogas sludge
338 significantly increased plant height, total dry weight, nutrient uptake of total-N and available-
339 P, and also crop growth rate of upland rice on ultisols with the highest increase at a dose of
340 630 mL polybag⁻¹ by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively.
341 Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland
342 rice with the highest increase at the dose of 315 mL polybag⁻¹ by 41.81%. The interaction of
343 isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop
344 growth rate of upland rice on ultisols with the highest increase found in the B1S3 by 5.76-
345 folds.

346

347 **Conflict of interest statement**

348 The authors declare that there is no conflict of interest regarding the publication of this article.

349

350 **Author's contributions**

351 All authors formulated the overarching research goals and aims, provided the study materials,
352 developed or designed the methodology. NEM analyze and interpretation study data. NEM
353 and MS wrote the initial draft, managed and coordinated the research activity in field, data
354 collection. ZN and I verified the overall reproducibility of results and other research outputs.
355 All authors conducted the critical review/commentary/revision of the manuscript.

356

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Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols

Rendimiento de aislados bacterianos superiores seleccionados de lodos de biogás en el crecimiento de arroz de tierras altas en ultisoles

ABSTRACT

The study searched to obtain the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice in ultisols. This study was conducted from October 2020 to April 2021 in a Randomized Block Design within two factors and seven replicates. The first factor was selected SBI (B0= untreated, B1= nitrogen-fixing bacteria isolate (N3), B2= phosphate solubilizing bacteria isolate (P7), B3= isolates combination (N3+P7)). The second factor was dosage of biogas sludge (S0= untreated, S1= 157.5; S2= 315; S3= 630 mL polybag⁻¹). The parameters were determined by ANOVA and followed by DMRT at $P < 0.05$. The results showed that the P7 isolate significantly increased total-N uptake and the highest crop growth rate (CGR) of upland rice by 20.77% and 2.81-folds, respectively. Biogas sludge dosage from 315 to 630 ml polybag⁻¹ significantly increased plant height, uptake of total-N and available-P, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 ml polybag⁻¹ significantly increased the CGR of upland rice. The application of N3 and P7 isolates and their combination within biogas sludge of 630 mL polybag⁻¹ has the potential to archive the CGR of upland rice in acidic soils.

Keywords: acidic soil, crop growth rate, dosage, sludge potential, upland rice.

RESUMEN

El estudio se realizó para obtener la influencia de aislados bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones en el crecimiento, la biomasa y la

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Commented [I2]: What the authors wanted to say? I don't understand this term.

Commented [I3]: The authors evaluated only the effect of bacteria and biogas sludge on rice growth. They evaluated also the effect of these treatments on rice nutrition!

Commented [I4]: Why is it necessary o specify the soil type?

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Commented [I6]: The abstract does not show the investigation novelty and the importance of results to agricultural management.

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28 absorción de nutrientes en el arroz de tierras altas en ultisoles. Este estudio utilizó un Diseño
 29 de Bloques Aleatorizados dentro de dos factores y siete repeticiones desde octubre de 2020
 30 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0= sin tratar, B1 = aislado de
 31 bacterias fijadoras de nitrógeno (N3), B2= aislado de bacterias solubilizantes de fosfato (P7),
 32 B3= combinación de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de
 33 biogás (S0= sin tratar, S1= 157.5; S2= 315; S3= 630 mL de polybag⁻¹). Los parámetros fueron
 34 determinados por ANOVA y seguidos por DMRT en $P < 0.05$. Los resultados mostraron que
 35 el aislado P7 aumentó significativamente la captación total de N y la mayor tasa de
 36 crecimiento del cultivo (TCC) de arroz de tierras altas en 20.77% y 2.81 pliegues,
 37 respectivamente. La dosificación de fangos de biogás de 315 a 630 mL de polybag⁻¹ aumentó
 38 significativamente la altura de la planta, la absorción de N total y P disponible, el peso fresco
 39 y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosificación de
 40 lodo de biogás de 630 mL de polybag⁻¹ aumentó significativamente el TCC del arroz de
 41 tierras altas. La aplicación de aislamientos de N3 y P7 y su combinación dentro de lodos de
 42 biogás de 630 mL de polybag⁻¹ tiene el potencial de archivar el TCC de arroz de tierras altas
 43 en suelos ácidos.

44 **Palabras clave:** arroz de tierras altas, dosis, potencial de lodo, suelo ácido, tasa de
 45 crecimiento de los cultivos.

46

47 Introduction

48 Biogas sludge is the waste by-product installation from an anaerobic processing system (Food
 49 and Agriculture Organization, 1997) and has a high nutrient content that can be used as
 50 organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). The biogas
 51 sludge from palm oil waste contained total-N of 490 mg L⁻¹; NH₃-N was 65 mg L⁻¹; total-P by
 52 110 mg L⁻¹; K of 1.9 mg L⁻¹; Ca by 23 mg L⁻¹; Mg of 256 mg L⁻¹; As <0.01 mg L⁻¹; Zn was
 53 0.61 mg L⁻¹; and Cr by 0.04 mg L⁻¹ (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour

Commented [I19]: Very much details. The authors must abbreviate since this will be said in Materials and methods.

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Commented [I11]: The isolate itself does not produce those effects. It is the application or inoculation of the isolate that produces the effects. Improve wording.

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54 *et al.*, 2019), NH₃-N ranged by 91 to 112 mg L⁻¹ (Choorit & Wisarnwan, 2007), pH ranged by
 55 6.8 to 8.3; and the highest bacterial populations was 7.21×10⁷ cells per ml and the lowest one
 56 was 3.15×10⁷ cells per ml (Alvionita *et al.*, 2019). Mustamu and Triyanto (2020) also
 57 reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria which
 58 have the potential to increase availability of nitrogen and phosphate in soils.

59 The diversity of beneficial bacteria, such as nitrogen-fixing and phosphate solubilizing, has
 60 greater potential for increasing soil fertility and plant growth. Zhang *et al.* (2013) reported that
 61 phosphate solubilizing bacteria play an important role in increasing soil fertility, plant yield,
 62 and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described that the *Bacillus*
 63 genera such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B. mycoides*,
 64 *B. coagulans*, *B. chitinolyticus*, and *B. subtilis* have been reported as having phosphate
 65 solubilizing activities. Ambrosini *et al.* (2016) reported that *B. cereus* showed the highest
 66 nitrogenase activity among 42 different strains of *Bacillus spp.* Lim *et al.* (2018) also reported
 67 the dominant bacteria found in the biogas sludge from anaerobic processing using the
 68 pyrosequencing and clone library methods, i.e. *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and
 69 *Thermotogae*.

70 Application of bacteriai isolates from biogas sludge for the improvement of upland rice
 71 growth on acidic soils has never been reported in Indonesia. Thus, it is necessary to test the
 72 potential of beneficial bacterial isolates from biogas sludge in increasing the availability of
 73 nitrogen, phosphate, and the growth responses of upland rice due to the biogas sludge and
 74 selected isolates in ultisols. The study was aimed at obtaining the influence of selected
 75 superior bacterial isolates, biogas sludge, and their interaction on the growth of upland rice in
 76 ultisols.

77 **Materials and methods**

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Commented [I17]: I think it is unnecessary to show all these *Bacillus* species. The authors will work with this genera in this investigation? In materials and methods the authors do not say that they work with this bacterial genera. I recommend to explain more the lines 66-69 since it is the essence of this work.

Commented [I18]: Are acid soils representative in Indonesia? What percentage of rice is cultivated upland in Indonesia? Is it representative? I think that the authors must state this information here for the enhancement of the investigation novelty. Furthermore, the authors must show the importance of using bio-products as an alternative to mineral fertilization in rice.

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78 Study area

79 The concentration of total-N and available-P in ultisols and the plant tissue were analyzed in
80 the Analytical Laboratory of PT. Socfin Indonesia, Medan. The bacterial isolates were applied
81 to upland rice in Padang Bulan Village, Medan Selayang Subdistrict, Medan City, North
82 Sumatra, Indonesia from October 2020 to April 2021.

83 Preparation of medium and upland rice seeds

84 The planting medium used a soil type of ultisols from the Simalingkar area, Medan
85 Tuntungan Subdistrict, Medan City with a soil depth of 0 to 20 cm. One hundred grams of
86 soil samples were taken and analyzed for chemical characteristics such as pH, organic-C,
87 available-P, total-N, CEC, and base saturation (K, Ca, Na, Mg) (Tab. 1). The soil was
88 sterilized by drying at 100°C for 2 h. After being incubated for 1 d, the soil was put into a
89 polybag of 10 kg. A basic fertilizer of NPK Mutiara at a dose of 300 kg ha⁻¹ was given by
90 stirring evenly with the soil. Concurrently, the seeds of upland rice were inbred varieties of
91 Inpago-8 then soaked in water for 24 h and followed by a propineb fungicide (70%) for 2 h.
92 Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth
93 of 2 cm.

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94 **TABLE 1.** Chemical characteristics of sterile ultisols

95

Chemical characteristics	Value	Category*
Soil pH (H ₂ O)	4.80	Acid
Organic-C (%)	0.44	Very low
Total-N (%)	0.04	Very low
Available-P (mg kg ⁻¹)	870.25	Very high
CEC (me/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (me/100 g)	0.60	High
Ca (me/100 g)	0.34	Very low
Mg (me/100 g)	0.32	Very low
Na (me/100 g)	0.09	Very low
Al (%)	0.02	Very low

96 Source: *Criteria for pH H₂O= 4.5-5.5 (acid); organik-C <1% (very low); total-N <0,1% (very
 97 low); available-P >60 mg kg⁻¹ (very high); CEC= 25-40 me/100 g (high); base saturation
 98 <20% (very low); exchangeable-K= 0.60-1.00 me/100 g (high); exchangeable-Ca <2 me/100
 99 g (very low); exchangeable-Mg <0.4 me/100 g (very low); exchangeable-Na <0.1 me/100 g
 100 (very low); exchangeable-Al <5% (very low) (Soil Research Institute, 2009).
 101

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102 Preparation of superior bacterial isolates suspension and biogas sludge

103 The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has
 104 been confirmed by Mustamu *et al.* (2021) and nitrogen-fixing bacteria or N3 (data
 105 unpublished). The isolates were grown on an NB medium, and incubated for 48 h. The
 106 ~~bacteria growth microbial mass~~ in the solution was measured using a spectrophotometer with
 107 a density of 10⁸ cells per mL. Ten ml was taken from the solution containing nitrogen-fixing
 108 bacteria (N3) and phosphate solubilizing bacteria (P7);

Commented [I23]: The authors must review the English. I don't understand what they wrote here.

Commented [I24]: What λ was use? The result that allows this determination is an optical density data. How the authors know that this date correspondis to 10⁸ cells per mL?

109 The biogas sludge was taken from an identical location in the first phase of the study (palm
 110 oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province,
 111 Indonesia). Bacterial isolate and biogas sludge were applied to the soil surface of the plants
 112 one week after planting (WAP). Biogas sludge of 500 ml samples were taken and then the
 113 chemical and biological characteristics were analyzed in the laboratory (Tab. 2).

Commented [I25]: Is this isolate a Bacilli? I consider very important that the authors declare this. The authors must give more data about both bacteria isolates.

Commented [I26]: Incomplete

Commented [I27]: I think that the authors must give more details about how biogas sludge was taken.

114 **TABLE 2.** Chemical and biological characteristics of biogas sludge
 115

Characteristics of biogas sludge	Value
Chemical	
pH	7.41
Chemical oxygen demand/COD (mg L ⁻¹)	4547.8
Biological oxygen demand/BOD (mg L ⁻¹)	1127.5
Total-N (%)	0.051
Total-P (%)	0.0097
Available-P (%)	0.013
Total-K (%)	0.18
Organic-C (%)	0.14
Ca (%)	0.04
Mg (%)	0.04
Na (ppm)	44.41
Cu (%)	0.0001
Biological	

Commented [I28]: The authors must say how the chemical and biological characteristics of biogas sludge were determinate, or make a bibliography cite.

Total nitrogen-fixing bacteria (CFU mL ⁻¹)	29.4×10 ⁵
Total phosphate solubilizing bacteria (CFU mL ⁻¹)	7.0×10 ⁴

116

117 **Treatments application**

118 This study used a Randomized Block Design with two factors and seven replicates. The first
 119 factor was the type of superior bacterial isolates at the similarly likewise dose, namely 10 mL
 120 polybag⁻¹ (B0= un-treated; B1= nitrogen-fixing bacterial isolate (N3); B2= phosphate
 121 solubilizing bacteria isolate (P7); B3= combination isolates N3+P7). The second factor was
 122 dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL polybag⁻¹). Each
 123 replicate was dismantled at 4, 8, and 12 Weeks After Isolate and Biogas Sludge Application
 124 (WAIBSA) for determination of the CGR.

Commented [I29]: Why the authors use these biogas sludge concentration. I think it is important!

Commented [I30]: It is necessary to say what it is. In abstract it is said but is necessary to repeat it here.

125 **Parameters and data analysis**

126 Variable observations were conducted by measuring the growth of upland rice (plant height,
 127 total fresh and dry weight), total-N and available-P content in the shoots, total uptake-N, and
 128 available-P. CGR was determined on plants at 4-8, 8-12, and 12-16 WAIBSA. Each polybag
 129 from each treatment and replicate was dismantled at the plants at 4, 8, 12, and 16 WAIBSA,
 130 then the plant height was measured, the total fresh weight was determined by weighing the
 131 roots and shoots. The total dry weight was measured by oven at 60°C for 48 h and weighed
 132 with analytical scales. A 200 g sample of the 2nd leaf from the shoots was taken by 200 g and
 133 analyzed for total-N using the Kjeldahl method and available-P by the dry ashing method with
 134 a UV-Vis Spectrophotometer. The total-N and available-P absorption were measured using
 135 equation (1). The CGR was calculated by the dry weight per unit area using equation (2)
 136 (Shon *et al.*, 1997):

$$137 \quad \text{Uptake nutrient} = \text{nutrient content in the shoots} \times \text{total dry weight} \quad (1)$$

$$138 \quad \text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W_2 - W_1}{t_2 - t_1} \quad (2)$$

139 where:

140 CGR = crop growth rate

141 W1 = dry weight per unit area at t1

142 W2 = dry weight per unit area at t2

143 t1 = first sampling

144 t2 = second sampling

145 The parameters of the second phase of the study were analyzed by ANOVA and if the
146 treatment had a significant effect, it was continued by DMRT at $P < 0.05$ with SPSS v.20
147 software.

148

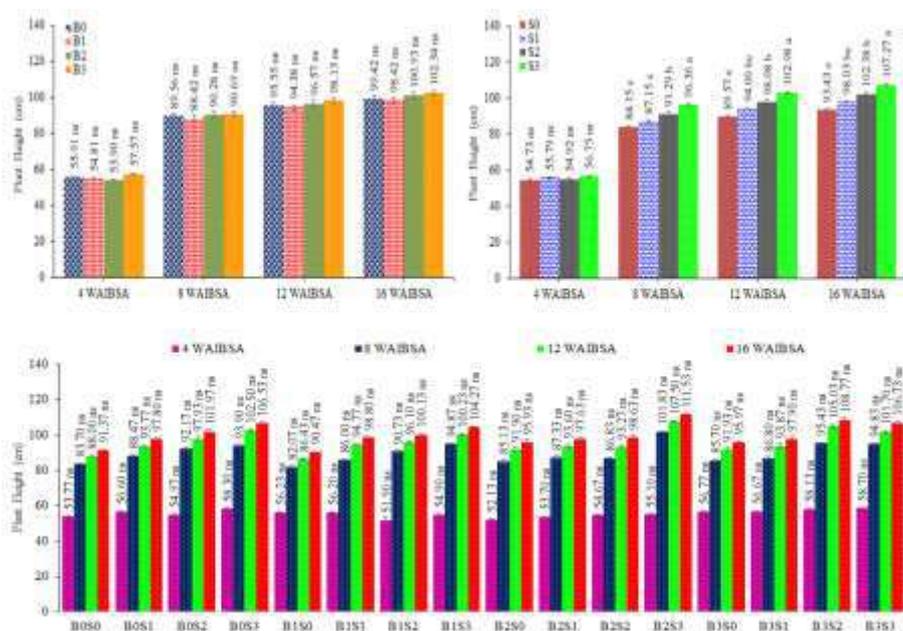
149 **Results and discussion**

150 **Plant height of upland rice**

151 The effect of biogas sludge application on the plant height of upland rice was significant at 8,
152 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant
153 effect on the plant height of upland rice at 4, 8, 12, and 16 WAIBSA (Fig. 1). A significant
154 increase in plant height of upland rice along with increased doses of biogas sludge to 630 ml
155 polybag⁻¹ at 8, 12, and 16 WAIBSA with the highest increase of 14.81% compared to the
156 control at 16 WAIBSA. Although the effect was insignificant, the isolates combination of B3
157 and the interaction of B2S3 showed the highest increase plant height of upland rice by 2.94%
158 and 22.06%, respectively, compared to the control.

Commented [I31]: It is necessary to say what is it. In the abstract it is said but it's necessary to repeat it here.

Commented [I32]: I consider that it is necessary to write gain Results and discussion in other form. The information of this section must be re-organized in two aspect: (1) Effect of bacterial isolates and biogas sludge on upland rice growth. (2) Effect of bacterial isolates and biogas sludge on upland rice nutrition. The first point could include:
-Plant height of upland rice
-Biomass of upland rice
-Crop growth rate of upland rice
The second point could include:
-Content of total-N and available-P nutrient of upland rice
-Uptake of total-N and available-P nutrient of upland rice
Furthermore, for both points the analysis made from lines 249-332 must be undertook.



159 **FIGURE 1.** Effect of superior bacterial isolates, dosage of biogas sludge, and their
 160 interactions on plant height of upland rice at 4, 8, 12, and 16 WAIBSA. Values followed by
 161 the different letter in the graph indicated significantly by DMRT at $P < 0.05$. ns= not
 162 significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630 mL
 163 polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7, B3=
 164 combination isolates N3+P7).

165

166 Biomass of upland rice (g)

167 The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8,
 168 12, and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant
 169 effect on the total fresh weight of upland rice at 4-16 WAIBSA (Tab. 3).

170 **TABLE 3.** Effect of superior bacterial isolates, biogas sludge, and their interactions on total
 171 fresh weight of upland rice at 4, 8, 12, and 16 WAIBSA.

172

Treatments	Total fresh weight \pm SE (g)			
	4 WAIBSA	8 WAIBSA	12 WAIBSA	16 WAIBSA
Superior bacterial isolates (B)				
B0	4.15 \pm 0.21ns	169.31 \pm 8.90ns	215.27 \pm 8.42ns	229.82 \pm 8.94ns
B1	3.12 \pm 0.12ns	194.50 \pm 9.35ns	235.08 \pm 10.32 ns	252.02 \pm 10.22ns
B2	4.52 \pm 0.23ns	162.89 \pm 11.15ns	201.85 \pm 9.89ns	230.70 \pm 9.28ns

Commented [I33]: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures. The authors could explore the possibility that gives them not interaction between the factors for discussing the result.

Commented [I34]: I think that it is redundant to show the variables values on the bars. I recommend to remove it.

Commented [I35]: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the interaction.

B3	3.30±0.25ns	173.91±12.55ns	220.40±15.96ns	245.03±16.32ns
Biogas sludge (S)				
S0	3.72±0.24ns	144.07±9.37b	182.67±7.14b	197.56±6.58b
S1	3.58±0.27ns	153.41±7.93b	190.70±8.90b	215.65±7.03b
S2	3.64±0.27ns	199.68±10.30a	258.70±9.63a	280.15±9.25a
S3	4.15±0.25ns	203.45±1.36a	240.52±2.81a	264.21±2.42a
Interactions (B×S)				
B0S0	4.99±0.33ns	124.08±5.60ns	185.64±3.32ns	192.78±2.96ns
B0S1	3.47±0.26ns	160.43±1.16ns	188.60±5.76ns	207.05±3.97ns
B0S2	3.42±0.42ns	185.97±6.80ns	232.60±8.75ns	250.84±7.40ns
B0S3	4.71±0.42ns	206.76±5.49ns	254.23±10.27ns	268.61±8.85ns
B1S0	2.80±0.18ns	155.79±1.12ns	183.96±5.20ns	202.88±2.88ns
B1S1	3.74±0.29ns	174.82±9.01ns	227.91±6.38ns	236.60±6.32ns
B1S2	3.28±0.40ns	241.17±5.25ns	283.60±7.76ns	296.08±8.05ns
B1S3	2.67±0.22ns	206.20±7.23ns	244.85±6.26ns	272.52±4.34ns
B2S0	3.19±0.18ns	190.90±7.77ns	215.36±7.67ns	229.11±6.75ns
B2S1	4.85±0.38ns	106.74±13.42ns	143.16±13.02ns	179.61±10.36ns
B2S2	5.20±0.24ns	148.40±11.59ns	219.65±5.26ns	248.72±6.94ns
B2S3	4.82±0.45ns	205.53±10.50ns	229.21±16.57ns	265.34±9.58ns
B3S0	3.91±0.30ns	105.53±3.94ns	145.72±1.96ns	165.45±1.11ns
B3S1	2.25±0.09ns	171.63±4.90ns	203.14±7.07ns	239.34±12.07ns
B3S2	2.66±0.14ns	223.17±7.84ns	298.95±1.51ns	324.94±3.03ns
B3S3	4.37±0.07ns	195.31±6.77ns	233.79±8.40ns	250.38±8.16ns
CV (%)	56.09	29.68	26.31	20.78

173 Note: values followed by the different letter in the column indicated significantly by DMRT
 174 at $P < 0.05 \pm SE$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 175 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 176 isolate P7, B3= combination isolates N3+P7).
 177

178 A significant increase in total fresh weight of upland rice along with the increase in the
 179 dosage of biogas sludge to 315 ml polybag⁻¹ at 16 WAIBSA with the highest increase by
 180 41.81% compared to the control. Although the effect was insignificant, the B1 and the
 181 interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with
 182 9.66% and 68.55%, respectively, compared to the control.

183 The effect of biogas sludge significantly increased the total dry weight of upland rice at 12
 184 and 16 WAIBSA. Superior bacterial isolates and their interactions had an insignificant effect
 185 on the total dry weight of upland rice at 4-16 WAIBSA (Tab. 4).

186 **TABLE 4.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the
 187 total dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Commented [I36]: If all the treatments (B x S) are NS, there was not interaction between the factors. Then it is unnecessary to show the interaction.

Treatments	Total dry weight \pm SE (g)			
	4 WAIBSA	8 WAIBSA	12 WAIBSA	16 WAIBSA
Superior bacterial isolates (B)				
B0	1.38 \pm 0.06ns	48.01 \pm 1.29ns	73.60 \pm 3.99ns	82.52 \pm 4.18ns
B1	1.13 \pm 0.05ns	54.09 \pm 2.41ns	76.83 \pm 2.66ns	99.72 \pm 4.15ns
B2	1.49 \pm 0.06ns	47.30 \pm 3.30ns	73.20 \pm 2.28ns	98.25 \pm 3.90ns
B3	1.15 \pm 0.07ns	52.32 \pm 3.39ns	77.18 \pm 4.90ns	98.47 \pm 4.56ns
Biogas sludge (S)				
S0	1.26 \pm 0.06ns	45.51 \pm 2.63ns	62.88 \pm 2.19b	76.78 \pm 1.63c
S1	1.23 \pm 0.08ns	44.47 \pm 1.71ns	68.52 \pm 2.00ab	87.65 \pm 2.84bc
S2	1.26 \pm 0.08ns	55.36 \pm 3.43ns	85.69 \pm 1.08a	98.95 \pm 1.86b
S3	1.40 \pm 0.06ns	56.38 \pm 1.05ns	83.73 \pm 3.44a	115.59 \pm 2.11a
Interactions (B \times S)				
B0S0	1.58 \pm 0.08ns	41.73 \pm 2.78ns	58.08 \pm 1.54ns	67.23 \pm 0.96ns
B0S1	1.12 \pm 0.08ns	45.87 \pm 0.83ns	62.74 \pm 1.83ns	71.08 \pm 1.91ns
B0S2	1.20 \pm 0.12ns	52.25 \pm 2.07ns	81.39 \pm 5.48ns	88.28 \pm 5.02ns
B0S3	1.60 \pm 0.12ns	52.18 \pm 0.29ns	92.20 \pm 3.05ns	103.49 \pm 2.43ns
B1S0	0.97 \pm 0.04ns	46.64 \pm 1.39ns	69.53 \pm 4.90ns	80.30 \pm 4.51ns
B1S1	1.40 \pm 0.07ns	48.13 \pm 2.78ns	78.91 \pm 0.53ns	96.23 \pm 1.50ns
B1S2	1.12 \pm 0.10ns	67.79 \pm 1.44ns	91.05 \pm 2.25ns	101.80 \pm 2.40ns
B1S3	1.02 \pm 0.08ns	53.81 \pm 3.76ns	67.84 \pm 1.77ns	120.54 \pm 2.15ns
B2S0	1.17 \pm 0.05ns	59.32 \pm 2.33ns	70.92 \pm 4.20ns	81.43 \pm 3.82ns
B2S1	1.54 \pm 0.10ns	34.47 \pm 2.16ns	61.69 \pm 1.97ns	89.84 \pm 1.41ns
B2S2	1.73 \pm 0.05ns	37.37 \pm 3.74ns	83.10 \pm 1.19ns	105.46 \pm 1.37ns
B2S3	1.53 \pm 0.10ns	58.05 \pm 1.76ns	77.07 \pm 4.27ns	116.28 \pm 1.30ns
B3S0	1.30 \pm 0.07ns	34.35 \pm 7.04ns	52.98 \pm 0.73ns	78.16 \pm 0.48ns
B3S1	0.85 \pm 0.03ns	49.40 \pm 0.08ns	70.72 \pm 1.29ns	93.44 \pm 2.19ns
B3S2	0.99 \pm 0.05ns	64.05 \pm 4.68ns	87.22 \pm 2.90ns	100.26 \pm 1.93ns
B3S3	1.44 \pm 0.02ns	61.48 \pm 2.47ns	97.80 \pm 0.77ns	122.04 \pm 0.20ns
CV (%)	43.80	31.22	26.54	18.38

189 Note: values followed by the different letter in the column indicated significantly by DMRT
 190 at $P < 0.05 \pm SE$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 191 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 192 isolate P7, B3= combination isolates N3+P7).
 193

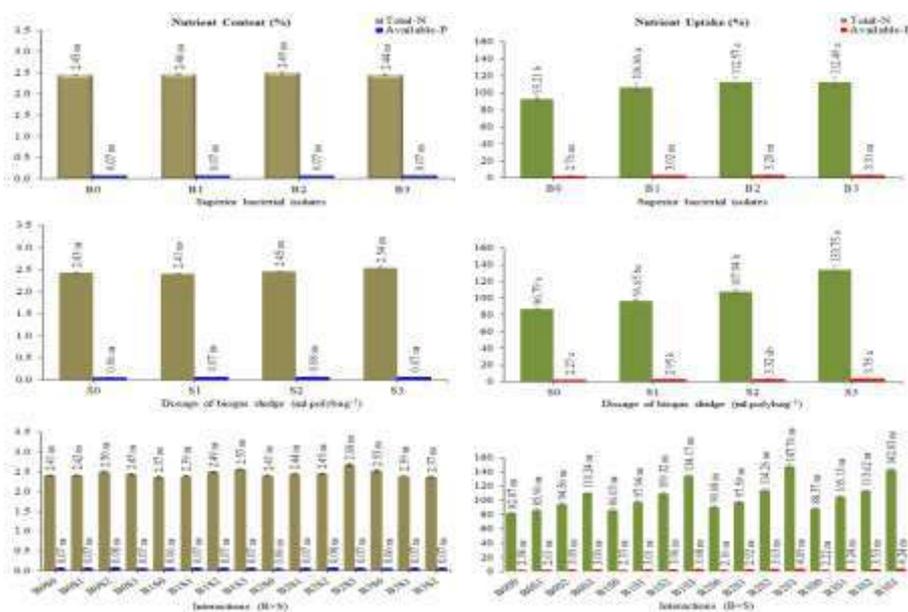
194 A significant increase in total dry weight of upland rice along with the increase in the dosage
 195 of biogas sludge to 630 ml polybag⁻¹ at 16 WAIBSA with the highest increase of 50.55%
 196 compared to the control. Although the effect was insignificant, the B1 and the interaction of
 197 B3S3 showed the highest increase in the total dry weight of upland rice, with 20.84% and
 198 81.53%, respectively, compared to the control.

199

200 **Content and uptake of total-N and available-P nutrient of upland rice**

201 The application effect of biogas sludge, superior bacterial isolates, and their interactions had
 202 an insignificant effect on the nutrient content of total-N and available-P in upland rice. The
 203 effect of biogas sludge significantly increased the nutrient uptake of total-N and available-P.
 204 Superior bacterial isolates significantly increased the nutrient uptake of total-N. The
 205 interaction of biogas sludge with superior bacterial isolates had an insignificant effect on the
 206 nutrient uptake of total-N and available-P of upland rice (Fig. 2).

Commented [I37]: He authors could explain that there was increase in uptake of nutrient ; however, there was not increase in nutrient content in the rice plants.



207 **FIGURE 2.** The effect of superior bacterial isolates, dosage of biogas sludge, and their
 208 interactions in the content and uptake of total-N and available-P nutrient of upland rice.
 209 Values followed by the different letter in graph indicated significantly by DMRT at $P < 0.05$.
 210 ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2= 315; S3= 630
 211 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2= isolate P7,
 212 B3= combination isolates N3+P7).

213
 214 A significant increase in the nutrient uptake of total-N and available-P in upland rice along
 215 with the increase in the dose of biogas sludge to 630 mL polybag⁻¹ with the highest increase
 216 of 54.11% and 65.20%, respectively compared to the control. The superior bacterial isolates
 217 (B1-B3) also significantly increased the nutrient uptake of total-N for upland rice with the
 218 highest increase in the B2 by 20.77% compared to the control. Although the effect was

Commented [I38]: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures.

219 insignificant, the B3 showed the highest increase in nutrient uptake of available-P in upland
 220 rice, with 19.93% compared to the control. Likewise, the interaction of B2S3 and B3S3
 221 showed the highest increase in nutrient uptake of total-N and available-P in upland rice by
 222 80.04% and 79.41%, respectively, compared to the control.

223 The biogas sludge doses of 315 and 630 ml polybag⁻¹ (S2 and S3) explained that the nutrient
 224 content of available-P and total-N in the plant tissue of upland rice were higher by 33.33%
 225 and 4.53%, respectively, compared to the control. The B2 isolate showed the highest nutrient
 226 content of total-N in the plant tissue of upland rice by 1.63% compared to the control, but all
 227 isolates (B1-B3) showed a similar level of available-P in the plant tissue of upland rice with
 228 the control. The interactions of B2S3 and B2S2 also showed the highest nutrient content of
 229 total-N and available-P compared to other interactions.

230

231 **Crop growth rate of upland rice**

232 The effect of superior bacterial isolates, biogas sludge, and their interactions significantly
 233 increased the crop growth rate of upland rice at 12 to 16 WAIBSA, but it had an insignificant
 234 effect at 4-8 and 8-12 WAIBSA (Tab. 5).

235 The biogas sludge dose of 630 ml polybag⁻¹ (S3) significantly increased the highest crop
 236 growth rate for upland rice at 12 to 16 WAIBSA by 129.44% compared to the control. The
 237 ability of B1-B3 isolates significantly increased the crop growth rate of upland rice with the
 238 highest increase in the B2 by 181.45% compared to the controls at 12 to 16 WAIBSA. The
 239 interaction of the B1S3 significantly increased the crop growth rate of upland rice by 5.76-
 240 folds greater compared to the control.

241 **TABLE 5.** The effect of superior bacterial isolates, biogas sludge, and their interactions on
 242 the crop growth rate of upland rice.

243

Superior bacterial	Biogas sludge (S)	Average
--------------------	-------------------	---------

isolates (B)	S0	S1	S2	S3	
4-8 WAIBSA					
B0	1.434ns	1.598ns	1.823ns	1.806ns	1.665ns
B1	1.631ns	1.669ns	2.381ns	1.885ns	1.892ns
B2	2.077ns	1.176ns	1.273ns	2.019ns	1.636ns
B3	1.180ns	1.734ns	2.252ns	2.144ns	1.828ns
Average	1.580ns	1.544ns	1.932ns	1.964ns	CV= 32.28%
8-12 WAIBSA					
B0	0.584ns	0.602ns	1.041ns	1.430ns	0.914ns
B1	0.818ns	1.099ns	0.831ns	0.501ns	0.812ns
B2	0.414ns	0.972ns	1.633ns	0.679ns	0.925ns
B3	0.665ns	0.761ns	0.828ns	1.297ns	0.888ns
Average	0.620ns	0.859ns	1.083ns	0.977ns	CV= 56.17%
12-16 WAIBSA					
B0	0.327fgh	0.298gh	0.246h	0.403b-h	0.318b
B1	0.385c-h	0.619a-h	0.384d-h	1.882a	0.817a
B2	0.375e-h	1.005a-h	0.798a-h	1.400a-h	0.895a
B3	0.899a-h	0.811a-h	0.466a-h	0.866a-h	0.761a
Average	0.496b	0.683b	0.474b	1.138a	CV= 51.07%

244 Note: values followed by the different letter in the column indicated significantly by DMRT
 245 at $P < 0.05$. ns= not significantly. Dosage of biogas sludge (S0= untreated; S1= 157.5; S2=
 246 315; S3= 630 mL polybag⁻¹). Superior bacterial isolates (B0= un-treated; B1= isolate N3, B2=
 247 isolate P7, B3= combination isolates N3+P7).
 248

249 **The effect of selected superior bacterial isolates**

250 The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake
 251 of total-N and crop growth rate of upland rice on ultisols at 12 to 16 WAIBSA, but it had an
 252 insignificant effect on plant height, total fresh weight, total dry weight, nutrient content (total-
 253 N and available-P) in leaf tissue, nutrient uptake of available-P, and crop growth rate of
 254 upland rice at 4 to 8 and 8 to 12 WAIBSA. The superior bacterial isolates (N3, P7, and
 255 N3+P7) could increase the nutrient uptake of total-N in upland rice by 14.64%, 20.77%, and
 256 20.68%, respectively compared to control (Fig. 2). Similar results are also shown in Table 5,
 257 that the crop growth rate of upland rice at 12 to 16 WAIBSA due to selected superior bacterial
 258 isolates (N3, P7, N3+P7) has increased by 2.57; 2.81; and 2.39-folds, respectively compared
 259 to the control. The finding results indicated that the ability of a single isolate by P7 bacteria
 260 was greater in increasing total-N and crop growth rate of upland rice compared to a single
 261 isolate by N3 bacteria and a combination of N3+P7 bacterial isolates. It was due to the

262 presence of several organic acids and hormones produced by P7 that can increase the nutrient
263 uptake of total-N and crop growth rate of upland rice. This result was supported by Mustamu
264 *et al.* (2021) that the phosphate solubilizing bacteria isolate (P7) from the biogas sludge
265 contains organic acids produced such as lactic, oxalic, acetic, citric acids, and it had the
266 highest ability to solubilize phosphate from calcium triphosphate and rock phosphate was
267 4.62 and 2.66-folds, respectively compared to the control. Meena *et al.* (2016) reported that
268 the availability of nitrogen and phosphorus slightly increased in the application of bio
269 fertilization with *Bacillus cereus*, it was due to the production of organic acids and other
270 chemicals such as citric, tartaric, and oxalic acids which can stimulate plant growth and
271 nutrients availability. Youssef and Eissa (2017) reported that the increase in vegetative growth
272 and total biomass was due to increased photosynthesis, translocation, and accumulation of
273 nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the
274 gibberellins, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that
275 gibberellins, IAA, and other hormones can increase plant growth under stressful conditions.
276 Kang *et al.* (2014) said that PGPB has several mechanisms to increase plant growth with
277 nitrogen-fixation, phosphate solubilizing, increasing nutrient availability. Suksong *et al.*
278 (2016) reported that bacteria of palm oil solid waste from anaerobic digester include:
279 *Ruminococcus sp.*, *Thiomargarita sp.*, *Clostridium sp.*, *Anaerobacter sp.*, *Bacillus sp.*,
280 *Sporobacterium sp.*, *Saccharofermentans sp.*, *Oscillibacter sp.*, *Sporobacter sp.*, and
281 *Enterobacter sp.* Liaquat *et al.* (2017) also reported that an abundance of *Bacillus*,
282 *Clostridium*, and *Enterobacter spp* in anaerobic digester of wastewater in producing biogas.

283

284 **The effect of biogas sludge**

285 The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and
286 16 WAIBSA), total dry weight (12 and 16 WAIBSA), nutrient uptake (total-N and available-

287 P), and the crop growth rate of upland rice at 8 to 12 WAIBSA, but it had an insignificant
288 effect on nutrient content (total-N and available-P) in leaf tissue, and crop growth rate of
289 upland rice (4-8 and 8-12 WAIBSA). An increase in plant height, total dry weight, nutrient
290 uptake of total-N and available-P, and also crop growth rate of upland rice on ultisols along
291 with the increase in the dose of biogas sludge to 630 mL polybag⁻¹ at the end of this study (16
292 WAIBSA). However, in contrast to the total fresh weight, an increase along with the increase
293 in the biogas sludge dose to 315 mL polybag⁻¹ then decreased at the dose of 630 mL polybag⁻¹.
294 It was caused the biogas sludge had chemical characteristics such as pH (7.41), total-N
295 (0.051%), available-P (0.013%), organic-C (0.14%), total-K (0.18%), and biological
296 characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU mL⁻¹) and total phosphate
297 solubilizing bacteria (7.0×10^4 CFU mL⁻¹) (Tab. 2). C-organic content and the total population
298 of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could be
299 increased the nutrient uptake of total-N and available-P in upland rice along with increasing
300 the dose of biogas sludge to 630 mL polybag⁻¹ (Fig. 2). Therefore, the nutrients absorbed are
301 used for plant metabolic processes and stimulate the growth of plant height, biomass, and crop
302 growth rate of upland rice. A similar result was reported by Mustamu and Triyanto (2020)
303 that the macro and micronutrients from the biogas sludge and also had the population of
304 nitrogen-fixing and phosphate solubilizing bacteria by 480×10^4 and 42×10^4 CFU mL⁻¹,
305 respectively. Ndubuisi-Nnaji *et al.* (2020) reported that total phosphate solubilizing bacteria
306 (1.6 to 2.5 CFU mL⁻¹) was significantly higher compared to nitrogen-fixing bacteria (0.5 – 1.4
307 CFU mL⁻¹) and a significant increase in nutrient concentration in the order of
308 N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported
309 that an increase in concentrations of NH₄⁺-N ranged from 45 to 80% after anaerobic waste.

310

311 **The interaction effect of selected superior bacterial isolates and biogas sludge**

312 The interaction of biogas sludge and superior bacterial isolates only significantly increased
 313 the crop growth rate of upland rice on ultisols at 8 to 12 WAIBSA, but it had an insignificant
 314 effect on other parameters in this study. The interaction of NFB *Bacillus paramycoides* with
 315 biogas sludge at the dose of 630 ml polybag⁻¹ (B1S3) showed the highest crop growth rate of
 316 upland rice compared to other interactions and 5.76-fold greater compared to the control. It
 317 was caused by the application of biogas sludge that could increase the contents of soil organic
 318 matter and the total population of beneficial bacteria. Likewise, the characteristics of the
 319 biogas sludge had the organic-C was 0.14%, total nitrogen-fixing bacteria was 29.4×10^5 CFU
 320 mL⁻¹, and total phosphate solubilizing bacteria was 7.0×10^4 CFU mL⁻¹ (Tab. 2) could improve
 321 soil quality and support the crop growth rate. This result is supported by Urrea *et al.* (2019)
 322 that the application of sewage sludge in the long-term significantly increases the organic
 323 matter in the soil, which causes a decrease in soil pH due to the nitrification of ammonium in
 324 sewage sludge and the production of organic acids along with the decomposition of organic
 325 matter. Bhardwaj *et al.* (2014); Carvajal-Muñoz *et al.* (2012) reported that the application of
 326 biofertilizer had advantages for plants, such as availability of nutrients that are balanced for
 327 plant health, stimulating nutrient mobilization that can increase soil biological activity,
 328 availability of microbial food to encourage the growth of beneficial microorganisms,
 329 increasing the soil organic matter content thereby increasing the cation exchange capacity.
 330 Siswanti and Lestari (2019) reported that the interaction of biogas sludge+biofertilizer (36
 331 mL+10 L ha⁻¹) significantly increased the plant height, number of leaves, and capcaisin
 332 content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

333

334 **Conclusions**

335 The isolates of N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient
 336 uptake of total-N and crop growth rate of upland rice on ultisols with the highest increase

Commented [I39]: The authors must review the English in lines 335-337.

The conclusions aren't the repetition of results. The authors could make an effort to show emerging properties accounted for in the results of this work and enhance the practical application and the investigation novelty.

337 found in the P7 isolate of 20.77% and 2.81-folds, respectively. The dose of biogas sludge
338 significantly increased plant height, total dry weight, nutrient uptake of total-N and available-
339 P, and also crop growth rate of upland rice on ultisols with the highest increase at a dose of
340 630 mL polybag⁻¹ by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively.
341 Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland
342 rice with the highest increase at the dose of 315 mL polybag⁻¹ by 41.81%. The interaction of
343 isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop
344 growth rate of upland rice on ultisols with the highest increase found in the B1S3 by 5.76-
345 folds.

346

347 **Conflict of interest statement**

348 The authors declare that there is no conflict of interest regarding the publication of this article.

349

350 **Author's contributions**

351 All authors formulated the overarching research goals and aims, provided the study materials,
352 developed or designed the methodology. NEM analyze and interpretation study data. NEM
353 and MS wrote the initial draft, managed and coordinated the research activity in field, data
354 collection. ZN and I verified the overall reproducibility of results and other research outputs.
355 All authors conducted the critical review/commentary/revision of the manuscript.

356

357 **Literature cited**

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Novilda Elisabeth Mustamu <nemustamu@gmail.com>

📧 Min, 24 Okt 2021 09.13 ☆ ↶ ☰

kepada Revista ▾

Dear editor of Agronomia Colombiana

attached file of the revised manuscript

Hopefully our revision can be considered by the editor

thanks a lot, Prof

Pada tanggal Sen, 4 Okt 2021 pukul 22.05 Novilda Elisabeth Mustamu

<nemustamu@gmail.com> menulis:

>



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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de secano

Novilda Elizabeth Mustamu^{1*}, Zulkifli Nasution², Irvan³, and Mariani Sembiring²

¹Doctoral Program ~~in~~ Agricultural Sciences, Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara, (Indonesia).

²Program ~~Study in~~ Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara, (Indonesia).

³Program ~~Study in~~ Chemical Engineering, Faculty of Engineering, Universitas Sumatera Utara, Sumatera Utara, (Indonesia).

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ABSTRACT

This ~~study e~~ ~~investigation~~ evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth, biomass, and nutrient uptake in upland rice ~~grown?~~ in ultisols. ~~This study~~ We used a ~~r~~Randomized ~~b~~Block ~~d~~Design with ~~in~~ two factors and seven replicat~~esions~~ from October 2020 ~~ountil~~ April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolat~~s~~ combination (N3+P7). The second factor was ~~the~~ dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ~~mL/ml~~ polybag⁺). The parameters were determined by ANOVA and followed by ~~Duncan's multiple range test~~ (DMRT) at $P < 0.05$. The results showed that the ~~isolate~~ P7 ~~isolate~~ significantly increased ~~the~~ total ~~-N~~ uptake ~~by 20.77%~~ and ~~the~~ highest crop growth rate (CGR) of upland rice ~~by 20.77%~~ and 2.81 ~~times~~, ~~respectively~~. Biogas sludge ~~doses~~ dosage from 315 to 630 ~~mL/ml~~ polybag⁺ significantly increased plant height, uptake of total ~~-N~~ and available ~~-P~~, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 ~~mL/ml~~ polybag⁺ significantly increased the CGR of upland rice. The application of ~~isolates~~ N3 and P7

Commented [EDTR1]: Reviewer 1 wrote: I recommend "Evaluation of selected bacterial isolates from biogas sludge in upland rice"

Reviewer 3 wrote: The title don't show effectively the results of investigation. I recommend the title: Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice. Regarding the term "superior bacterial isolates" reviewer 3 wrote: What the authors wanted to say? I don't understand this term. This reviewer also wrote: The authors evaluated only the effect of bacteria and biogas sludge on rice growth. They evaluated also the effect of these treatments on rice nutrition! Finally, regarding the word "ultisols" reviewer 3 wrote: Why is it necessary to specify the soil type?

Commented [EDTR2R1]: The authors only considered the suggestion of reviewer 3 but did not reply to any of the reviewer's comments.

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Commented [EDTR4]: Reviewer 3 wrote: It is necessary to review the English and to enhance the investigation novelty. The authors show unnecessary dates and must remove word abbreviations.

29 ~~isolates~~ and their combination within biogas sludge of 630 ~~mL/ml~~-polybag⁺ has the potential
30 to ~~archive~~ the CGR of upland rice in acidic soils.

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32 **Key words:** acidic soil, crop growth rate, dosage, sludge potential, ~~upland rice~~.

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34 RESUMEN

35 ~~La investigación~~El presente estudio evaluó la influencia de aisla~~mientos~~ bacterianos
36 superiores seleccionados (ABS), lodos de biogás y sus interacciones ~~sobre~~ el crecimiento, la
37 biomasa y la absorción de nutrientes en el arroz de tierras altas ~~cultivado~~ en ultisoles. Este
38 estudio ~~Se~~ utilizó un ~~d~~Diseño de ~~b~~Bloques ~~al azar~~ Aleatorizados ~~dentro de~~ ~~con~~ dos factores y
39 siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado
40 seleccionó ABS (~~B0~~ = sin ~~tratamiento~~, B1 = aisla~~miento~~ de bacterias fijadoras de nitrógeno
41 (N3), B2 = aisla~~miento~~ de bacterias solubilizantes de fosfato (P7), B3 = combinación de
42 aislados (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (~~S0~~ = sin
43 ~~tratamiento~~, S1 = 157,5; S2 = 315; S3 = 630 ~~mL/ml~~ de polybag⁺). Los parámetros fueron
44 determinados por ANOVA ~~análisis de varianza~~ y seguidos de la prueba de rangos múltiples de
45 Duncan (~~DMRT~~) ~~a~~ $P < 0,05$. Los resultados mostraron que el aisla~~miento~~ P7 aumentó
46 significativamente la captación total de N ~~en 20.77%~~ y la mayor tasa de crecimiento del cultivo
47 (TCC) de arroz de tierras altas ~~en 20.77% y 2.81 veces, respectivamente~~. La ~~dosificación~~
48 de ~~fangos lodos~~ de biogás de 315 a 630 ~~mL/ml~~ de polybag⁺ ~~aumentaron~~ significativamente la
49 altura de la planta, la absorción de N total y P disponible, el peso fresco y seco total y el TCC
50 de arroz de tierras altas. La interacción de N3 con la ~~dosificación~~ dosis de lodos de biogás de
51 630 ~~mL/ml~~ de polybag⁺ aumentó significativamente el TCC del arroz de tierras altas. La
52 aplicación de ~~los~~ aislamientos ~~de~~ N3 y P7 y su combinación dentro de lodos de biogás de 630

Commented [EDTR8]: Reviewer 3 wrote: The abstract does not show the investigation novelty and the importance of results to agricultural management.

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Commented [EDTR10]: Reviewer 3 wrote: Biomass is a part of growth

Commented [EDTR11R10]: The authors did not reply to this comment.

Commented [EDTR12]: Reviewer 3 wrote: Too many details. The authors must abbreviate since this will be said in Materials and methods.

Commented [EDTR13R12]: The authors neither replied to this comment nor changed this section.

Commented [EDTR14]: Reviewer 3 wrote: Only state the concentration value.

Commented [EDTR15R14]: The authors neither replied to this comment nor changed this section.

Commented [EDTR16]: Reviewer 3 wrote: The isolate itself does not produce those effects. It is the application or inoculation of the isolate that produces the effects. Improve wording. I recommend: "Los resultados mostraron que la inoculación aumentó... pues el aislado por sí solo no produce el efecto, sino que hay que inocularlo".

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Commented [EDTR18]: Reviewer 3 wrote: I recommend "absorción"

Commented [EDTR19R18]: The authors neither replied to the comment nor changed the term.

Commented [EDTR20]: Reviewer 3 wrote: Do not use abbreviations in the abstract.

Commented [EDTR21R20]: The authors did not reply to this comment.

53 ~~mL/ml~~-de polybag⁺ tiene el potencial de ~~archivar~~ el TCC de arroz de tierras altas en suelos
54 ácidos.

55 ~~Palavras-chave~~ **Palabras clave:** ~~arroz de tierras altas, suelo ácido, tasa de crecimiento de~~
56 ~~cultivos,~~ dosis, potencial de lodo, ~~suelo ácido, tasa de crecimiento de los cultivos.~~

58 **Introduction**

59 Biogas sludge is the waste by-product ~~installation~~ from an anaerobic processing system (~~Food~~
60 ~~and Agriculture Organization~~FAO, 1997) and has a high nutrient content that can be used as
61 organic fertilizer to increase soil fertility and plant yields (Adela *et al.*, 2014). ~~It has been~~
62 ~~reported that~~ The following characteristics of the biogas sludge from palm oil waste ~~have been~~
63 ~~were reported: such as~~ total-N of 490 mg L⁻¹, ~~total-P by of~~ 110 mg L⁻¹, ~~and total K of was~~ 1.9
64 mg L⁻¹ (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), ~~and NH₃-N of~~
65 ~~ranged by~~ 91 to 112 mg L⁻¹ (Choorit & Wisarnwan, 2007). The pH ~~may could~~ ~~ranged by from~~
66 6.8 to 8.3, ~~and with~~ the highest bacterial populations ~~was of~~ 7.21×10⁷ cells per ~~mL/ml~~ and the
67 lowest ~~one of was~~ 3.15×10⁷ cells per ~~mL/ml~~ (Alvionita *et al.*, 2019). ~~Additionally,~~ Mustamu
68 ~~&and~~ Triyanto (2020) ~~also~~ reported that the biogas sludge has nitrogen-fixing and phosphate
69 solubilizing ~~bacteria? That which~~ have the potential ~~to~~ availability of nitrogen and phosphate
70 in ~~the~~ soils.

71 The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing ~~bacteria~~
72 has a greater potential ~~to in~~ ~~increasing~~ soil fertility and plant growth. Zhang *et al.* (2013)
73 reported that phosphate solubilizing bacteria play an important role in increasing soil fertility,
74 ~~and~~ plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described ~~that~~
75 ~~the different~~ *Bacillus* ~~species, genera~~ such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*,
76 *B. megaterium*, *B. mycoides*, *B. coagulans*, *B. chitinolyticus*, ~~and~~ *B. subtilis*, ~~have been reported~~
77 as phosphate solubilizing ~~microorganisms~~. Ambrosini *et al.* (2016) ~~reported that~~ *Bacillus cereus*

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Commented [EDTR23]: Reviewer 3 wrote: The keywords aren't in the same order in both abstracts. The authors must review the Spanish version since there are words incorrectly used like as: obtener, captación, and archivar.

Commented [EDTR24R23]: The authors neither replied to the comments nor corrected the terms.

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Commented [EDTR25]: Reviewer 3 wrote: It is necessary to include relevant information about acid soil and the representative upland rice in Indonesia and its impact in the economy of the country.

Furthermore, the authors must enhance the investigation novelty.

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<https://www.fao.org/publications/card/en/c/34d03d32-bd9f-5d08-aa08-ed2499349eb1/>

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Improve?
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78 showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus*
 79 spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from
 80 anaerobic processing using the pyrosequencing and clone library methods, *i.e.*, *Proteobacteria*,
 81 *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

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82 The application of bacteria from biogas sludge has never been reported in Indonesia on the
 83 application of bacterial isolates from biogas sludge in for improving upland rice growth on
 84 acidic soils. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas
 85 sludge to increase in increasing the availability of nitrogen and phosphate, and the growth
 86 response to the growth of upland rice due to application of the biogas sludge and selected
 87 isolates in ultisols. The study was aimed to evaluate the influence of selected superior bacterial
 88 isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown
 89 in ultisols.

Commented [EDTR29]: Reviewer 3 wrote: I think it is unnecessary to show all these *Bacillus* species. Will the authors work with these genera in this investigation? In materials and methods, the authors do not say that they work with these bacterial genera. I recommend explaining more the lines 66-69 since it is the essence of this work.

Commented [EDTR30R29]: The authors did not reply to this comment.

Commented [EDTR31]: Reviewer 3 wrote: Are acid soils representative in Indonesia? What percentage of rice is cultivated upland in Indonesia? Is it representative? I think that the authors must state this information here for the enhancement of the investigation novelty. Furthermore, the authors must show the importance of using bio-products as an alternative to mineral fertilization in rice.

Commented [EDTR32R31]: The authors did not reply to any of these comments.

90 Materials and methods

91 Study area

92 The concentration of total -N and available -P in ultisols and the plant tissue were analyzed in
 93 the Analytical Laboratory of PT. Socfin Indonesia, Medan (Indonesia). The bacterial isolates
 94 were applied to upland rice in the village of Padang Bulan Village (3°37.760' N; 98°38.898' E;
 95 altitude 18 m a.s.l. above sea level), Medan Selayang Subdistrict, Medan City, North Sumatra,
 96 Indonesia, from October 2020 to April 2021. Furthermore, The average temperature was 27.4°C,
 97 the average air humidity was 82%, temperature was 27.4°C and the average rainfall was
 98 recorded 228.5 mm by per month.

Commented [EDTR33]: Reviewer 3 wrote: The description of the methodology is brief and doesn't have details, which does not allow it to be reproduced. The authors must review the English.

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Commented [SM35]: What do you mean by measuring concentration of available P in the plant tissue?

Commented [SM36]: Please, provide the bibliographic reference of the manual used for these analyses

Commented [SM37]: What does the PT. abbreviation stand for?

99 Preparation of medium and upland rice seeds

100 The medium to grow upland rice plants used ultisols order from the Simalingkar area, Medan
 101 Tuntungan Subdistrict, Medan City, with at a depth of 0 to 20 cm. One hundred 100 g of soil

102 samples were taken and analyzed for chemical characteristics such as pH using HCl 25%
 103 ~~method with spectrophotometer~~, organic C by Walkley-Black ~~method with spectrophotometer~~,
 104 available P by Bray-II ~~method with spectrophotometer~~, total N using Kjeldahl method ~~with~~
 105 ~~spectrophotometer~~, and cation exchange capacity (CEC) and base saturation (K, Ca, Na,
 106 Mg) by ammonium acetate pH 7 method ~~with atomic absorption spectrophotometry~~ (Tab. 1).

107 The soil was sterilized by ~~burning/drying~~ at 100°C for 2 ~~hours~~. After being incubated for 1
 108 ~~day~~, the soil was ~~put~~ placed into a 10 kg polybag ~~with a size of 10 kg~~. A basic NPK fertilizer
 109 of NPK-Mutiara at a dose of 300 kg ha⁻¹ was ~~applied-given~~ by stirring evenly with the soil.
 110 ~~Concurrently~~, the seeds of upland rice (*Oryza sativa* L.) used ~~was-were of an?~~ inbred
 111 variety of Inpago-8, then soaked in water for 24 ~~hours~~ and followed by a pPropineb fungicide
 112 (70%) ~~application~~ for 2 ~~hours~~. Upland rice was planted after 1 ~~day~~ of basic fertilization
 113 with two seeds per polybag at a depth of 2 cm.

114 **TABLE 1.** ~~The~~ Chemical characteristics of ~~the sterile~~ ultisols soil samples after sterilization at 100°C.

Chemical characteristics	Value	Category*
Soil pH (H ₂ O)	4.80	Acid
Organic C (%)	0.44	Very low
Total N (%)	0.04	Very low
Available P (mg kg ⁻¹)	870.25	Very high
CEC (meq/100 g)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (meq/100 g)	0.60	High
Ca (meq/100 g)	0.34	Very low
Mg (meq/100 g)	0.32	Very low
Na (meq/100 g)	0.09	Very low
Al (%)	0.02	Very low

116 ~~Source:~~*Criteria for pH H₂O = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available
 117 P >60 mg kg⁻¹ (very high); Cation exchange capacity (CEC) = 25-40 meq/100 g⁻¹ (high); base saturation <20%
 118 (very low); exchangeable K = 0.60-1.00 meq/100 g⁻¹ (high); exchangeable Ca <2 meq/100 g⁻¹ (very low);
 119 exchangeable Mg <0.4 meq/100 g⁻¹ (very low); exchangeable Na <0.1 meq/100 g⁻¹ (very low); exchangeable
 120 Al <5% (very low) (Soil Research Institute, 2009).
 121

122 Preparation of superior bacterial isolates suspension and biogas sludge

123 The selected superior bacterial isolates used phosphate solubilizing bacteria (P7) which has
 124 been confirmed by Mustamu *et al.* (2021a) and nitrogen-fixing bacteria or N3 (Mustamu *et al.*,

Commented [SM38]: Does this correct? The Note for the table 1 indicates that the pH was measured in H2O

Commented [EDTR39]: Reviewer 1 asked the authors why the soil was incubated but the authors did not answer.

Commented [SM40]: What do you mean by soil incubation?

Commented [SM41]: What were the dimensions of a polybag?

Commented [SM42]: Please, indicate the composition of the fertilizer (percentages of N, P and K), the company and the country of the fertilizer manufacture

Commented [SM43]: What was the dose of the fertilizer per bag?

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Commented [EDTR44]: Please check this reference and add it in the original language.

Commented [EDTR45]: Reviewer 3 wrote: I think that it is irrelevant. I recommend to write the techniques that allow the soil characteristics and then state the bibliographic cites correspondingly.

Commented [EDTR46R45]: The authors neither replied to this comment nor changed the table's footnote.

Commented [SM47]: According to which criteria these are called "superior"?

Commented [SM48]: The species or the genera of the microorganisms should be named for each of the treatments P7, N3, etc., so that the differences between the treatments could be appreciated.
 Without these data, this research may not be reproduced in future since no information is provided about the bacterial composition of these isolates.

Commented [EDTR49]: Reviewer 1 asked: why are they superior bacteria? How were they selected? what species are they?

Commented [EDTR50R49]: The authors did not answer these questions

2021b). The isolates were grown on NB medium, and incubated for 48 h. The bacterial growth in the solution was measured using an spectrophotometer with a density of 10^8 cells per mL.

10 mL was taken from solution containing nitrogen-fixing bacteria (N3) and phosphate solubilizing (P7) bacteria.

The biogas sludge was taken collected from the digester tank at the palm oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. Bacterial isolates

and biogas sludge were applied to the soil surface at the base of the plants one week after planting (WAP). Biogas sludge samples were taken at 500 mL volume were used to analyze

the then analyzed the chemical and biological characteristics in the laboratory (Tab. 2).

TABLE 2. The chemical and biological characteristics of the biogas sludge.

Characteristics of biogas sludge	Method	Value
Chemical		
pH	Electrometry	7.41
Chemical oxygen demand (COD) (mg L ⁻¹)	Spectrophotometry	4547.8
Biological oxygen demand (BOD) (mg L ⁻¹)	Titrimetry	1127.5
Total-N (%)	Spectrophotometry	0.051
Total-P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total-K (%)	Graphite furnace - atomic absorption spectrophotometry AAS	0.18
Organic-C (%)	Atomic absorption spectrophotometry (AAS)	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (ppm mg L ⁻¹)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Biological		
Total nitrogen-fixing bacteria (CFU mL ⁻¹)	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU mL ⁻¹)	Plate count	7.0×10^4

Treatments application

This study used a randomized block design with two factors and seven replications.

The first factor was the type of superior bacterial isolates (B0 = un-treated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at the a similar dose, namely 10 mL/polybag⁺. The second factor was

Commented [EDTR51]: Please add the brand, model, city and country.

Commented [EDTR52]: Reviewer 3 wrote: What λ was use? The result that allows this determination is an optical density data. How the authors know that this date correspondis to 10^8 cells per mL?

Commented [EDTR53R52]: The authors did not reply.

Commented [EDTR54]: Reviewer 3 wrote: incomplete. What did you do with this?

Commented [EDTR55R54]: The authors did not add any information.

Commented [EDTR56]: Reviewer 3 wrote: Is this isolate a Bacilli? I consider very important that the authors declare this. The authors must give more data about both bacteria isolates.

Commented [EDTR57R56]: The authors did not reply to this comment.

Commented [SM58]: Could you indicate which species or hybrid of palm was employed? This may have an effect on the sludge composition

Commented [SM59]: What does this abbreviation PT. stand for?

Commented [EDTR60]: Reviewer 3 wrote: I think that the authors must give more details about how biogas sludge was taken.

Commented [EDTR61R60]: The authors did not provide any other detail.

Commented [SM62]: Please, provide the bibliographic reference of the manual used for the biogas sludge analysis

Commented [EDTR63]: Reviewer 3 wrote: The authors must say how the chemical and biological characteristics of biogas sludge were determined, or make a bibliography cite.

Commented [EDTR64R63]: The authors did not add a citation.

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Commented [SM65]: Kjeldahl?

Commented [SM66]: Did you measure the organic C with atomic absorption spectrophotometry?

Commented [EDTR67]: Reviewer 1 asked: Is it correct? It can be a very high amount of sodium

Commented [EDTR68R67]: The authors did not answer.

dosage-dose of biogas sludge (S₀ = untreated; S₁ = 157.5; S₂ = 315; S₃ = 630 mL ml polybag¹). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m³ ha⁻¹ equal to 126,000 L ha⁻¹ (Sutarta *et al.*, 2003), then converted to soil weight per polybag (Equation 1). Each replication was disassembled at 4, 8, and 12 weeks after the application of the isolates and biogas sludge application (WAIBSAWAA) for determination of the crop growth rate (CGR).

$$\text{Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer per ha}}{\text{soil weight per ha}} \times \text{soil weight per polybag} \quad (1)$$

$$= \frac{126,000 \text{ L ha}^{-1}}{2,000,000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 630 \text{ mL ml}$$

Parameters and data analysis

The observations of the variables were conducted by measuring the growth of upland rice (plant height, and total fresh and dry weight), total -N and available -P contents in the shoots, total N uptake -N, and available -P. The CGR were conducted was determined on plants at 4-8, 8-12, and 12-16 WAIBSAWAA. Each polybag from each treatment and replication was disassembled at when the plants were 4, 8, 12, and 16 WAIBSAWAA, then measured the plant height was measured, and the total fresh weight was conducted-obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was measured after using an by oven at 60°C for 48 hours and weighed using by the analytical scales. A 200 g sample of the second leaf from the shoots was collected taken by 200 g and analyzed to determine for the total -N using the Kjeldahl method and the available -P was estimated using by the dry ashing method through UV-Vis Spectrophotometer. The total -N and available -P absorption were measured using Equation (2). The CGR was calculated by as the dry weight related to the per unit area using Equation (3) (Shon *et al.*, 1997):

$$\text{Uptake N nutrient uptake} = \text{nutrient content in the shoots} \times \text{total dry weight} \quad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W_2 - W_1}{t_2 - t_1} \quad (3)$$

Commented [EDTR69]: Reviewer 3 wrote: Why the authors use these biogas sludge concentrations. I think it is important!

Commented [EDTR70R69]: The authors did not reply.

Commented [EDTR71]: What do you mean by disassembled?

Commented [SM72]: Corresponds to weeks after application

Commented [EDTR73]: Please change per ha for ha⁻¹. Also, add spaces between the words because they are written together. Please use the equation editor in Word to write these equations. It is not possible to edit them.

Commented [EDTR74]: Delete the space between ha and ⁻¹

Commented [SM75]: How exactly the plant height was measured?

Commented [SM76]: The total weight includes aerial parts + plant roots?

Commented [EDTR77]: What do you mean by disassembled?

Commented [SM78]: individual culm?

Commented [EDTR79]: Please add the model, brand, city and country.

Commented [SM80]: Please revise the name of this method in English

Commented [SM81]: What do you mean by the absorption of the "total N"? How is it possible to absorb the total nitrogen? Please check the English spelling

166 ~~where~~Note:

167 CGR ~~—=~~ crop growth rate;

168 W1 ~~—=~~ dry weight per unit area at t1;

169 W2 ~~—=~~ dry weight per unit area at t2;

170 t1 ~~—=~~ first sampling;

171 t2 ~~—=~~ second sampling;

172 The parameters of the second phase of the study were analyzed by an ANOVA and if the
173 treatment had a significant effect, ~~then continued followed by~~ Duncan's multiple range test
174 (DMRT) at $P < 0.05$ using with SPSS v.20 software.

Commented [SM82]: Please, provide the bibliographic reference or the web page for this software

176 **Results**

177 **Effect of bacterial isolates and biogas sludge on upland rice growth**

178 Plant height of upland rice (cm)

179 The effect of biogas sludge application was significant on the plant height of upland rice at 8,
180 12, and 16 WAIBSAWAA. Superior bacterial isolates and their interactions did not have a
181 significant had an insignificant effect on the plant height of upland rice at 4, 8, 12, and 16
182 WAIBSAWAA (Fig. 1). A significant increase in plant height of upland rice was observed
183 ~~along~~ with increased doses of biogas sludge ~~of to~~ 630 ~~mL~~ ~~m~~ ~~l~~ ~~-~~ /polybag⁻¹ at 8, 12, and 16
184 WAIBSAWAA with the highest increase of 14.81% compared to the control at 16
185 WAIBSAWAA. Although the effect was insignificant, not significant, it was seen that the
186 ~~isolates~~ combination of isolates B3 and the interaction of B2S3 showed the highest increase in
187 plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

Commented [EDTR83]: Reviewer 1 wrote: I recommend that the results part be restructured in its figures and tables. It could only show figures that show a significant difference, without showing those that do not have a significant difference. Reviewer 3 wrote: It is necessary write Results and discussion again in other form. All the information of this section must be re-organized. Account of the statistical analysis made and some information in figures and tables is irrelevant. The figures have repeated information.

Commented [EDTR84R83]: The authors did not reply to these comments.

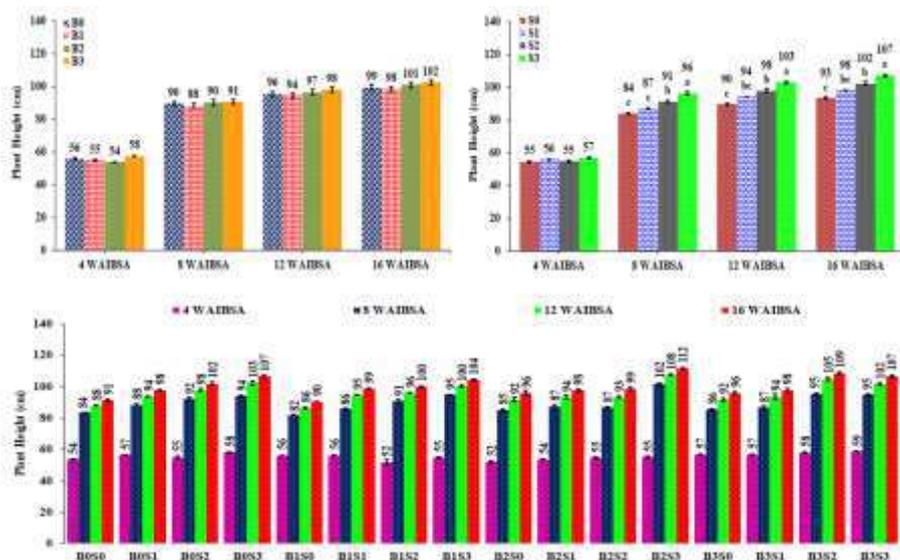
Commented [EDTR85]: Reviewer 3 wrote: I consider that it is necessary to write again Results and discussion in other form. The information of this section must be re-organized in two aspects: (1) Effect of bacterial isolates and biogas sludge on upland rice growth. (2) Effect of bacterial isolates and biogas sludge on upland rice nutrition. The first point could include:
- Plant height of upland rice
- Biomass of upland rice
- Crop growth rate of upland rice
The second point could include:
- Content of total-N and available-P nutrient of upland rice
- Uptake of total-N and available-P nutrient of upland rice
Furthermore, for both points the analysis made from lines 249-332 must be undertaken.

Commented [EDTR86R85]: It seems the authors reorganized these sections following the reviewer's suggestion. However, they did not reply to the comment.

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188 **FIGURE 1.** Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of
 189 upland rice at 4, 8, 12, and 16 WAIBSAWAA. Values followed by the different letter in the graph significantly
 190 differed according to the Duncan test indicated significantly by DMRT at $P < 0.05$. ns = not significant. Dosage
 191 of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 mL/ml-polybag⁺). Superior bacterial isolates
 192 (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).
 193

194 **Biomass of upland rice (g)**

195 The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12,
 196 and 16 WAIBSAWAA. Superior bacterial isolates and their interactions did not have a had an
 197 insignificant effect on the total fresh weight of upland rice at 4-16 WAIBSAWAA (Tab. 3).

198 **TABLE 3.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (aerial
 199 parts+shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application of the isolate and
 200 biogase sludge (WAA). WAIBSA.
 201

Treatments	Total fresh weight ± standard error SE (g)			
	4 <u>WAIBSAWAA</u>	8 <u>WAIBSAWAA</u>	12 <u>WAIBSAWAA</u>	16 <u>WAIBSAWAA</u>
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a

Commented [EDTR87]: The word Height in the Y axes must be in lowercase. Please add these graphs as independent files to ensure they have the appropriate resolution and quality.

Commented [EDTR88]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures. The authors could explore the possibility that gives them not interaction between the factors for discussing the result.

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Commented [EDTR90]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the interaction.

Commented [EDTR91R90]: The authors did not reply to this comment.

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S3	4.15±0.25	203.45±1.36 a	240.52±2.81 a	264.21±2.42 a
Interactions (B×S)				
B0S0	4.99±0.33	124.08±5.60	185.64±3.32	192.78±2.96
B0S1	3.47±0.26	160.43±1.16	188.60±5.76	207.05±3.97
B0S2	3.42±0.42	185.97±6.80	232.60±8.75	250.84±7.40
B0S3	4.71±0.42	206.76±5.49	254.23±10.27	268.61±8.85
B1S0	2.80±0.18	155.79±1.12	183.96±5.20	202.88±2.88
B1S1	3.74±0.29	174.82±9.01	227.91±6.38	236.60±6.32
B1S2	3.28±0.40	241.17±5.25	283.60±7.76	296.08±8.05
B1S3	2.67±0.22	206.20±7.23	244.85±6.26	272.52±4.34
B2S0	3.19±0.18	190.90±7.77	215.36±7.67	229.11±6.75
B2S1	4.85±0.38	106.74±13.42	143.16±13.02	179.61±10.36
B2S2	5.20±0.24	148.40±11.59	219.65±5.26	248.72±6.94
B2S3	4.82±0.45	205.53±10.50	229.21±16.57	265.34±9.58
B3S0	3.91±0.30	105.53±3.94	145.72±1.96	165.45±1.11
B3S1	2.25±0.09	171.63±4.90	203.14±7.07	239.34±12.07
B3S2	2.66±0.14	223.17±7.84	298.95±1.51	324.94±3.03
B3S3	4.37±0.07	195.31±6.77	233.79±8.40	250.38±8.16
CV (%)	56.09	29.68	26.31	20.78

Note: Values followed by the different letter in the column significantly differed according to the Duncan test indicated significantly by DMRT at $P < 0.05 \pm SE$. ns = not significantly. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 mL/mL/polybag⁻¹). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).

A significant increase in the total fresh weight of upland rice along was observed with the higher increase in the dosage dose of biogas sludge of 315 mL/mL/polybag⁻¹ at 16 WAIBSAWAA, with the highest increase by of 41.81% compared to the control. Although the effect was not insignificant, it was seen that the B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice were with -9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAIBSAWAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAIBSAWAA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (aerial parts + shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application of isolate and biogas sludge (WAA). the total dry weight of upland rice at 4, 8, 12, and 16 WAIBSA.

Treatments	Total dry weight ± standard error SE (g)			
	4 WAIBSAWAA	8 WAIBSAWAA	12 WAIBSAWAA	16 WAIBSAWAA
Superior bacterial isolates (B)				
B0	1.38±0.06	48.01±1.29	73.60±3.99	82.52±4.18
B1	1.13±0.05	54.09±2.41	76.83±2.66	99.72±4.15

Commented [EDTR93]: Reviewer 1 wrote: This is not an interaction assessment, it is the treatments. In the ANOVA of this trial there are 16 treatments and 112 experimental units. The sources of variation evaluated would be (treatments, bacteria, sludge, and bacteria x sludge interaction). And there may be a significant difference in treatments without any interaction between the factors.

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Commented [EDTR96]: Reviewer 3 wrote: If all the treatments (B x S) are NS, there was not interaction between the factors. Then it is unnecessary to show the interaction.

Commented [EDTR97R96]: The authors did not reply to this comment.

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B2	1.49±0.06	47.30±3.30	73.20±2.28	98.25±3.90
B3	1.15±0.07	52.32±3.39	77.18±4.90	98.47±4.56
Biogas sludge (S)				
S0	1.26±0.06	45.51±2.63	62.88±2.19 b	76.78±1.63 c
S1	1.23±0.08	44.47±1.71	68.52±2.00 ab	87.65±2.84 bc
S2	1.26±0.08	55.36±3.43	85.69±1.08 a	98.95±1.86 b
S3	1.40±0.06	56.38±1.05	83.73±3.44 a	115.59±2.11 a
Interactions (B×S)				
B0S0	1.58±0.08	41.73±2.78	58.08±1.54	67.23±0.96
B0S1	1.12±0.08	45.87±0.83	62.74±1.83	71.08±1.91
B0S2	1.20±0.12	52.25±2.07	81.39±5.48	88.28±5.02
B0S3	1.60±0.12	52.18±0.29	92.20±3.05	103.49±2.43
B1S0	0.97±0.04	46.64±1.39	69.53±4.90	80.30±4.51
B1S1	1.40±0.07	48.13±2.78	78.91±0.53	96.23±1.50
B1S2	1.12±0.10	67.79±1.44	91.05±2.25	101.80±2.40
B1S3	1.02±0.08	53.81±3.76	67.84±1.77	120.54±2.15
B2S0	1.17±0.05	59.32±2.33	70.92±4.20	81.43±3.82
B2S1	1.54±0.10	34.47±2.16	61.69±1.97	89.84±1.41
B2S2	1.73±0.05	37.37±3.74	83.10±1.19	105.46±1.37
B2S3	1.53±0.10	58.05±1.76	77.07±4.27	116.28±1.30
B3S0	1.30±0.07	34.35±7.04	52.98±0.73	78.16±0.48
B3S1	0.85±0.03	49.40±0.08	70.72±1.29	93.44±2.19
B3S2	0.99±0.05	64.05±4.68	87.22±2.90	100.26±1.93
B3S3	1.44±0.02	61.48±2.47	97.80±0.77	122.04±0.20
CV (%)	43.80	31.22	26.54	18.38

Commented [EDTR99]: Reviewer 1 wrote: Idem table 3

Commented [EDTR100R99]: The authors did not reply to this comment.

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Note: Values followed by the different letter in the column significantly differed according to the Duncan test indicated significantly by DMRT at $P < 0.05$ SE. ns = not significantly. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 mL/ml/polybag⁻¹). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).

A significant increase in total dry weight of upland rice was observed along with the increase in the dosage of biogas sludge of 630 mL/ml/polybag⁻¹ at 16 WAIBSAWAA, with the highest increase of 50.55% compared to the control. Although the effect was not insignificant, it was seen that the B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice with were 20.84% and 81.53%, respectively, compared to the control.

Commented [EDTR102]: Reviewer 1 wrote: The interaction was insignificant

Commented [EDTR103R102]: The authors did not reply to this comment.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 WAIBSAWAA, but it did not have had a n insignificant effect at 4-8 and 8-12 WAIBSAWAA (Tab. 5).

Commented [EDTR104]: Reviewer 1 wrote: This table can generate confusion, take into account the recommendations of the previous tables

TABLE 5. The effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice.

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Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
		4-8 WAIBSAWAA			
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
		8-12 WAIBSAWAA			
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
		12-16 WAIBSAWAA			
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

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238 Note: ν values followed by the different letter in the column significantly differed according to the Duncan test
 239 indicated significantly by DMRT at $P < 0.05$. ns = not significantly. Dosage of biogas sludge (S0 = untreated; S1
 240 = 157.5; S2 = 315; S3 = 630 mL/polybag⁻¹). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 =
 241 isolate P7, B3 = combination of isolates N3+P7).

242

243 The biogas sludge dose of 630 mL/polybag⁻¹ (S3) significantly increased the highest crop
 244 growth rate for upland rice at 12 to 16 WAIBSAWAA by 129.44% compared to the control.

245 The ability of isolates B1-B3 isolates significantly increased the crop growth rate of upland rice
 246 with the highest increase in the for B2 of by 181.45% compared to the controls at 12 to 16
 247 WAIBSAWAA. The interaction of the B1S3 significantly increased the crop growth rate of
 248 upland rice, showing values by 5.76 times greater compared to those of the control.

249

250 Effect of bacterial isolates and biogas sludge on upland rice nutrition

251 Content of total N and available P nutrient of upland rice

252 The effect of biogas sludge, superior bacterial isolates, and their interactions had and did not have
 253 a insignificant effect on the nutrient content of total N and available P in the upland rice (Fig.
 254 2). The biogas sludge doses of 315 and 630 mL/polybag⁻¹ (S2 and S3) explained that the
 255 nutrient contents of available P and total N in the plant tissue of upland rice were higher by
 256 33.33% and 4.53% higher, respectively, compared to the control. The isolate B2 isolate showed

Commented [EDTR107]: Reviewer 1 asked: how it was calculated and where it compares with the other treatments

Commented [EDTR108R107]: The authors did not reply.

Commented [EDTR109]: Reviewer 1 wrote: In this analysis, it is necessary to justify the reason why the bacteria achieve a greater absorption of nutrients such as nitrogen and a higher growth rate, but do not achieve a greater height, dry weight and content of N and P

Commented [EDTR110R109]: The authors did not reply to this comment.

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257 the highest ~~nutrient~~ content of total ~~_N~~ in the plant tissue of upland rice ~~with values by~~ 1.63%
 258 ~~higher than those of compared to~~ the control; ~~however, but~~ all isolates (B1-B3) showed a
 259 similar level of available ~~_P~~ in the plant tissue ~~of upland rice with compared to?~~ the control.
 260
 261 **Uptake of total-N and available-P nutrient of upland rice**
 262 The effect of biogas sludge significantly increased in the ~~nutrient~~ uptake of total ~~_N~~ and
 263 available ~~_P~~. The Superior bacterial isolates significantly increased in the ~~nutrient~~ uptake of
 264 total ~~_N~~. The interaction of biogas sludge with superior bacterial isolates ~~did not show a had an~~
 265 insignificant effect on the ~~nutrient~~ uptake of total ~~_N~~ and available ~~_P~~ of ~~the~~ upland rice (Fig.
 266 2).

Commented [SM111]: Do you refer to the contents of available P in soil or in the plant tissue? If plant tissue, what was the analytical method to determine the "available" P in the plant tissues?

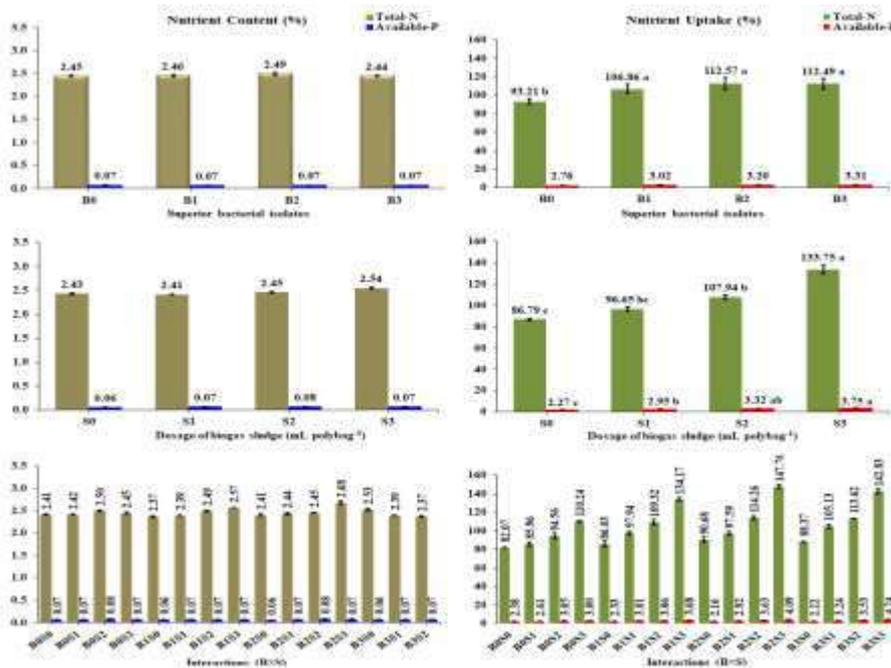
Commented [EDTR112]: Reviewer 1 wrote: there is no significant difference should not be analyzed, the differences are given only by chance.

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Commented [EDTR114]: Reviewer 3 wrote: He authors could explain that there was increase in uptake of nutrient; however, there was not increase in nutrient content in the rice plants.

Commented [EDTR115R114]: The authors shortened the sentence and modified it somehow, but they did not reply to the comment of the reviewer.



267 **FIGURE 2.** The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the content and uptake of total ~~_N~~ and available ~~_P~~ ~~nutrient~~ of the upland rice. Values followed by the different letters in graph
 268 significantly differed according to the Duncan test indicated significantly by DMRT at $P < 0.05$. ns = not
 269 significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 mL/polybag⁻¹). Superior
 270 bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = combination of isolates N3+P7).
 271
 272

Commented [EDTR116]: Please add the names of the Y axes. They should appear next to the axes. Please add these graphs as independent files to ensure they have the appropriate quality and resolution.

Commented [EDTR117]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures.

Commented [EDTR118R117]: The authors did not reply to this comment.

273 A significant increase in the ~~nutrient uptake of total -N~~ and available -P in upland rice ~~was~~
 274 ~~observed along with the increase a higher in the~~ dose of biogas sludge ~~of~~ 630 ~~ml/ml~~/polybag,
 275 ⁺ with the highest increases of 54.11% and 65.20%, respectively, compared to the control. The
 276 superior bacterial isolates (B1-B3) also significantly increased the ~~nutrient uptake of total -N~~
 277 ~~for in~~ upland rice with the highest increase ~~in with~~ the B2 ~~of by~~ 20.77% compared to the control.
 278 Although the effect was ~~not insignificant~~, ~~it was seen that the~~ B3 showed the highest increase
 279 in ~~nutrient~~ uptake of available -P in upland rice ~~was of~~ 19.93% compared to the control.

Commented [SM119]: This phrase makes no physiological sense. Please check out the English spelling. What does the "nutrient uptake of total" element stand for?

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281 Discussion

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282 The effect of selected superior bacterial isolates

283 The selected superior bacterial isolates (N3 and P7) significantly increased ~~the nutrient uptake~~
 284 ~~of total -N~~ and crop growth rate of upland rice on ultisols at 12 to 16 ~~WAIBSAWAA~~, but it ~~did~~
 285 ~~not have~~ an insignificant effect on plant height, total fresh weight, total dry weight, nutrient
 286 content (total -N and available -P) in leaf tissue, nutrient uptake of available -P, and crop growth
 287 rate of upland rice at 4 to 8 and 8 to 12 ~~WAIBSAWAA~~. The superior bacterial isolates (N3, P7,
 288 and N3+P7) could increase the ~~nutrient~~ uptake of total -N in upland rice by 14.64%, 20.77%,
 289 and 20.68%, respectively, compared to ~~the~~ control (Fig. 2). Similar results are also shown in
 290 Table 5, ~~that where can be observed that~~ the crop growth rate of upland rice at 12 to 16
 291 ~~WAIBSAWAA due to selected superior bacterial isolates (N3, P7, N3+P7)~~ has increased ~~by~~
 292 2.57, 2.81, and 2.39 times, respectively ~~due to the selected superior bacterial isolates (N3, P7,~~
 293 ~~N3+P7)~~, compared to the control. The ~~finding~~ results indicated that the ability of a single ~~isolate~~
 294 ~~by P7 bacterial isolate~~ was greater in increasing total -N and crop growth rate of upland rice
 295 compared to a single ~~isolate by~~ N3 bacterial ~~isolate~~ and ~~the~~ combination of N3+P7 bacterial
 296 isolates. ~~This~~ was due to the presence of several organic acids and hormones produced by P7
 297 that can increase the nutrient uptake of total -N and crop growth rate of upland rice. This result

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Commented [SM121]: The phrase "nutrient uptake of total-N" has no physiological sense. Please re-write these phrases using different terms

298 ~~i~~was supported by Mustamu *et al.* (2021) who found that the phosphate solubilizing bacterial
 299 isolate (P7) from the biogas sludge contains organic acids ~~produced~~ such as lactic, oxalic,
 300 acetic, and citric acids, and ~~it~~ had the highest ability to solubilize phosphate from calcium
 301 triphosphate and rock phosphate with values was 4.62 and 2.66 -times higher, respectively,
 302 compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and
 303 phosphorus in soils slightly increased within the application of bio fertilization with *Bacillus*
 304 *cereus*; this, it was due to the production of organic acids and other chemicals such as citric,
 305 tartaric, and oxalic acids which-that can stimulate plant growth and nutrients availability.
 306 Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was
 307 due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et*
 308 *al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin,
 309 indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that the hormone
 310 gibberellin and, IAA, and other hormones can increase plant growth under stressful conditions.
 311 Kang *et al.* (2014) said that PGPB has several mechanisms to increase plant growth with
 312 nitrogen-fixation and, phosphate solubilizationing, increasing nutrient availability. Suksong *et*
 313 *al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include:
 314 *Ruminococcus sp.*, *Thiomargarita sp.*, *Clostridium sp.*, *Anaerobacter sp.*, *Bacillus sp.*,
 315 *Sporobacterium sp.*, *Saccharofermentans sp.*, *Oscillibacter sp.*, *Sporobacter sp.*, and
 316 *Enterobacter sp.* Liaquat *et al.* (2017) also reported that an abundance of *Bacillus*, *Clostridium*,
 317 and *Enterobacter spp.* in an anaerobic digester of wastewater whenin producing biogas.

318

319 ~~The e~~ffect of biogas sludge

320 The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16
 321 WAIBSAWAA), total dry weight (12 and 16 WAIBSAWAA), nutrient uptake (total -N and
 322 available -P), and the crop growth rate of upland rice at 8 to 12 WAIBSAWAA. However, but

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Commented [SM123]: What does this abbreviation mean?

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Commented [SM124]: Did you measure the "available P" in plant samples?
 What does the "available P" stand for in relation to the nutrient uptake by the plants in this experiment?

323 it ~~did not have had~~ an insignificant effect on nutrient content (total₋N and available₋P) in leaf
 324 tissue, and crop growth rate of upland rice (4-8 and 8-12 ~~WAIBSAWAA~~). An increase in plant
 325 height, total dry weight, nutrient uptake ~~in terms of~~ total₋N and available₋P, and also crop
 326 growth rate of upland rice on ultisols ~~along~~ with ~~the increase in a higher the~~ dose of biogas
 327 sludge ~~to of~~ 630 mL₋ml⁻¹/polybag⁺ at the end of this study (16 ~~WAIBSAWAA~~). However, in
 328 contrast to the total fresh weight, an increase along with the increase in the biogas sludge dose
 329 to 315 mL₋ml⁻¹/polybag⁺ then decreased at the dose of 630 mL₋ml⁻¹/polybag⁺. ~~It was caused~~ ~~T~~the
 330 biogas sludge had chemical characteristics such as pH (7.41), total₋N (0.051%), available₋P
 331 (0.013%), organic₋C (0.14%), total₋K (0.18%), and biological characteristics such as total
 332 nitrogen-fixing bacteria (29.4×10⁵ CFU mL₋ml⁻¹) and total phosphate solubilizing bacteria
 333 (7.0×10⁴ CFU mL₋ml⁻¹) (Tab₋le 2). ~~The~~ ~~C~~organic ~~C~~ content and the total population of nitrogen-
 334 fixing and phosphate solubilizing bacteria from the biogas sludge could ~~be~~ ~~increased~~ the
 335 nutrient uptake ~~in terms of~~ total₋N and available₋P in upland rice ~~along with~~ ~~an~~ increasing
 336 ~~the~~ dose of biogas sludge ~~of to~~ 630 mL₋ml⁻¹/polybag⁺ (Fig. 2). Therefore, the nutrients absorbed
 337 are used for plant metabolic processes and stimulate the ~~growth of~~ plant height, biomass, and
 338 crop growth rate of ~~the~~ upland rice. A similar result was reported by Mustamu and Triyanto
 339 (2020) ~~who determined? that~~ the macro and micronutrients from the biogas sludge and ~~also had~~
 340 the population of nitrogen-fixing and phosphate solubilizing bacteria ~~of by~~ 480×10⁴ and 42×10⁴
 341 CFU mL₋ml⁻¹, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that ~~the~~ total phosphate
 342 solubilizing bacteria (1.6 to 2.5 CFU mL₋ml⁻¹) was significantly higher compared to nitrogen-
 343 fixing bacteria (0.5–1.4 CFU mL₋ml⁻¹) ~~showing and~~ a significant increase in nutrient
 344 concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller
 345 and Müller (2012) reported that an increase in concentrations of NH₄⁺-N ranged from 45 to
 346 80% ~~after~~ anaerobic waste.

Commented [EDTR125]: This sentence is poorly written. It is not clear what increased/decreased. Please rewrite this sentence. The manuscript needs to be checked by a professional translator or a native speaker to ensure its quality.

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347

The interaction effect of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on ultisols at 12-16 WAIBSAWAA, but it did not have had a significant effect on the other parameters in this study. The interaction of NFB *Bacillus paramycoides* with biogas sludge at the dose of 630 mL/ml/polybag⁺ (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and was 5.76-times greater compared to the control. This ~~It~~ was caused by the application of biogas sludge that could ~~have be~~ increased soil organic matter and the total population of beneficial bacteria. Likewise, ~~the characteristics of~~ the biogas sludge ~~contained had the~~ organic ~~C was~~ (0.14%), total nitrogen-fixing bacteria ~~was~~ (29.4×10^5 CFU mL⁻¹), and total phosphate solubilizing bacteria ~~was~~ (7.0×10^4 CFU mL⁻¹) (Tab. 2) that could improve soil quality and support the crop growth rate. This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long-term significantly increases the organic matter contents in the soil, ~~causing which causes~~ a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014) ~~and~~ Carvajal-Muñoz ~~and~~ Carmona-Garcia *et al.* (2012) ~~showed reported~~ that the application of a biofertilizer had advantages in the plant such as availability of nutrients that are balanced for plant health. It also ~~stimulates~~ ~~ing~~ nutrient mobilization that can increase soil biological activity ~~and the~~ availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content ~~and, therefore, thereby~~ ~~increasing~~ the cation exchange capacity. Siswanti and Lestari (2019) ~~indicated reported~~ that the interaction of biogas sludge+biofertilizer (36 mL/ml+10 L ha⁻¹) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

Commented [EDTR127]: Reviewer 1 wrote: This bacterium is named for the first time, what is its function and characteristics, because the characteristics of the bacteria do not appear in the methodology

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Commented [EDTR129]: Reviewer 3 wrote: The authors must review the English in lines 359-361. The conclusions aren't the repetition of results. The authors could make an effort to show emerging properties accounted for in the results of this work and enhance the practical application and the investigation novelty.

Commented [EDTR130R129]: The authors did not reply to these comments and kept the same conclusions.

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373 The isolates ~~of~~ N3, P7, N3+P7 from the biogas sludge significantly increased the ~~nutrient~~-uptake
 374 of total ~~-~~N (20.77%) and crop growth rate (2.81 times higher than the control?) of upland rice
 375 on ultisols with the highest increase found ~~in-with~~ the P7 isolate ~~of 20.77% and 2.81 times,~~
 376 ~~respectively.~~ The dose of biogas sludge significantly increased plant height (14.81%), total dry
 377 weight (50.55%), ~~nutrient~~-uptake of total ~~-~~N (54.11%) and available ~~-~~P (65.20%), and also crop
 378 growth rate (129.44%) of upland rice on ultisols with the highest increase at a dose of 630 ~~mL~~
 379 ~~/polybag⁺ by 14.81%; 50.55%; 54.11%; 65.20%; and 129.44%, respectively.~~ Likewise, the
 380 dose of biogas sludge significantly increased the total fresh weight of upland rice ~~by 41.81%~~
 381 with the highest increase at the dose of 315 ~~mL~~
 382 isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop
 383 growth rate of upland rice on ultisols 5.76 times with the highest increase found ~~within the~~
 384 B1S3 ~~by 5.76 times.~~

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385 **Conflict of interest statement**

386 The authors declare that there is no conflict of interest regarding the publication of this article.

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387 **Author's contributions**

388 All authors formulated the overarching research goals and aims, provided the study materials,
 389 developed or designed the methodology. NEM analyzed ~~d~~ and ~~interpretation~~ ~~interpreted the~~ study
 390 data. NEM and MS wrote the initial draft, managed and coordinated the research activity in ~~the~~
 391 field, ~~data collection~~ ~~and collected the data.~~ ZN and I verified the overall reproducibility of
 392 results and ~~the~~ other research outputs. All authors conducted the critical
 393 review/commentary/revision of the manuscript.

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395 **Literature cited**

Commented [EDTR131]: Reviewer 3 wrote: The literature used in the introduction must be updated.

Commented [EDTR132R131]: The authors either replied to this comment nor changed the citations in the text (only added two).

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<https://www.fao.org/publications/card/en/c/34d03d32-bd9f-5d08-aa08-ed2499349eb1/>

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 493 sludge to agricultural soil increases the abundance of antibiotic resistance genes without

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- 494 altering the composition of prokaryotic communities. *Science of the Total Environment*,
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501 soil microorganism with biological control potential. *African Journal of Microbiology*
502 *Research*, 7(1), 41–47. <https://doi.org/10.5897/AJMR12.1485>.

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Bogotá, November 22nd, 2021

Certificate of acceptance for publication

This certificate was issued to certify that the article entitled “Performance of selected superior bacterial isolates from biogas sludge on the growth of upland rice in ultisols” submitted on August 1st, 2021 by the authors Novilda Elizabeth Mustamu, Zulkifli Nasution, Irvan, and Mariani Sembiring, has been accepted for publication in volume 39 No. 3 (2021) of the journal *Agronomía Colombiana*.

Sincerely,

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Dear author:

The Editor in Chief checked the paper one last time before sending it to proofreading and layout design. We enclose the manuscript with some final comments we consider that should be addressed before final acceptance. We look forward to getting the revised version as soon as possible in order to continue with its editorial process.

Kind regards,

Stanislav Magnitskiy

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de secano

Novilda Elizabeth Mustamu^{1*}, Zulkifli Nasution^{2*}, Irvan³, and Mariani Sembiring²

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²Program Study of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara (Indonesia).

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ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P < 0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and ~~the highest~~ crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, ~~nutrient~~ uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction ~~of between~~ N3 ~~with and~~ biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination within biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Commented [EDTR1]: Reviewer 1 wrote: I recommend "Evaluation of selected bacterial isolates from biogas sludge in upland rice"

Reviewer 3 wrote: The title don't show effectively the results of investigation. I recommend the title: Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice. Regarding the term "superior bacterial isolates" reviewer 3 wrote: What the authors wanted to say? I don't understand this term. This reviewer also wrote: The authors evaluated only the effect of bacteria and biogas sludge on rice growth. They evaluated also the effect of these treatments on rice nutrition! Finally, regarding the word "ultisols" reviewer 3 wrote: Why is it necessary to specify the soil type?

Commented [EDTR2R1]: The authors only considered the suggestion of reviewer 3 but did not reply to any of the reviewer's comments.

Commented [EDTR3]: Please do not change the corresponding author because all emails and communication has been addressed to Novilda Elizabeth Mustamu. If this author does not have an institutional email address the personal address should be used.

Commented [EDTR4]: This word is in the resumen in Spanish, it should be here, too.

30 **Key words:** acidic soil, crop growth rate, dosage, sludge potential.

31

32 **RESUMEN**

33 El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados
 34 (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes en
 35 el arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos
 36 factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor
 37 utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de
 38 nitrógeno (N3), B2 = aislamiento de bacterias solubilizantes de fosfato (P7), B3 = combinación
 39 de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin
 40 tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados
 41 por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P < 0.05$. Los
 42 resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en
 43 un 20.77% y la ~~mayor~~ tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81_
 44 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la
 45 altura de la planta, la absorción ~~de nutrientes~~ de N y P, el peso fresco y seco total y el TCC de
 46 arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag
 47 aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos
 48 N3 y P7 y su combinación dentro de lodos de biogás de 630 ml/polybag tiene el potencial de
 49 aumentar el TCC de arroz de tierras altas en suelos ácidos.

50

51 **Palabras clave:** suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

52

53 **Introduction**

Commented [EDTR5]: Reviewer 3 wrote: The abstract does not show the investigation novelty and the importance of results to agricultural management.

Commented [EDTR6R5]: The authors did not reply to this comment.

Commented [EDTR7]: Reviewer 3 wrote: Only state the concentration value.

Commented [EDTR8R7]: The authors neither replied to this comment nor changed this section.

Commented [EDTR9]: Reviewer 3 wrote: The isolate itself does not produce those effects. It is the application or inoculation of the isolate that produces the effects. Improve wording. I recommend: "Los resultados mostraron que la inoculación aumentó... pues el aislado por sí solo no produce el efecto, sino que hay que inocularlo".

Commented [EDTR10R9]: The authors neither replied to these comments nor changed this section.

Commented [EDTR11]: Reviewer 3 wrote: It is necessary to include relevant information about acid soil and the representative upland rice in Indonesia and its impact in the economy of the country. Furthermore, the authors must enhance the investigation novelty.

Commented [EDTR12R11]: The authors did not reply to these comments.

54 Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and
 55 has a high nutrient content that can be used as organic fertilizer to increase soil fertility and the
 56 plants yield (Adela *et al.*, 2014). The following characteristics of the biogas sludge from palm
 57 oil waste have been reported: total N of 490 mg L⁻¹, total P of 110 mg L⁻¹, total K of 1.9 mg L⁻¹
 58 (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), and NH₃-N of 91 -112
 59 mg L⁻¹ (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest
 60 bacterial population of 7.21×10⁷ cells per ml and the lowest one of 3.15×10⁷ cells per ml
 61 (Alvionita *et al.*, 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas
 62 sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase
 63 the availability of nitrogen and phosphate in soils.

64 The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria
 65 has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported
 66 that phosphate solubilizing bacteria play an important role in increasing soil fertility, and plant
 67 yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different
 68 *Bacillus* species, such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B.*
 69 *mycooides*, *B. coagulans*, *B. chitinolyticus*, and *B. subtilis* as phosphate solubilizing
 70 microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus*
 71 *cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant
 72 bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and
 73 clone library methods, *i.e.*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

74 The application of bacteria from biogas sludge has never been reported in Indonesia for
 75 improving upland rice growth on acidic soils, including Ultisols. According to the Pusat
 76 Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research), (2000), found
 77 that the area in Indonesia covered by Ultisols was 45.8 million ha, or 24% of the total area of
 78 Indonesia. Furthermore, according to the Ministry of Agriculture, the area of dedicated to rice

Commented [EDTR13]: Reviewer 3 wrote: I think it is unnecessary to show all these *Bacillus* species. Will the authors work with these genera in this investigation? In materials and methods, the authors do not say that they work with these bacterial genera. I recommend explaining more the lines 66-69 since it is the essence of this work.

Commented [EDTR14R13]: The authors did not reply to this comment.

79 ~~growing cultivation~~ in Indonesia was 15,712,025 ha with ~~a the~~ yield of 81,148,617 ~~ton~~ ha⁻¹ in
 80 2017 and the contribution of upland rice yield reacheds 4.66% (~~Kementerian Pertanian,~~
 81 ~~Ministry of Agriculture,~~ 2017). The yield contribution of upland rice was classified as low and,
 82 ~~therefore,~~ it is necessary to ~~develop find options in order to increase yield through biogas~~
 83 ~~sludge.~~ Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas
 84 sludge to increase the availability of nitrogen and phosphate, and the growth response of upland
 85 rice due to application of the biogas sludge and selected isolates in Ultisols. The study aimed
 86 to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their
 87 interaction on the mineral nutrition of the upland rice grown in Ultisols.

Commented [EDTR15]: This should not be written because this assures biogas sludge increases yield. It may be an option to increase it and for that reason it was evaluated in this study.

89 **Materials and methods**

90 **Study area**

91 The concentration of total N and ~~available P in Ultisols and in the plant tissue~~ (N and P uptake)
 92 were ~~analyzed~~ in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The
 93 bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N;
 94 98°38.898' E; altitude 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from
 95 October 2020 to April 2021. The average temperature ~~was 27.4°C, the average~~ air humidity,
 96 ~~was 82% and average rainfall was in this study were 27.4°C, 82%, and~~ 228.5 mm per month,
 97 ~~respectively.~~

Commented [EDTR16]: The Editor in Chief asked: What do you mean by measuring concentration of available P in the plant tissue?

Commented [EDTR17R16]: The authors did not reply.

Commented [EDTR18]: The Editor in Chief wrote: Please, provide the bibliographic reference of the manual used for these analyses

Commented [EDTR19R18]: The authors neither responded nor added the requested information.

98 **Preparation of medium and upland rice seeds**

99 The medium to grow upland rice plants used the Ultisols from the Simalingkar area, Medan
 100 Tuntungan Subdistrict, Medan City, at a depth of 0 to 20 cm. One hundred g of soil samples
 101 were taken and analyzed for chemical characteristics such as pH using H₂O, organic C by
 102 Walkley-Black, available P by Bray-II, total N using Kjeldahl method, and cation exchange

103 capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH 7 method (Tab.
 104 1). The soil was sterilized by drying at 100°C for 2 h. For preventing heat from the sterilization
 105 process, the soil ~~was~~ incubated for 1 d ~~and~~ then placed into a 10 kg polybag (18 cm × 18 cm).
 106 A basic NPK fertilizer (16-16-16) by ~~Meroke Tetap Jaya Inc.~~, at a dose of 1.5 g/polybag was
 107 applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) ~~used in~~
 108 ~~the~~ ~~were of the inbred variety~~ Inpago-8 ~~inbred variety were off from the~~ Indonesian Agency for
 109 Agricultural Research and Development, ~~then were~~ soaked in water for 24 h, followed by ~~the~~
 110 ~~application of the fungicide~~ Propineb ~~fungicide~~ (70%) ~~application~~ for 2 h. Upland rice was
 111 planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

Commented [EDTR20]: Please add the city and country.

112 **TABLE 1.** Chemical characteristics of the Ultisols soil samples after sterilization at 100°C.

Chemical characteristics	Value	Category*
Soil pH (H ₂ O)	4.80	Acid
Organic C (%)	0.44	Very low
Total N (%)	0.04	Very low
Available P (mg kg ⁻¹)	870.25	Very high
CEC (meq 100 g ⁻¹)	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (meq 100 g ⁻¹)	0.60	High
Ca (meq 100 g ⁻¹)	0.34	Very low
Mg (meq 100 g ⁻¹)	0.32	Very low
Na (meq 100 g ⁻¹)	0.09	Very low
Al (%)	0.02	Very low

114 *Criteria for pH (H₂O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg
 115 kg⁻¹ (very high); Cation exchange capacity (CEC) = 25-40 meq 100 g⁻¹ (high); base saturation <20% (very low);
 116 exchangeable K= 0.60-1.00 meq 100 g⁻¹ (high); exchangeable Ca <2 meq 100 g⁻¹ (very low); exchangeable Mg
 117 <0.4 meq 100 g⁻¹ (very low); exchangeable Na <0.1 meq 100 g⁻¹ (very low); exchangeable Al <5% (very low)
 118 (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Commented [EDTR21]: Reviewer 3 wrote: I think that it is irrelevant. I recommend to write the techniques that allow the soil characteristics and then state the bibliographic cites correspondingly.

Commented [EDTR22R21]: The authors neither replied to this comment nor changed the table's footnote.

120 Preparation of superior bacterial isolates suspension and biogas sludge

121 A total of 1 ml of the bacterial isolate suspension obtained from the characteristic stage was put
 122 into a test tube containing 9 ml of distilled water and homogenized. ~~It put a total of 1 ml of the~~
 123 ~~suspension from the dilution into 9 ml of distilled water.~~ The dilution was made to 10⁻⁵. A total
 124 of 0.1 ml of the suspension from the last dilution was spread over the James ~~n~~Nitrogen ~~f~~Free
 125 ~~m~~Malat ~~b~~Bromothymol ~~b~~Blue (JNFB) medium for the nitrogen-fixing bacterial isolates test.

Commented [EDTR23]: This was said in the first line.

Commented [EDTR24]: ?

126 While the suspension phosphate solubilizing bacteria isolates, the test was spread over
 127 Pikovskaya (PVK) medium. The culture medium was incubated for 2 to 3 days at room
 128 temperature. The nitrogen-fixing bacterial isolate test was characterized by the presence of
 129 colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates
 130 was indicated by a halo zone around the microbial colonies on the PVK medium. The result
 131 was found in seven nitrogen-fixing and seven phosphate-solubilizing isolates were found to
 132 produce total-N and available-P. The isolates of superior bacteria were selected which that had
 133 showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate
 134 solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu
 135 *et al.* (2021a, 2021b).

Commented [EDTR25]: This does not make any sense, please check the writing here.

Commented [EDTR26]: What medium is this?

Commented [EDTR27]: In the previous version of the manuscript the authors wrote something different: The isolates were grown on NB medium, and incubated for 48 h. The bacterial growth in the solution was measured using an spectrophotometer with a density of 108 cells per ml.

136 The biogas sludge was collected by the fixed tank of digester at the palm oil mill of Nubika
 137 Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia, and then
 138 handled with tongs. Bacterial isolates and biogas sludge were applied to the soil surface at the
 139 base of the plants at one week after planting. Biogas sludge samples at a 500 ml volume were
 140 used to analyze the chemical and biological characteristics (Tab. 2).

Commented [EDTR28]: The Editor in Chief asked: Could you indicate which species or hybrid of palm was employed? This may have an effect on the sludge composition.

Commented [EDTR29R28]: The authors did not reply.

Commented [EDTR30]: Reviewer 3 wrote: I think that the authors must give more details about how biogas sludge was taken.

Commented [EDTR31R30]: The authors did not add any information.

Commented [EDTR32]: Reviewer 3 wrote: The authors must say how the chemical and biological characteristics of biogas sludge were determined, or make a bibliography cite.

The Editor in Chief also wrote: Please, provide the bibliographic reference of the manual used for the biogas sludge analysis.

Commented [EDTR33R32]: The authors did not add a citation.

141 **TABLE 2.** The chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L ⁻¹)	Spectrophotometry	4547.8
Biological oxygen demand (mg L ⁻¹)	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L ⁻¹)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml ⁻¹)	Plate count	29.4×10 ⁵
Total phosphate solubilizing bacteria (CFU ml ⁻¹)	Plate count	7.0×10 ⁴

Commented [EDTR34]: Reviewer 1 asked: Is it correct? It can be a very high amount of sodium.

Commented [EDTR35R34]: The authors did not answer.

143 Source: laboratory analysis

144

145 Treatment application

146 This study used a randomized block design with two factors and seven replicates. The first
 147 factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial
 148 isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates
 149 N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was dose of biogas sludge
 150 (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge
 151 based on the dose of liquid organic fertilizer at the oil palm was 126 m³ ha⁻¹ equal to 126,000
 152 L ha⁻¹ (Sutarta *et al.*, 2003), then converted to soil weight per polybag (Eq. 1). Each replicate
 153 was disassembled at 4, 8, and 12 weeks after the application (WAA) of what? for determination
 154 of the crop growth rate (CGR).

$$155 \text{ Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \quad (1)$$

$$156 = \frac{26,000 \text{ L ha}^{-1}}{2,000,000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 630 \text{ ml}$$

157 Parameters and data analysis

158 The observations of the variables were conducted by measuring the growth of upland rice (plant
 159 height, and total fresh and dry weight), nutrient contents and uptake of N and P in the shoots,
 160 and CGR. The plant height was measured from by the base of the roots to the tip of leaves using
 161 a measuring tape?, and the total fresh weight was obtained by weighing the roots and shoots.
 162 The total dry weight (roots + shoots) was measured after using an oven (model VS-1202D3,
 163 Vision Scientific Co., Ltd., Korea) at 60°C for 48 h and weighed using the analytical scales. A
 164 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N
 165 content using the Kjeldahl and the P content was estimated using the destruction method
 166 through dry ashing. The N and P uptake were measured using Equation 2. The CGR was
 167 calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation
 168 3 (Shon *et al.*, 1997):

Commented [EDTR36]: Reviewer 3 wrote: Why the authors use these biogas sludge concentrations. I think it is important!

Commented [EDTR37R36]: The authors did not reply.

Commented [EDTR38]: What do you mean by disassembled?

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Commented [EDTR39]: The Editor in Chief asked: individual culm?

Commented [EDTR40R39]: The authors did not answer.

169 Nutrient uptake = nutrient content in the shoots \times total dry weight (2)

$$170 \text{ CGR} = \frac{\Delta W}{\Delta t} = \frac{W_2 - W_1}{t_2 - t_1} \quad (3)$$

171 where:

172 CGR = crop growth rate;

173 W1 = dry weight per unit area at t1;

174 W2 = dry weight per unit area at t2;

175 t1 = first sampling;

176 t2 = second sampling;

177 The parameters of the second phase of the study were analyzed by an ANOVA and if the
178 treatment had a significant effect, followed by Duncan's multiple range test at $P < 0.05$ using
179 SPSS v.20 software (IBM, 2011).

180

181 **Results**

182 **Effect of bacterial isolates and biogas sludge on upland rice growth**

183 Plant height of upland rice

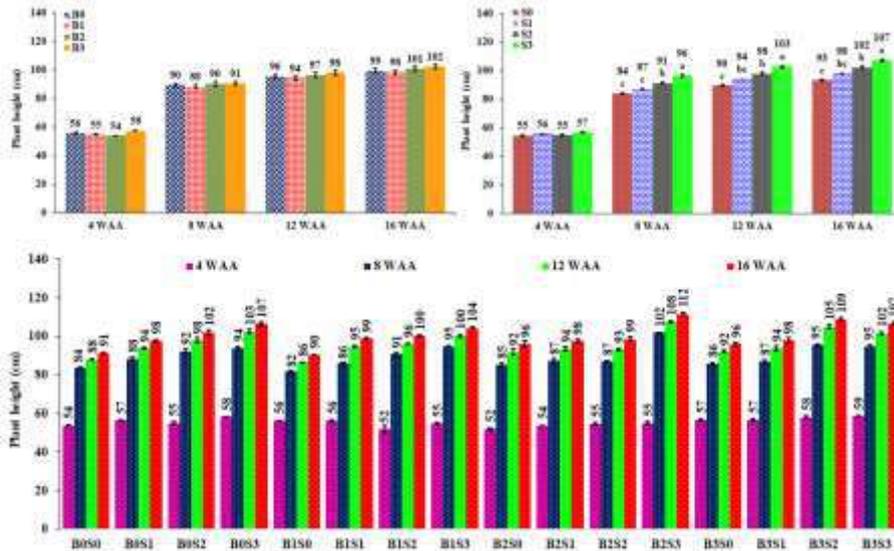
184 The effect of biogas sludge application was significant on the plant height of upland rice at 8,
185 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant
186 effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1). A significant increase
187 in plant height of upland rice was observed with ~~increased~~ higher doses of biogas sludge of 630
188 ml/polybag at 8, 12, and 16 WAA with the highest increase of 14.81% compared to the control
189 at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the
190 interaction of B2S3 showed the highest in plant height of upland rice by 2.94 and 22.06%,
191 respectively, compared to the control.

Commented [EDTR41]: Reviewer 1 wrote: I recommend that the results part be restructured in its figures and tables. It could only show figures that show a significant difference, without showing those that do not have a significant difference.
Reviewer 3 wrote: It is necessary write Results and discussion again in other form. All the information of this section must be re-organized. Account of the statistical analysis made and some information in figures and tables is irrelevant. The figures have repeated information.

Commented [EDTR42R41]: The authors did not reply to these comments.

Commented [EDTR43]: Reviewer 3 wrote: I consider that it is necessary to write again Results and discussion in other form. The information of this section must be re-organized in two aspects: (1) Effect of bacterial isolates and biogas sludge on upland rice growth. (2) Effect of bacterial isolates and biogas sludge on upland rice nutrition. The first point could include:
- Plant height of upland rice
- Biomass of upland rice
- Crop growth rate of upland rice
The second point could include:
- Content of total-N and available-P nutrient of upland rice
- Uptake of total-N and available-P nutrient of upland rice
Furthermore, for both points the analysis made from lines 249-332 must be undertaken.

Commented [EDTR44R43]: It seems the authors reorganized these sections following the reviewer's suggestion. However, they did not reply to the comment.



192 **FIGURE 1.** Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of
 193 upland rice at 4, 8, 12, and 16 WAA. Values followed by the different letter in the graph significantly differed
 194 according to the Duncan test at $P < 0.05$. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5;
 195 S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7,
 196 B3 = isolates N3+P7-isolates).
 197

198 Biomass of upland rice

199 The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12,
 200 and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect
 201 on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

202 **TABLE 3.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight
 203 (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).
 204

Treatments	Total fresh weight \pm standard error (g)			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 \pm 0.21	169.31 \pm 8.90	215.27 \pm 8.42	229.82 \pm 8.94
B1	3.12 \pm 0.12	194.50 \pm 9.35	235.08 \pm 10.32	252.02 \pm 10.22
B2	4.52 \pm 0.23	162.89 \pm 11.15	201.85 \pm 9.89	230.70 \pm 9.28
B3	3.30 \pm 0.25	173.91 \pm 12.55	220.40 \pm 15.96	245.03 \pm 16.32
Biogas sludge (S)				
S0	3.72 \pm 0.24	144.07 \pm 9.37 b	182.67 \pm 7.14 b	197.56 \pm 6.58 b
S1	3.58 \pm 0.27	153.41 \pm 7.93 b	190.70 \pm 8.90 b	215.65 \pm 7.03 b
S2	3.64 \pm 0.27	199.68 \pm 10.30 a	258.70 \pm 9.63 a	280.15 \pm 9.25 a
S3	4.15 \pm 0.25	203.45 \pm 1.36 a	240.52 \pm 2.81 a	264.21 \pm 2.42 a
Interactions (B \times S)				
B0S0	4.99 \pm 0.33	124.08 \pm 5.60	185.64 \pm 3.32	192.78 \pm 2.96

Commented [EDTR45]: Please add these graphs as independent files to ensure they have the appropriate resolution and quality.

Commented [EDTR46]: Reviewer 3 wrote: If all the treatments (B \times S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures. The authors could explore the possibility that gives them not interaction between the factors for discussing the result.

Commented [EDTR47R46]: The authors did not reply to this comment.

Commented [EDTR48]: Reviewer 3 wrote: If all the treatments (B \times S) are NS then there was not interaction between the factors. Then it is unnecessary to show the interaction.

Commented [EDTR49R48]: The authors did not reply to this comment.

B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by the different letter in the column significantly differed according to the Duncan test at $P < 0.05$. ns - not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7-isolates).

Commented [EDTR50]: Reviewer 1 wrote: This is not an interaction assessment, it is the treatments. In the ANOVA of this trial there are 16 treatments and 112 experimental units. The sources of variation evaluated would be (treatments, bacteria, sludge, and bacteria x sludge interaction). And there may be a significant difference in treatments without any interaction between the factors.

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A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest in the total fresh weight of upland rice with 9.66 and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4 -16 WAA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight ± standard error (g)			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				

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B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

221 Values followed by the different letter in the column significantly differed according to the Duncan test at $P < 0.05$.
 222 ns= not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 =
 223 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 =
 224 isolates N3+P7-isolates).
 225

226 A significant increase in total dry weight of upland rice was observed with the increase in the
 227 dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55%
 228 compared to the control. Although the effect was not significant, B1 and the interaction of B3S3
 229 showed the highest in the total dry weight of upland rice with 20.84 and 81.53%, respectively,
 230 compared to the control.

231

232 Crop growth rate of upland rice

233 The effect of superior bacterial isolates, biogas sludge, and their interactions significantly
 234 increased the crop growth rate of upland rice at 12 to 16 WAA, but it did not have a significant
 235 effect at 4-8 and 8-12 WAA (Tab. 5).

236 **TABLE 5.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the
 237 upland rice- 4, 8, 12, and 16 weeks after the application (WAA).
 238

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
	4-8 WAA				
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%

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Commented [EDTR56]: Reviewer 1 wrote: The interaction was insignificant

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Commented [EDTR58]: Reviewer 1 wrote: This table can generate confusion, take into account the recommendations of the previous tables.

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8-12 WAA					
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
12-16 WAA					
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

239 Values followed by the different letter in the column significantly differed according to the Duncan test at $P < 0.05$.
 240 ns - not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 =
 241 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 =
 242 isolates N3+P7-isolates).

243
 244 The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth
 245 rate for upland rice at 12 to 16 WAA by 129.44% compared to the control. The isolates B1-B3
 246 significantly increased the crop growth rate of upland rice with the highest increase for B2 of
 247 181.45% compared to the controls at 12 to 16 WAA. The interaction of the B1S3 significantly
 248 increased the crop growth rate of upland rice, showing values 5.76-times greater than those of
 249 the control.

250

251 **Effect of bacterial isolates and biogas sludge on upland rice nutrition**

252 **Nutrient Content** of N and P in the upland rice

253 The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a
 254 significant effect on the **nutrient** content of N and P in the upland rice (Fig. 2). **The biogas**
 255 **sludge doses of 315 and 630 ml/polybag (S2 and S3) explained that the contents of P and N in**
 256 **the plant tissue of upland rice were 33.33 and 4.53% higher, respectively, compared to the**
 257 **control. The isolate B2 showed the highest content of N in the plant tissue of upland rice with**
 258 **values 1.63% higher than those of the control; however, all isolates (B1-B3) showed a similar**
 259 **level of P in the plant tissue of upland rice compared to the control.**

260

261 **Nutrient uptake** of N and P in the upland rice

Commented [EDTR60]: Reviewer 1 asked: how it was calculated and where it compares with the other treatments

Commented [EDTR61R60]: The authors did not reply.

Commented [EDTR62]: Reviewer 1 wrote: In this analysis, it is necessary to justify the reason why the bacteria achieve a greater absorption of nutrients such as nitrogen and a higher growth rate, but do not achieve a greater height, dry weight and content of N and P.

Commented [EDTR63R62]: The authors did not reply to this comment.

Commented [EDTR64]: This expression does not make sense. The right phrase is content of N and P.

Commented [EDTR65]: The Editor in Chief asked: Do you refer to the contents of available P in soil or in the plant tissue? If plant tissue, what was the analytical method to determine the "available" P in the plant tissues?

Commented [EDTR66R65]: The authors did not answer.

Commented [EDTR67]: Reviewer 1 wrote: there is no significant difference should not be analyzed, the differences are given only by chance.

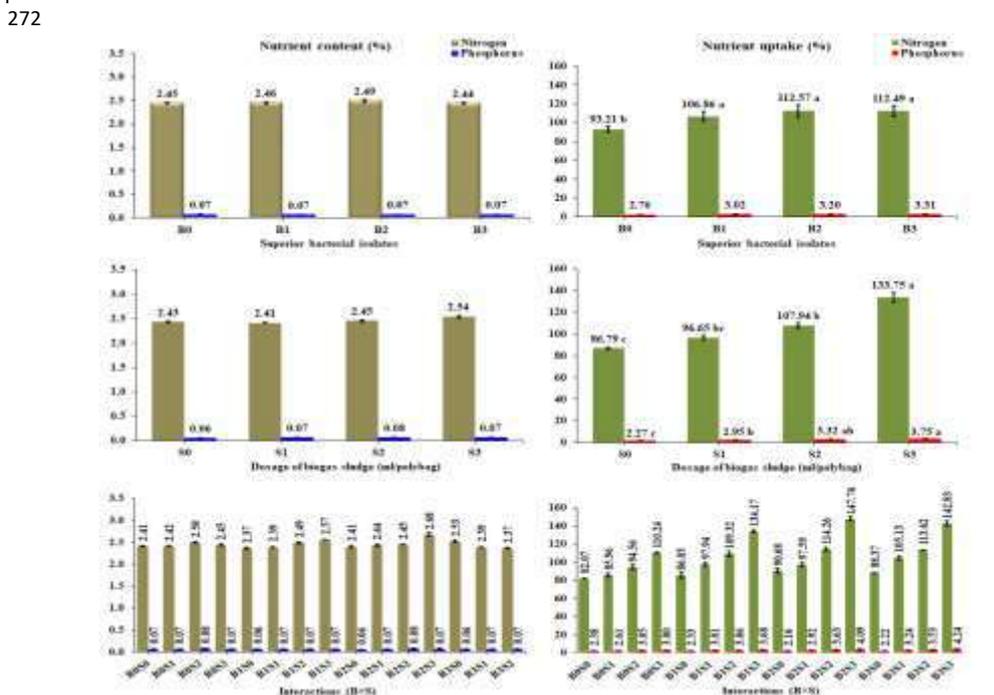
Commented [EDTR68R67]: The authors did not reply to this comment.

262 The effect of biogas sludge significantly increased in the nutrient-uptake of N and P. The
 263 superior bacterial isolates significantly increased in the nutrient-uptake of nitrogen. The
 264 interaction of biogas sludge with superior bacterial isolates did not show a significant effect on
 265 the nutrient uptake of N and P in the upland rice (Fig. 2).

266 A significant increase in the nutrient-uptake of N and P in upland rice was observed with a
 267 higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11 and
 268 65.20%, respectively, compared to the control. The superior-bacterial isolates (B1-B3) also
 269 significantly increased the nutrient-uptake of N in the upland rice with the highest increase with
 270 the B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed
 271 the highest in nutrient-uptake of P in the upland rice of 19.93% compared to the control.

Commented [EDTR69]: The Editor in Chief wrote: This phrase makes no physiological sense. Please check out the English spelling. What does the "nutrient uptake of total" element stand for?

You can use the phrase "nutrient uptake" when referring to the nutrients the plant absorbs in general. Nutrient uptake of N and P is redundant and does not make any sense. Please use only "uptake of N and/or P"



273 **FIGURE 2.** The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient
 274 content and uptake of N and P in the upland rice. Values followed by different letters significantly differed
 275 according to the Duncan test at $P < 0.05$. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5;

Commented [EDTR70]: Please add the names of the Y axes. They should appear next to the axes. Please add these graphs as independent files to ensure they have the appropriate quality and resolution.

Commented [EDTR71]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures.

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276 S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3
 277 = N3+P7 isolates).
 278

279 Discussion

280 Effect of selected superior bacterial isolates

281 The selected superior bacterial isolates (N3 and P7) significantly increased the ~~nutrient~~ uptake
 282 of nitrogen and crop growth rate of upland rice on Ultisols at 12 to 16 WAA, but it did not have
 283 a significant effect on plant height, total fresh weight, total dry weight, nutrient content (N and
 284 P) in leaf tissue, ~~nutrient~~-uptake of phosphorus, and crop growth rate of upland rice at 4 to 8
 285 and 8 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the
 286 ~~nutrient~~-uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively,
 287 compared to the control (Fig. 2). Similar results are also shown in Table 5, where can be
 288 observed that the crop growth rate of upland rice at 12 to 16 WAA has increased 2.57, 2.81,
 289 and 2.39 times, respectively due to the selected superior bacterial isolates (N3, P7, N3+P7),
 290 compared to the control. The results indicate that the ability of a single P7 bacterial isolate was
 291 greater in increasing the nitrogen and crop growth rate of upland rice compared to a single N3
 292 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic
 293 acids and hormones produced by P7 that can increase the ~~nutrient~~-uptake of nitrogen and crop
 294 growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that
 295 the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids
 296 such as lactic, oxalic, acetic, and citric acids, and had the highest ability to solubilize phosphate
 297 from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher,
 298 respectively, compared to the control. Meena *et al.* (2016) reported that the availability of
 299 nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with
 300 *Bacillus cereus*; this was due to the production of organic acids and other chemicals such as
 301 citric, tartaric, and oxalic acids that can stimulate plant growth and nutrient availability. Youssef

Commented [EDTR73]: The Editor in Chief wrote: The text of the manuscript should be revised with the help of a professional English proofreader.

Commented [EDTR74]: The phrase "nutrient uptake of N" has no physiological sense. Please re-write these phrases using different terms

302 and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to
 303 increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.*
 304 (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-
 305 acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that the hormone gibberellin
 306 and IAA, can increase plant growth under stressful conditions. Kang *et al.* (2014) said that the
 307 **pPlant gGrowth-pPromoting bBacteria** (PGPB) has several mechanisms to increase plant
 308 growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability.
 309 Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester
 310 include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp., *Anaerobacter* sp., *Bacillus* sp.,
 311 *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and
 312 *Enterobacter* sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus*, *Clostridium*, and
 313 *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

314

315 **Effect of biogas sludge**

316 The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16
 317 WAA), total dry weight (12 and 16 WAA), **nutrient uptake (N and P)**, and the crop growth rate
 318 of upland rice at 8 to 12 WAA. However, it did not have a significant effect on nutrient content
 319 (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase
 320 in plant height, total dry weight, nutrient uptake in terms of nitrogen and phosphorus, and also
 321 crop growth rate of upland rice on Ultisols with a higher dose of biogas sludge of 630
 322 ml/polybag at the end of this study (16 WAA). In contrast, the total fresh weight ~~had an~~
 323 ~~increas~~ed along with the ~~increase at the~~higher dose of biogas sludge to 315 ml/polybag ~~and~~
 324 then decreased at the dose of 630 ml/polybag. This result is supported the biogas sludge had
 325 chemical characteristics such as pH (7.41), total N (0.051%), available P (0.013%), organic C
 326 (0.14%), total K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria

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327 (29.4×10⁵ CFU ml⁻¹) and total phosphate solubilizing bacteria (7.0×10⁴ CFU ml⁻¹) (Tab. 2). The
328 organic C content and the total population of nitrogen-fixing and phosphate solubilizing
329 bacteria from the biogas sludge could increase the nutrient uptake in terms of nitrogen and
330 phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2).
331 Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant
332 height, biomass, and crop growth rate of the upland rice. A similar result was reported by
333 Mustamu and Triyanto (2020) who determined the macro and micronutrients from the biogas
334 sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10⁴
335 and 42×10⁴ CFU ml⁻¹, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total
336 phosphate solubilizing bacteria (1.6 to 2.5 CFU ml⁻¹) was significantly higher compared to
337 nitrogen-fixing bacteria (0.5-1.4 CFU ml⁻¹) showing a significant increase in nutrient
338 concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller
339 and Müller (2012) reported that an increase in concentrations of NH₄⁺-N ranged from 45 to
340 80% in the anaerobic waste.

341

342 **Interaction of selected superior bacterial isolates and biogas sludge**

343 The interaction of biogas sludge and superior bacterial isolates only significantly increased the
344 crop growth rate of upland rice on Ultisols at 12-16 WAA, but it did not have a significant
345 effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose
346 of 630 ml/polybag (B1S3) showed the highest crop growth rate of upland rice compared to
347 other interactions and was 5.76-times greater compared to the control. This was caused by the
348 application of biogas sludge that could have increased soil organic matter and the total
349 population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%),
350 total nitrogen-fixing bacteria (29.4×10⁵ CFU ml⁻¹), and total phosphate solubilizing bacteria
351 (7.0×10⁴ CFU ml⁻¹) (Tab. 2) that could improve soil quality and support the crop growth rate.

352 This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge
353 in the long term significantly increases the organic matter contents in the soil, causing a
354 decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production
355 of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014);
356 Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had
357 advantages in the plant such as availability of nutrients that are balanced for plant health. It also
358 stimulates nutrient mobilization that can increase soil biological activity and the availability of
359 microbial food to encourage the growth of beneficial microorganisms, increasing the soil
360 organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019)
361 indicated that the interaction of biogas sludge+biofertilizer (36 ml+10 L ha⁻¹) significantly
362 increased the plant height, number of leaves, and capsaicin content in chili pepper compared
363 to a single treatment of biogas sludge and biofertilizer.

364 **Conclusions**

365 The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the ~~nutrient~~-uptake
366 of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice
367 on Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge
368 significantly increased plant height (14.81%), total dry weight (50.55%), ~~nutrient~~-uptake of
369 nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland
370 rice on Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of
371 biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the
372 highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with
373 the dose of biogas sludge only significantly increased the crop growth rate of upland rice on
374 Ultisols 5.76 times with the highest increase found with B1S3.

375 **Conflict of interest statement**

376 The authors declare that there is no conflict of interest regarding the publication of this article.

377 **Author's contributions**

378 All authors formulated the overarching research goals and aims, provided the study materials,
379 developed or designed the methodology. NEM analyzed and interpreted the study data. NEM
380 and MS wrote the initial draft, managed and coordinated the research activity in the field, and
381 collected the data. ZN and I verified the overall reproducibility of results and the other research
382 outputs. All authors conducted the critical review/commentary/revision of the manuscript.

383

384 **Literature cited**

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y
la nutrición del arroz de secano

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ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P < 0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination within biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

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Reviewer 3 wrote: The title don't show effectively the results of investigation. I recommend the title: Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice. Regarding the term "superior bacterial isolates" reviewer 3 wrote: What the authors wanted to say? I don't understand this term. This reviewer also wrote: The authors evaluated only the effect of bacteria and biogas sludge on rice growth. They evaluated also the effect of these treatments on rice nutrition! Finally, regarding the word "ultisols" reviewer 3 wrote: Why is it necessary to specify the soil type?

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We agree with reviewer 3 suggestion, the title of the manuscript doesn't need to have the word superior and soil type because it is clarified in the materials and methods

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Corresponding author is required by my promotor, so that this manuscript can be used in completing my doctoral education

I appeal to the editor, the correspondent of the author remains Zulkifli Nasution (zulnasution@usu.ac.id)

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30 **Key words:** acidic soil, crop growth rate, dosage, sludge potential.

31

32 **RESUMEN**

33 El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados
 34 (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes en
 35 el arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos
 36 factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor
 37 utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de
 38 nitrógeno (N3), B2 = aislamiento de bacterias solubilizantes de fosfato (P7), B3 = combinación
 39 de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin
 40 tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados
 41 por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P < 0.05$. Los
 42 resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en
 43 un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las
 44 dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de
 45 la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas.
 46 La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó
 47 significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7
 48 y su combinación dentro de lodos de biogás de 630 ml/polybag tiene el potencial de aumentar
 49 el TCC de arroz de tierras altas en suelos ácidos.

50

51 **Palabras clave:** suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

52

53 **Introduction**

Commented [EDTR7]: Reviewer 3 wrote: The abstract does not show the investigation novelty and the importance of results to agricultural management.

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This sentence has been written at the end of the abstract

Commented [EDTR10]: Reviewer 3 wrote: Only state the concentration value.

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Commented [EDTR12]: Reviewer 3 wrote: It is necessary to include relevant information about acid soil and the representative upland rice in Indonesia and its impact in the economy of the country.
Furthermore, the authors must enhance the investigation novelty.

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54 Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and
 55 has a high nutrient content that can be used as organic fertilizer to increase soil fertility and the
 56 plants yield (Adela *et al.*, 2014). The following characteristics of the biogas sludge from palm
 57 oil waste have been reported: total N of 490 mg L⁻¹, total P of 110 mg L⁻¹, total K of 1.9 mg L⁻¹
 58 (Lubis *et al.*, 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al.*, 2019), and NH₃-N of 91 -112
 59 mg L⁻¹ (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest
 60 bacterial population of 7.21×10⁷ cells per ml and the lowest one of 3.15×10⁷ cells per ml
 61 (Alvionita *et al.*, 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas
 62 sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase
 63 the availability of nitrogen and phosphate in soils.

64 The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria
 65 has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported
 66 that phosphate solubilizing bacteria play an important role in increasing soil fertility, and plant
 67 yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different
 68 *Bacillus* species, such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B.*
 69 *mycooides*, *B. coagulans*, *B. chitinolyticus*, and *B. subtilis* as phosphate solubilizing
 70 microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus*
 71 *cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant
 72 bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and
 73 clone library methods, *i.e.*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

74 The application of bacteria from biogas sludge has never been reported in Indonesia for
 75 improving upland rice growth on acidic soils, including Ultisols. According to the Pusat
 76 Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area
 77 in Indonesia covered by Ultisols was 45.8 million ha, or 24% of the total area of Indonesia.
 78 Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in

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79 Indonesia was 15,712,025 ha with a yield of 81,148,617 t ha⁻¹ in 2017 and the contribution of
 80 upland rice yield reached 4.66% (Kementerian Pertanian, 2017). The yield contribution of
 81 upland rice was classified as low and, therefore, it is necessary to find options in order to
 82 increase it. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas
 83 sludge to increase the availability of nitrogen and phosphate, and the growth response of upland
 84 rice due to application of the biogas sludge and selected isolates in Ultisols. The study aimed
 85 to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their
 86 interaction on the mineral nutrition of the upland rice grown in Ultisols.

88 Materials and methods

89 Study area

90 The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake)
 91 were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The
 92 bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N;
 93 98°38.898' E; altitude 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from
 94 October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity,
 95 was 82% and average rainfall was 228.5 mm per month.

96 Preparation of medium and upland rice seeds

97 The medium to grow upland rice plants used the Ultisols from the Simalingkar area, Medan
 98 Tuntungan Subdistrict, Medan City, at a depth of 0 to 20 cm. One hundred g of soil samples
 99 were taken and analyzed for chemical characteristics such as pH using H₂O, organic C by
 100 Walkley-Black, available P by Bray-II, total N using Kjeldahl method, and cation exchange
 101 capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH 7 method (Tab.
 102 1). The soil was sterilized by drying at 100°C for 2 h. For preventing heat from the sterilization

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Commented [EDTR19R18]: The authors did not reply.

Commented [NEM20R18]: we apologize for this typo, we mean to measure available P in soil and uptake P in plants

Commented [EDTR21]: The Editor in Chief wrote: Please, provide the bibliographic reference of the manual used for these analyses

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103 process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm).
 104 A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc., Medan (Indonesia) at a dose of
 105 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza*
 106 *sativa* L.) were of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural
 107 Research and Development were soaked in water for 24 h, followed by the application of the
 108 fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with
 109 two seeds per polybag at a depth of 2 cm.

110 **TABLE 1.** Chemical characteristics of the Ultisols soil samples after sterilization at 100°C.
 111

Chemical characteristics	Methods*	Value	Category*
Soil pH (H ₂ O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg ⁻¹)	Spectrophotometry	870.25	Very high
CEC (meq 100 g ⁻¹)	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
Exchangeable cations			
K (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

112 *Criteria for pH (H₂O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg
 113 kg⁻¹ (very high); Cation exchange capacity (CEC) = 25-40 meq 100 g⁻¹ (high); base saturation <20% (very low);
 114 exchangeable K= 0.60-1.00 meq 100 g⁻¹ (high); exchangeable Ca <2 meq 100 g⁻¹ (very low); exchangeable Mg
 115 <0.4 meq 100 g⁻¹ (very low); exchangeable Na <0.1 meq 100 g⁻¹ (very low); exchangeable Al <5% (very low)
 116 (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).
 117

118 Preparation of superior bacterial isolates suspension and biogas sludge

119 A total of 1 ml of the bacterial isolate suspension obtained from the characteristic stage was put
 120 into a test tube containing 9 ml of distilled water and homogenized. The dilution was made to
 121 10⁻⁵. A total of 0.1 ml of the suspension from the last dilution was spread over the James
 122 nitrogen free malat bromothymol blue (JNFB) medium for the nitrogen-fixing bacterial isolates
 123 test and Pikovskaya (PVK) medium for the phosphate solubilizing bacteria isolates. The culture
 124 medium was incubated for 2 to 3 d at room temperature. The nitrogen-fixing bacterial isolate
 125 test was characterized by the presence of colonies growing on the JNFB medium. The growth

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126 of phosphate solubilizing bacterial isolates was indicated by a halo zone around the microbial
 127 colonies on the Pikovskaya medium. Seven nitrogen-fixing and seven phosphate-solubilizing
 128 isolates were found to produce total-N and available-P. The isolates that showed the highest
 129 phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing
 130 bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.*
 131 (2021a, 2021b).

132 The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District,
 133 North Sumatra Province, Indonesia. The procedure for processing biogas sludge can be
 134 explained that the palm oil mill removes POME (Palm Oil Mill Effluent) waste from the second
 135 pond which has been mixed with oil and then separated at an optimal temperature of 35°C.
 136 Liquid waste is pumped into the receiver tank with a volume of 10 m³ and filtered on a fiber
 137 tank screen for separated the solid waste such as fiber and others. Liquid waste from the receiver
 138 tank is pumped to the tower tank. Then it is distributed evenly to the fixed tank with a
 139 temperature of 35 to 37°C and a flow rate of 20 to 30 m³/h. The biogas sludge is taken from a
 140 fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of
 141 the plants at one week after planting. Biogas sludge samples at a 500 ml volume were used to
 142 analyze the chemical and biological characteristics (Tab. 2).

143 **TABLE 2.** The chemical and biological characteristics of the biogas sludge.
 144

Characteristics	Method*	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L ⁻¹)	Spectrophotometry	4547.8
Biological oxygen demand (mg L ⁻¹)	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (ppm)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml ⁻¹)	Plate count	29.4×10 ⁵

Commented [EDTR27]: Reviewer 3 wrote: I think that the authors must give more details about how biogas sludge was taken.

Commented [NEM28R27]: The authors has been added the processing for taking biogas sludge

Commented [EDTR29]: Reviewer 3 wrote: The authors must say how the chemical and biological characteristics of biogas sludge were determined, or make a bibliography cite.

The Editor in Chief also wrote: Please, provide the bibliographic reference of the manual used for the biogas sludge analysis.

Commented [EDTR30R29]: The authors did not add a citation.

Commented [NEM31R29]: The authors has been added the bibliography cite

Commented [EDTR32]: Reviewer 1 asked: Is it correct? It can be a very high amount of sodium.

Commented [EDTR33R32]: The authors did not answer.

Commented [NEM34R32]: The results of the analysis from the laboratory of 44.41 ppm

Total phosphate solubilizing bacteria (CFU ml⁻¹) Plate count 7.0×10⁴

Note: *laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

145

146

147 Treatment application

148 This study used a randomized block design with two factors and seven replicates. The first
149 factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial
150 isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates
151 N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was dose of biogas sludge

152 (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge

153 based on the dose of liquid organic fertilizer at the oil palm was 126 m³ ha⁻¹ equal to 126,000

154 L ha⁻¹ (Sutarta *et al.*, 2003), then converted to soil weight per polybag (Eq. 1). Each replicate

155 was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop

156 growth rate (CGR).

$$157 \text{ Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \quad (1)$$

$$158 = \frac{26,000 \text{ L ha}^{-1}}{2,000,000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 630 \text{ ml}$$

159 Parameters and data analysis

160 The observations of the variables were conducted by measuring the growth of upland rice (plant
161 height, and total fresh and dry weight), contents and uptake of N and P in the shoots, and CGR.

162 The plant height was measured from the base of the roots to the tip of leaves using a meter, and
163 the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots

164 + shoots) was measured after using an oven (model VS-1202D3, Vision Scientific Co., Korea)

165 at 60°C for 48 h and weighed using the analytical scales. A 200 g sample of the second leaf

166 from the shoots was collected and analyzed to determine the N content using the Kjeldahl and

167 the P content was estimated using the destruction method through dry ashing. The N and P

Commented [EDTR35]: Reviewer 3 wrote: Why the authors use these biogas sludge concentrations. I think it is important!

Commented [EDTR36R35]: The authors did not reply.

Commented [NEM37R35]: The author has answered based on the dose of liquid organic fertilizer (yellow mark)

Commented [EDTR38]: What do you mean by disassembled?

Commented [NEM39R38]: harvested

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Commented [EDTR40]: The Editor in Chief asked: individual culm?

Commented [EDTR41R40]: The authors did not answer.

Commented [NEM42R40]: Clump

168 uptake were measured using Equation 2. The CGR was calculated as the dry weight related to
 169 the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$170 \quad \text{Nutrient uptake} = \text{nutrient content in the shoots} \times \text{total dry weight} \quad (2)$$

$$171 \quad \text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W_2 - W_1}{t_2 - t_1} \quad (3)$$

172 where:

173 CGR = crop growth rate;

174 W1 = dry weight per unit area at t1;

175 W2 = dry weight per unit area at t2;

176 t1 = first sampling;

177 t2 = second sampling;

178 The parameters of the second phase of the study were analyzed by an ANOVA and if the
 179 treatment had a significant effect, followed by Duncan's multiple range test at $P < 0.05$ using
 180 SPSS v.20 software (IBM, 2011).

181

182 **Results**

183 **Effect of bacterial isolates and biogas sludge on upland rice growth**

184 **Plant height of upland rice**

185 The effect of biogas sludge application was significant on the plant height of upland rice at 8,
 186 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant
 187 effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1). A significant increase
 188 in plant height of upland rice was observed with higher doses of biogas sludge of 630
 189 ml/polybag at 8, 12, and 16 WAA with the highest increase of 14.81% compared to the control
 190 at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the
 191 interaction of B2S3 showed the highest in plant height of upland rice by 2.94 and 22.06%,
 192 respectively, compared to the control.

Commented [EDTR43]: Reviewer 1 wrote: I recommend that the results part be restructured in its figures and tables. It could only show figures that show a significant difference, without showing those that do not have a significant difference. Reviewer 3 wrote: It is necessary write Results and discussion again in other form. All the information of this section must be re-organized. Account of the statistical analysis made and some information in figures and tables is irrelevant. The figures have repeated information.

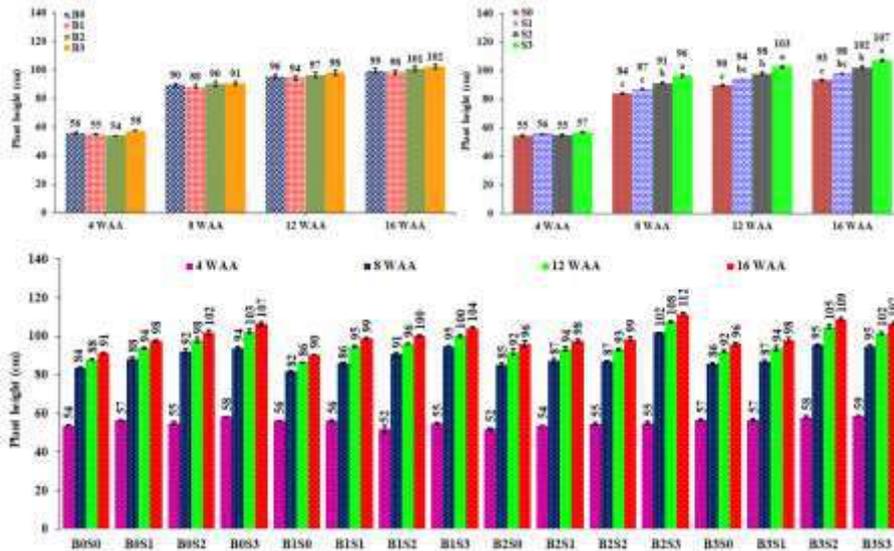
Commented [EDTR44R43]: The authors did not reply to these comments.

Commented [NEM45R43]: According to the authors opinion, the author is still displayed because the results show that there is an effect of treatment a, b, and interaction compared to the control even though the results of the treatment are not significantly different

Commented [EDTR46]: Reviewer 3 wrote: I consider that it is necessary to write again Results and discussion in other form. The information of this section must be re-organized in two aspects: (1) Effect of bacterial isolates and biogas sludge on upland rice growth. (2) Effect of bacterial isolates and biogas sludge on upland rice nutrition. The first point could include:
 - Plant height of upland rice
 - Biomass of upland rice
 - Crop growth rate of upland rice
 The second point could include:
 - Content of total-N and available-P nutrient of upland rice
 - Uptake of total-N and available-P nutrient of upland rice
 Furthermore, for both points the analysis made from lines 249-332 must be undertaken.

Commented [EDTR47R46]: It seems the authors reorganized these sections following the reviewer's suggestion. However, they did not reply to the comment.

Commented [NEM48R46]: The author has followed the reviewer's suggestion



193 **FIGURE 1.** Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of
 194 upland rice at 4, 8, 12, and 16 WAA. Values followed by the different letter in the graph significantly differed
 195 according to the Duncan test at $P < 0.05$. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5;
 196 S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3
 197 = isolates N3+P7).

198

199 Biomass of upland rice

200 The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12,
 201 and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect
 202 on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

203 **TABLE 3.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight
 204 (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).
 205

Treatments	Total fresh weight \pm standard error (g)			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 \pm 0.21	169.31 \pm 8.90	215.27 \pm 8.42	229.82 \pm 8.94
B1	3.12 \pm 0.12	194.50 \pm 9.35	235.08 \pm 10.32	252.02 \pm 10.22
B2	4.52 \pm 0.23	162.89 \pm 11.15	201.85 \pm 9.89	230.70 \pm 9.28
B3	3.30 \pm 0.25	173.91 \pm 12.55	220.40 \pm 15.96	245.03 \pm 16.32
Biogas sludge (S)				
S0	3.72 \pm 0.24	144.07 \pm 9.37 b	182.67 \pm 7.14 b	197.56 \pm 6.58 b
S1	3.58 \pm 0.27	153.41 \pm 7.93 b	190.70 \pm 8.90 b	215.65 \pm 7.03 b
S2	3.64 \pm 0.27	199.68 \pm 10.30 a	258.70 \pm 9.63 a	280.15 \pm 9.25 a
S3	4.15 \pm 0.25	203.45 \pm 1.36 a	240.52 \pm 2.81 a	264.21 \pm 2.42 a
Interactions (B x S)				
B0S0	4.99 \pm 0.33	124.08 \pm 5.60	185.64 \pm 3.32	192.78 \pm 2.96

Commented [EDTR49]: Please add these graphs as independent files to ensure they have the appropriate resolution and quality.

Commented [NEM50R49]: We will send excel file of this figure

Commented [EDTR51]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures. The authors could explore the possibility that gives them not interaction between the factors for discussing the result.

Commented [EDTR52R51]: The authors did not reply to this comment.

Commented [NEM53R51]: According to the authors opinion, the author is still displayed because the results show that there is an effect of interaction compared to the control even though the results of the treatment are not significantly different

Commented [EDTR54]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the interaction.

Commented [EDTR55R54]: The authors did not reply to this comment.

Commented [NEM56R54]: According to the authors opinion, the author is still displayed because the results show that there is an effect of interaction compared to the control even though the results of the treatment are not significantly different

B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

206 Values followed by the different letter in the column significantly differed according to the Duncan test at $P < 0.05$.
 207 ns - not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315;
 208 S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates
 209 N3+P7).

210
 211 A significant increase in the total fresh weight of upland rice was observed with the higher dose
 212 of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared
 213 to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed
 214 the highest in the total fresh weight of upland rice with 9.66 and 68.55%, respectively,
 215 compared to the control.

216 The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and
 217 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the
 218 total dry weight of upland rice at 4 -16 WAA (Tab. 4).

219 **TABLE 4.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight
 220 (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).
 221

Treatments	Total dry weight ± standard error (g)			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				

Commented [EDTR57]: Reviewer 1 wrote: This is not an interaction assessment, it is the treatments. In the ANOVA of this trial there are 16 treatments and 112 experimental units. The sources of variation evaluated would be (treatments, bacteria, sludge, and bacteria x sludge interaction). And there may be a significant difference in treatments without any interaction between the factors.

Commented [EDTR58R57]: The authors did not reply to this comment.

Commented [NEM59R57]: The data has been rechecked and reprocessed, but the results are the same as the table

Commented [EDTR60]: Reviewer 3 wrote: If all the treatments (B x S) are NS, there was not interaction between the factors. Then it is unnecessary to show the interaction.

Commented [EDTR61R60]: The authors did not reply to this comment.

Commented [NEM62R60]: According to the authors opinion, the author is still displayed because the results show that there is an effect of interaction compared to the control even though the results of the treatment are not significantly different

B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

222 Values followed by the different letter in the column significantly differed according to the Duncan test at $P < 0.05$.
 223 ns= not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315;
 224 S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates
 225 N3+P7).
 226

227 A significant increase in total dry weight of upland rice was observed with the increase in the
 228 dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55%
 229 compared to the control. Although the effect was not significant, B1 and the interaction of B3S3
 230 showed the highest in the total dry weight of upland rice with 20.84 and 81.53%, respectively,
 231 compared to the control.
 232

233 Crop growth rate of upland rice

234 The effect of superior bacterial isolates, biogas sludge, and their interactions significantly
 235 increased the crop growth rate of upland rice at 12 to 16 WAA, but it did not have a significant
 236 effect at 4-8 and 8-12 WAA (Tab. 5).

237 **TABLE 5.** Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the
 238 upland rice 4, 8, 12, and 16 weeks after the application (WAA).
 239

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
	4-8 WAA				
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%

Commented [EDTR63]: Reviewer 1 wrote: Idem table 3

Commented [EDTR64R63]: The authors did not reply to this comment.

Commented [NEM65R63]: The data has been rechecked and reprocessed, but the results are the same as the table

Commented [EDTR66]: Reviewer 1 wrote: The interaction was insignificant

Commented [EDTR67R66]: The authors did not reply to this comment.

Commented [NEM68R66]: The results showed that there was an interaction effect compared to the control although it was not significantly different

Commented [EDTR69]: Reviewer 1 wrote: This table can generate confusion, take into account the recommendations of the previous tables.

Commented [EDTR70R69]: The authors did not reply to this comment.

Commented [NEM71R69]: The author made a more efficient table for crop growth rates to be more varied see readers

8-12 WAA					
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
12-16 WAA					
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

240 Values followed by the different letter in the column significantly differed according to the Duncan test at $P < 0.05$.
 241 ns - not significant; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315;
 242 S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = isolates
 243 N3+P7).
 244

245 The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth
 246 rate for upland rice at 12 to 16 WAA by 129.44% compared to the control. The isolates B1-B3
 247 significantly increased the crop growth rate of upland rice with the highest increase for B2 of
 248 181.45% compared to the controls at 12 to 16 WAA. The interaction of the B1S3 significantly
 249 increased the crop growth rate of upland rice, showing values 5.76 times greater than those of
 250 the control.

251

252 **Effect of bacterial isolates and biogas sludge on upland rice nutrition**

253 **Content of N and P in the upland rice**

254 The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a
 255 significant effect on the content of N and P in the upland rice (Fig. 2). The biogas sludge doses
 256 of 315 and 630 ml/polybag (S2 and S3) explained that the contents of P and N in the plant tissue
 257 of upland rice were 33.33 and 4.53% higher, respectively, compared to the control. The isolate
 258 B2 showed the highest content of N in the plant tissue of upland rice with values 1.63% higher
 259 than those of the control; however, all isolates (B1-B3) showed a similar level of P in the plant
 260 tissue of upland rice compared to the control.

261

262 **Uptake of N and P in the upland rice**

Commented [EDTR72]: Reviewer 1 asked: how it was calculated and where it compares with the other treatments

Commented [EDTR73R72]: The authors did not reply.

Commented [NEM74R72]: These results were compared with the control of the interaction (B0S0)

Commented [EDTR75]: Reviewer 1 wrote: In this analysis, it is necessary to justify the reason why the bacteria achieve a greater absorption of nutrients such as nitrogen and a higher growth rate, but do not achieve a greater height, dry weight and content of N and P.

Commented [EDTR76R75]: The authors did not reply to this comment.

Commented [EDTR77]: This expression does not make sense. The right phrase is content of N and P.

Commented [NEM78R77]: It has been changed

Commented [EDTR79]: The Editor in Chief asked: Do you refer to the contents of available P in soil or in the plant tissue? If plant tissue, what was the analytical method to determine the "available" P in the plant tissues?

Commented [EDTR80R79]: The authors did not answer.

Commented [NEM81R79]: The content P in plant tissue and analyzed using Dry Ashing with Spectrophotometer

Commented [EDTR82]: Reviewer 1 wrote: there is no significant difference should not be analyzed, the differences are given only by chance.

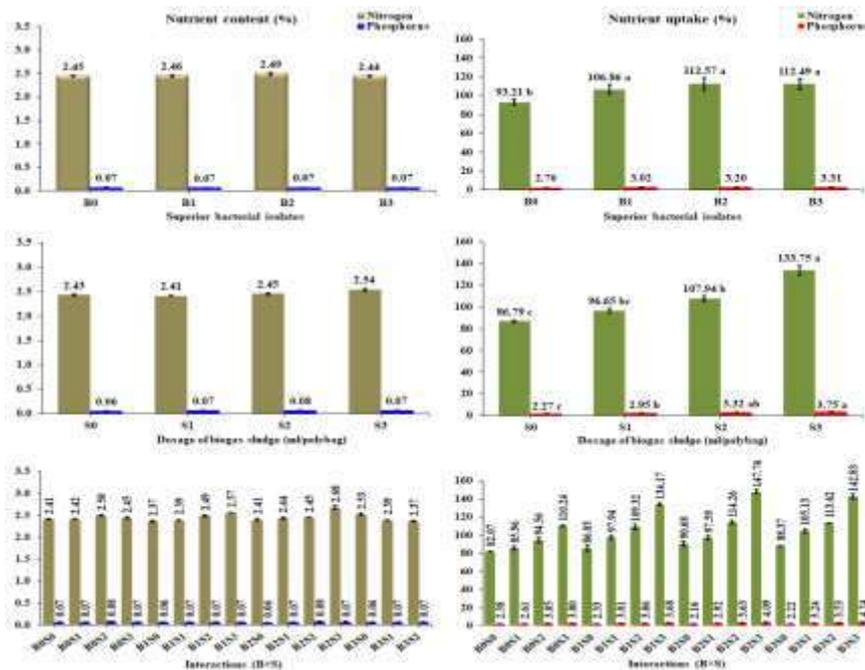
Commented [EDTR83R82]: The authors did not reply to this comment.

Commented [NEM84R82]: The results showed that treatment biogas sludge, bacterial isolates, and interaction had an effect compared to the control although not significantly different

263 The effect of biogas sludge significantly increased in the uptake of N and P. The superior
 264 bacterial isolates significantly increased in the uptake of nitrogen. The interaction of biogas
 265 sludge with superior bacterial isolates did not show a significant effect on the uptake of N and
 266 P in the upland rice (Fig. 2).

267 A significant increase in the uptake of N and P in upland rice was observed with a higher dose
 268 of biogas sludge of 630 ml/polybag, with the highest increases of 54.11 and 65.20%,
 269 respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased
 270 the uptake of N in the upland rice with the highest increase with the B2 of 20.77% compared to
 271 the control. Although the effect was not significant, B3 showed the highest uptake of P in the
 272 upland rice of 19.93% compared to the control.

273



274 **FIGURE 2.** The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient
 275 content and uptake of N and P in the upland rice. Values followed by different letters significantly differed
 276 according to the Duncan test at $P < 0.05$. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5;

Commented [EDTR85]: The Editor in Chief wrote: This phrase makes no physiological sense. Please check out the English spelling. What does the "nutrient uptake of total" element stand for?

You can use the phrase "nutrient uptake" when referring to the nutrients the plant absorbs in general. Nutrient uptake of N and P is redundant and does not make any sense. Please use only "uptake of N and/or P"

Commented [NEM86R85]: We agree with the editor's suggestion, and we've changed it

Commented [EDTR87]: Please add the names of the Y axes. They should appear next to the axes. Please add these graphs as independent files to ensure they have the appropriate quality and resolution.

Commented [NEM88R87]: We will send excel file of this figure

Commented [EDTR89]: Reviewer 3 wrote: If all the treatments (B x S) are NS then there was not interaction between the factors. Then it is unnecessary to show the third figure. I recommend to show only first and second figures.

Commented [EDTR90R89]: The authors did not reply to this comment.

Commented [NEM91R89]: The results showed that there was an interaction effect compared to the control although it was not significantly different

277 S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3
278 = N3+P7 isolates).
279

280 Discussion

281 Effect of selected superior bacterial isolates

282 The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of
283 nitrogen and crop growth rate of upland rice on Ultisols at 12 to 16 WAA, but it did not have a
284 significant effect on plant height, total fresh weight, total dry weight, content (N and P) in leaf
285 tissue, uptake of phosphorus, and crop growth rate of upland rice at 4 to 8 and 8 to 12 WAA.
286 The superior bacterial isolates (N3, P7, and N3+P7) could increase the uptake of nitrogen in
287 upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2).
288 Similar results are also shown in Table 5, where can be observed that the crop growth rate of
289 upland rice at 12 to 16 WAA has increased 2.57, 2.81, and 2.39 times, respectively due to the
290 selected superior bacterial isolates (N3, P7, N3+P7), compared to the control. The results
291 indicate that the ability of a single P7 bacterial isolate was greater in increasing the nitrogen
292 and crop growth rate of upland rice compared to a single N3 isolate and the combination of
293 N3+P7 isolates. This was due to the presence of several organic acids and hormones produced
294 by P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result
295 is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial
296 isolate (P7) from the biogas sludge contains organic acids such as lactic, oxalic, acetic, and
297 citric acids, and had the highest ability to solubilize phosphate from calcium triphosphate and
298 rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control.
299 Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly
300 increased with the application of bio fertilization with *Bacillus cereus*; this was due to the
301 production of organic acids and other chemicals such as citric, tartaric, and oxalic acids that can
302 stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the

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303 increase in vegetative growth and total biomass was due to increased photosynthesis,
304 translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus*
305 *cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic
306 acids. Ferrara *et al.* (2012) reported that the hormone gibberellin and IAA, can increase plant
307 growth under stressful conditions. Kang *et al.* (2014) said that the plant growth-promoting
308 bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and
309 phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that
310 bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp.,
311 *Thiomargarita* sp., *Clostridium* sp., *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp.,
312 *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al.*
313 (2017) also reported abundance of *Bacillus*, *Clostridium*, and *Enterobacter* spp. in an anaerobic
314 digester of wastewater when producing biogas.

315

316 **Effect of biogas sludge**

317 The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16
318 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and the crop growth rate of upland
319 rice at 8 to 12 WAA. However, it did not have a significant effect on content (N and P) in leaf
320 tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height,
321 total dry weight, uptake in terms of nitrogen and phosphorus, and also crop growth rate of
322 upland rice on Ultisols with a higher dose of biogas sludge of 630 ml/polybag at the end of this
323 study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of
324 biogas sludge to 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result
325 is supported the biogas sludge had chemical characteristics such as pH (7.41), total N (0.051%),
326 available P (0.013%), organic C (0.14%), total K (0.18%), and biological characteristics such
327 as total nitrogen-fixing bacteria (29.4×10^5 CFU ml⁻¹) and total phosphate solubilizing bacteria

328 (7.0×10^4 CFU ml⁻¹) (Tab. 2). The organic C content and the total population of nitrogen-fixing
329 and phosphate solubilizing bacteria from the biogas sludge could increase the uptake in terms
330 of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630
331 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes
332 and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result
333 was reported by Mustamu and Triyanto (2020) who determined the macro and micronutrients
334 from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing
335 bacteria of 480×10^4 and 42×10^4 CFU ml⁻¹, respectively. Ndubuisi-Nnaji *et al.* (2020) reported
336 that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml⁻¹) was significantly higher
337 compared to nitrogen-fixing bacteria (0.5-1.4 CFU ml⁻¹) showing a significant increase in
338 nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors.
339 Möller and Müller (2012) reported that an increase in concentrations of NH₄⁺-N ranged from
340 45 to 80% in the anaerobic waste.

341

342 **Interaction of selected superior bacterial isolates and biogas sludge**

343 The interaction of biogas sludge and superior bacterial isolates only significantly increased the
344 crop growth rate of upland rice on Ultisols at 12-16 WAA, but it did not have a significant
345 effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose
346 of 630 ml/polybag (B1S3) showed the highest crop growth rate of upland rice compared to
347 other interactions and was 5.76 times greater compared to the control. This was caused by the
348 application of biogas sludge that could have increased soil organic matter and the total
349 population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%),
350 total nitrogen-fixing bacteria (29.4×10^5 CFU ml⁻¹), and total phosphate solubilizing bacteria
351 (7.0×10^4 CFU ml⁻¹) (Tab. 2) that could improve soil quality and support the crop growth rate.
352 This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge

353 in the long term significantly increases the organic matter contents in the soil, causing a
354 decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production
355 of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014);
356 Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had
357 advantages in the plant such as availability of nutrients that are balanced for plant health. It also
358 stimulates nutrient mobilization that can increase soil biological activity and the availability of
359 microbial food to encourage the growth of beneficial microorganisms, increasing the soil
360 organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019)
361 indicated that the interaction of biogas sludge+biofertilizer (36 ml+10 L ha⁻¹) significantly
362 increased the plant height, number of leaves, and capsaicin content in chili pepper compared to
363 a single treatment of biogas sludge and biofertilizer.

364 **Conclusions**

365 The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of
366 nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice on
367 Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge
368 significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen
369 (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice on
370 Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas
371 sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest
372 increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose
373 of biogas sludge only significantly increased the crop growth rate of upland rice on Ultisols
374 5.76 times with the highest increase found with B1S3.

375 **Conflict of interest statement**

376 The authors declare that there is no conflict of interest regarding the publication of this article.

377 **Author's contributions**

378 All authors formulated the overarching research goals and aims, provided the study materials,
379 developed or designed the methodology. NEM analyzed and interpreted the study data. NEM
380 and MS wrote the initial draft, managed and coordinated the research activity in the field, and
381 collected the data. ZN and I verified the overall reproducibility of results and the other research
382 outputs. All authors conducted the critical review/commentary/revision of the manuscript.

383

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de tierras altas

Novilda Elizabeth Mustamu¹, Zulkifli Nasution^{2*}, Irvan³, and Mariani Sembiring²

ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P < 0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination with biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes del arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizadoras de fosfato (P7), B3 = combinación de aislamientos (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P < 0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación con lodos de biogás de 630 ml/polybag tiene el potencial de aumentar la TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

Introduction

Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L⁻¹, total P of 110 mg L⁻¹, total K of 1.9 mg L⁻¹ (Lubis *et al.*, 2014), C/N 8; 0.14% N,

1.12% C (Tepsour *et al.*, 2019), and N-NH₃ of 91-112 mg L⁻¹ (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10⁷ cells ml⁻¹ and the lowest of 3.15×10⁷ cells ml⁻¹ (Alvionita *et al.*, 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

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The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B. mycoides*, *B. coagulans*, *B. chitinolyticus*, and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e.*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area in Indonesia covered by Ultisols is 45.8 million ha or 24% of the total area of the country. Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in Indonesia is 15,712,025 ha with a yield of 81,148,617 t in 2017, and the contribution of upland rice yield is 4.66% (Kementerian Pertanian, 2017). The yield contribution of upland rice is classified as low and, therefore, it is necessary to find options to increase it. Therefore, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate and, thus, the growth response of upland rice in Ultisols. This study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N, 98°38.898' E, and altitude of 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity was 82%, and the average rainfall was 228.5 mm per month.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants was the Ultisol soil from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, collected at a depth of 0 to 20 cm. One hundred g of soil sample were taken and analyzed for chemical characteristics of pH H₂O, organic C by Walkley-Black, available P by Bray-II, total N using the Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. To prevent heat from the sterilization process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc. (Medan, Indonesia) at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural Research and Development were soaked in water for 24 h, followed by the application of the fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisol soil samples after sterilization at 100°C.

Chemical characteristics	Method	Value	Category
Soil pH (H ₂ O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg ⁻¹)	Spectrophotometry	870.25	Very high
CEC (meq 100 g ⁻¹)	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
Exchangeable cations			
K (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

Criteria for pH (H₂O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg⁻¹ (very high); cation exchange capacity (CEC) = 25-40 meq 100 g⁻¹ (high); base saturation <20% (very low); exchangeable K = 0.60-1.00 meq 100 g⁻¹ (high); exchangeable Ca <2 meq 100 g⁻¹ (very low); exchangeable Mg <0.4 meq 100 g⁻¹ (very low); exchangeable Na <0.1 meq 100 g⁻¹ (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

One ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water, homogenized and then diluted to 10⁻⁵. A total of 0.1 ml of the suspension from the last dilution was spread over James nitrogen free malat bromothymol blue

(JNFB) medium (Kirchhof *et al.*, 1997) for the nitrogen-fixing bacterial isolates test and Pikovskaya (PVK) medium (Pikovskaya, 1948) for the phosphate solubilizing bacteria isolates. The culture medium was incubated for 2 to 3 d at 37°C. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates was indicated by a halo zone around the microbial colonies on the PVK medium. Seven nitrogen-fixing and seven phosphate-solubilizing isolates were found to produce total N and available P. The isolates that showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.* (2021a; 2021b).

The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. The procedure for processing biogas sludge was the following: the palm oil mill removed the palm oil mill effluent (POME) waste from the second pond which has been mixed with oil and then separated at an optimal temperature of 35°C. Liquid waste was then pumped into the receiver tank of 10 m³ volume and filtered on a fiber tank screen to separate solid waste such as fiber and other materials. Liquid waste from the receiver tank was further pumped into the tower tank. Then, it was distributed evenly to the fixed tank at a temperature of 35 to 37°C and a flow rate of 20 to 30 m³/h. Finally, the biogas sludge was taken from the fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at

one week after planting. Biogas sludge samples of 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was the dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m³ ha⁻¹ equal to 126,000 L ha⁻¹ (Sutarta *et al.*, 2000), then converted to soil weight per polybag (Eq. 1). Each replicate was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop growth rate (CGR).

$$\begin{aligned} \text{Biogas sludge} &= \frac{\text{Dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \quad (1) \\ &= \frac{26,000 \text{ L ha}^{-1}}{2,000,000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 130 \text{ ml} \end{aligned}$$

Plant growth variables and data analysis

The growth variables were assessed by measuring the growth of upland rice (plant height, and total fresh and dry weights), contents and uptake of N and P in the aerial

TABLE 2. Chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L ⁻¹)	Spectrophotometry	4547.8
Biological oxygen demand (mg L ⁻¹)	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L ⁻¹)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml ⁻¹)	Plate count	29.4×10 ⁵
Total phosphate solubilizing bacteria (CFU ml ⁻¹)	Plate count	7.0×10 ⁴

Laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute) (2009).

parts, and crop growth rate (CGR). The plant height was measured from the root apex to the tip of leaves using a measuring tape, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was obtained after plant drying in an oven (VS-1202D3, Vision Scientific Co., Korea) at 60°C for 48 h and weighed using analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl method, and the P content was recorded using the destruction method through dry ashing (Bertramson, 1942). The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$\text{Nutrient uptake} = \frac{\text{nutrient content in the shoots}}{\text{total dry weight}} \times \text{total dry weight} \quad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} \times \frac{W_2 - W_1}{t_2 - t_1} \quad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t₁ = first sampling;

t₂ = second sampling.

The parameters of the second phase of the study were analyzed by an ANOVA. If the treatment had a significant effect, the Duncan's multiple range test was applied at $P < 0.05$ using SPSS v.20 software (IBM, 2011).

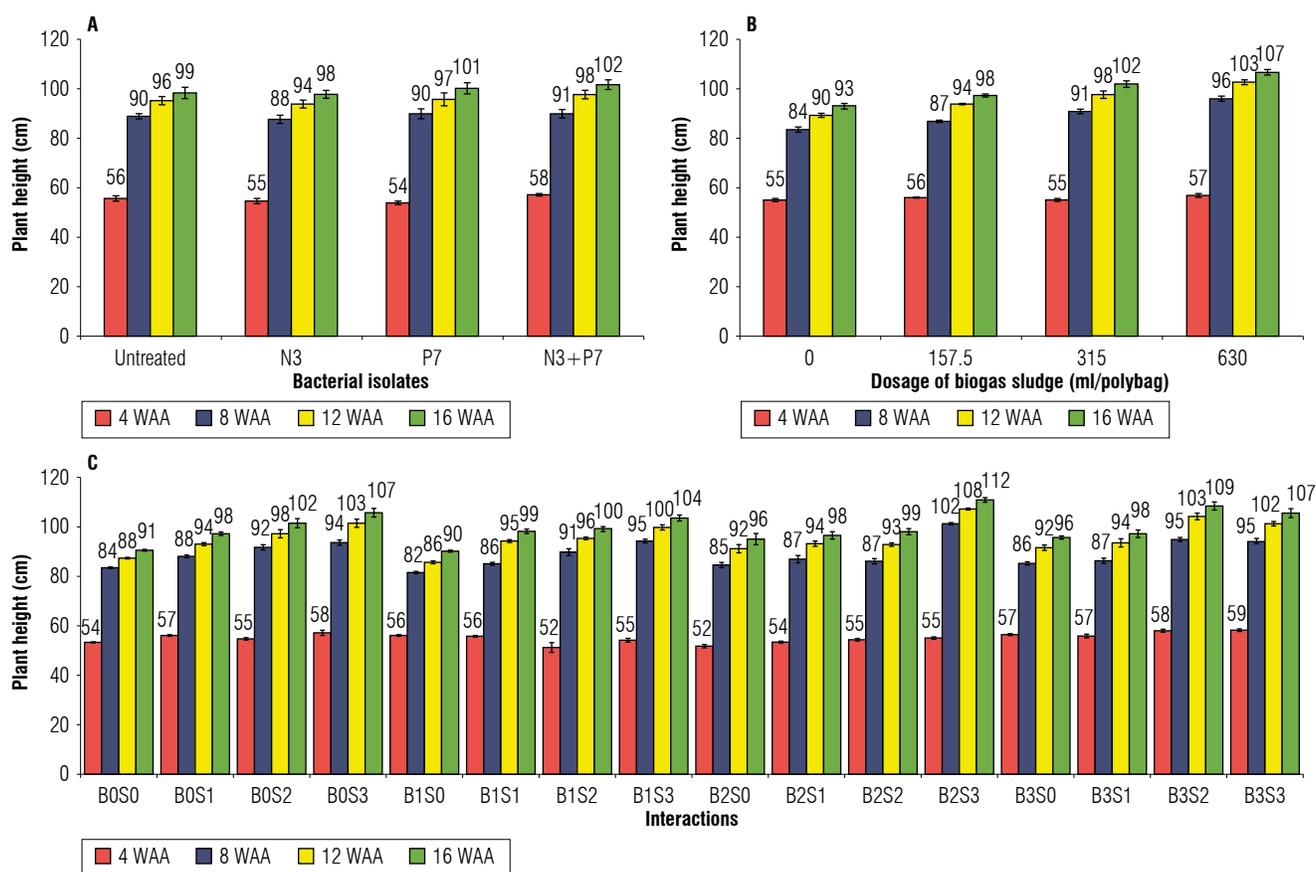


FIGURE 1. A) Effect of superior bacterial isolates, B) dosage of biogas sludge, and C) their interactions on plant height of upland rice at 4, 8, 12, and 16 weeks after application (WAA). Values followed by a different letter in the graph significantly differed according to the Duncan test at $P < 0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3 = isolates N3+P7).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1A-C). A significant increase in plant height of upland rice was observed with higher doses of biogas sludge of 630 ml/polybag at 8, 12,

and 16 WAA, with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest increase in plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total fresh weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96
B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAA (Tab. 4).

A significant increase in the total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice with 20.84% and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12-16 WAA but did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				
B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice 4, 8, 12, and 16 weeks after the application (WAA).

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
4-8 WAA					
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
8-12 WAA					
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
12-16 WAA					
B0	0.327 fgh	0.298 gh	0.246 h	0.403 b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882 a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400 a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866 a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12-16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12-16 WAA. The interaction of B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 times greater than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Contents of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the content of N and P in the upland rice (Fig. 2A-C). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) increased P and N in the plant tissue of upland rice by 33.33% and 4.53%, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissues of upland rice with values 1.63% higher than those of

the control. However, all isolates (B1-B3) showed a similar level of P in the plant tissues of upland rice compared to the control.

Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased the uptake of N and P. The superior bacterial isolates significantly increased the uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the uptake of N and P in the upland rice (Fig. 2D-F).

A significant increase in the uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11% and 65.20%, respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased the uptake of N in the upland rice with the highest increase with B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest uptake of P in the upland rice of 19.93% compared to the control.

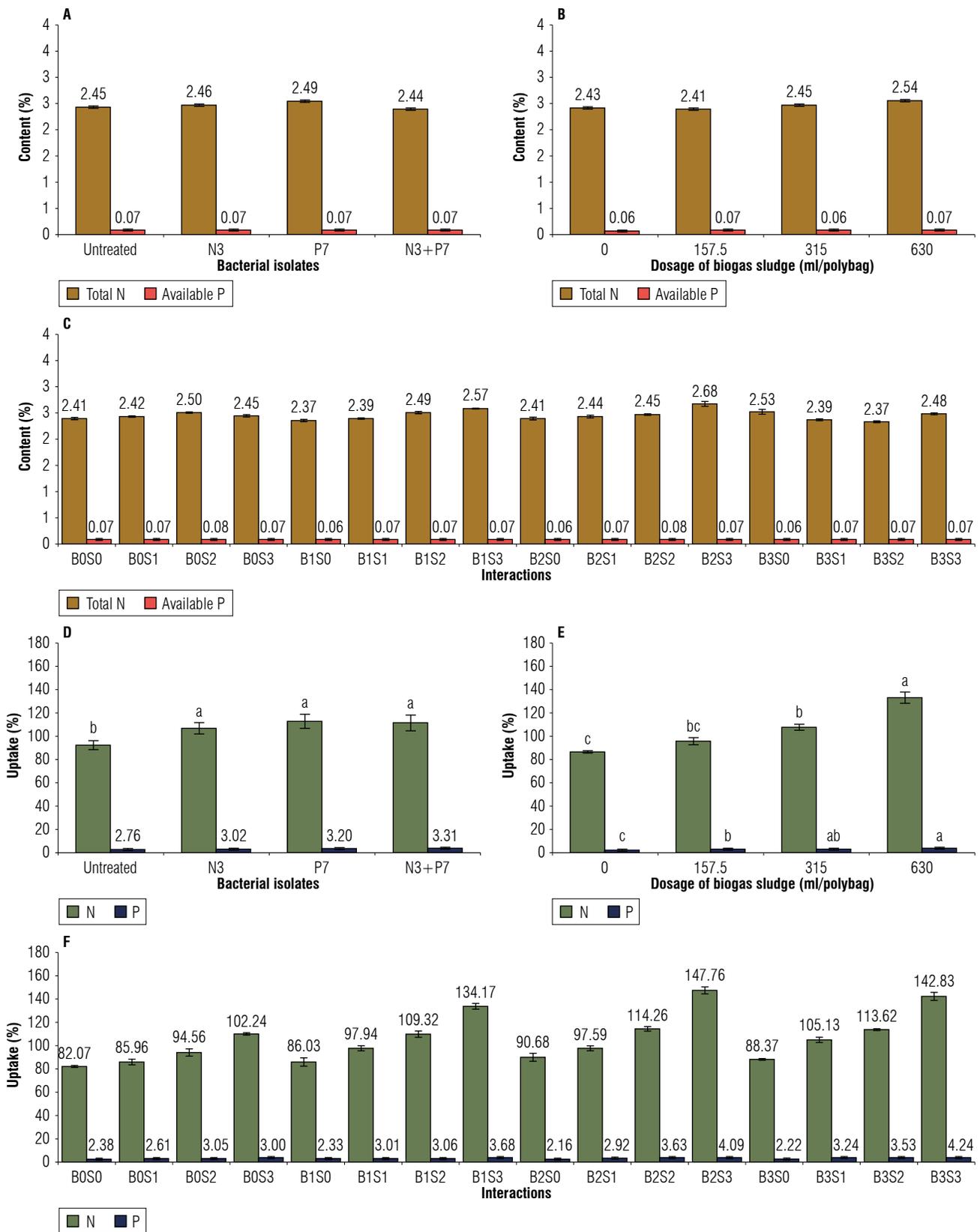


FIGURE 2. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content (A-C) and uptake of N and P (D-F) of upland rice. Values followed by different letters significantly differed according to the Duncan test at $P < 0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of nitrogen and the crop growth rate of upland rice in an Ultisol at 12-16 WAA but did not have a significant effect on plant height, total fresh weight, total dry weight, content of N and P in leaves, uptake of phosphorus by the plants, and crop growth rate of upland rice from 4 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) increased the uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, with the crop growth rate of upland rice at 12-16 WAA increased by 2.57, 2.81, and 2.39 times, respectively, due to the selected superior bacterial isolates (N3, P7, N3+P7) compared to the control. The results indicate that a single P7 bacterial isolate increased the nitrogen uptake and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by the isolate P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids, such as lactic, oxalic, acetic, and citric, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*. This was due to the production of organic acids and other substances, including citric, tartaric, and oxalic acids, that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellin and IAA can increase plant growth under stressful conditions. Kang *et al.* (2014) indicated that the plant growth-promoting bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp., *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and

Enterobacter sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus*, *Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased the plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and crop growth rate of upland rice at 8-12 WAA. However, it did not have a significant effect on the content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, uptake of nitrogen and phosphorus, and also crop growth rate of upland rice in an Ultisol with a higher dose of biogas sludge of 630 ml/polybag was seen at the end of this study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of biogas sludge of 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is due to the chemical characteristics of the biogas sludge such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and the biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ml⁻¹) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml⁻¹) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the uptake of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020), who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml⁻¹, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml⁻¹) was significantly higher than nitrogen-fixing bacteria (0.5-1.4 CFU ml⁻¹), showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported an increase in concentrations of NH₄⁺-N from 45% to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice in Ultisols at 12-16 WAA but did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of

upland rice compared to other interactions and was 5.76 times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml⁻¹), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml⁻¹) (Tab. 2) that could improve soil quality and increase the CGR. This result is supported by Urrea *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014) and Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages for the plants such as availability of nutrients balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and nutrient availability for microorganisms to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge + biofertilizer (36 ml + 10 L ha⁻¹) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice in Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice in Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice in Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, and developed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of the results and the other research outputs. All authors conducted the critical review of the manuscript.

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de tierras altas

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ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P < 0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination with biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes del arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizadoras de fosfato (P7), B3 = combinación de aislamientos (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P < 0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación con lodos de biogás de 630 ml/polybag tiene el potencial de aumentar la TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

Introduction

Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L⁻¹, total P of 110 mg L⁻¹, total K of 1.9 mg L⁻¹ (Lubis *et al.*, 2014), C/N 8; 0.14% N,

1.12% C (Tepsour *et al.*, 2019), and N-NH₃ of 91-112 mg L⁻¹ (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10⁷ cells ml⁻¹ and the lowest of 3.15×10⁷ cells ml⁻¹ (Alvionita *et al.*, 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

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The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B. mycoides*, *B. coagulans*, *B. chitinolyticus*, and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e.*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area in Indonesia covered by Ultisols is 45.8 million ha or 24% of the total area of the country. Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in Indonesia is 15,712,025 ha with a yield of 81,148,617 t in 2017, and the contribution of upland rice yield is 4.66% (Kementerian Pertanian, 2017). The yield contribution of upland rice is classified as low and, therefore, it is necessary to find options to increase it. Therefore, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate and, thus, the growth response of upland rice in Ultisols. This study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N, 98°38.898' E, and altitude of 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity was 82%, and the average rainfall was 228.5 mm per month.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants was the Ultisol soil from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, collected at a depth of 0 to 20 cm. One hundred g of soil sample were taken and analyzed for chemical characteristics of pH H₂O, organic C by Walkley-Black, available P by Bray-II, total N using the Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. To prevent heat from the sterilization process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc. (Medan, Indonesia) at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural Research and Development were soaked in water for 24 h, followed by the application of the fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisol soil samples after sterilization at 100°C.

Chemical characteristics	Method	Value	Category
Soil pH (H ₂ O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg ⁻¹)	Spectrophotometry	870.25	Very high
CEC (meq 100 g ⁻¹)	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
Exchangeable cations			
K (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

Criteria for pH (H₂O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg⁻¹ (very high); cation exchange capacity (CEC) = 25-40 meq 100 g⁻¹ (high); base saturation <20% (very low); exchangeable K = 0.60-1.00 meq 100 g⁻¹ (high); exchangeable Ca <2 meq 100 g⁻¹ (very low); exchangeable Mg <0.4 meq 100 g⁻¹ (very low); exchangeable Na <0.1 meq 100 g⁻¹ (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

One ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water, homogenized and then diluted to 10⁻⁵. A total of 0.1 ml of the suspension from the last dilution was spread over James nitrogen free malat bromothymol blue

(JNFB) medium (Kirchhof *et al.*, 1997) for the nitrogen-fixing bacterial isolates test and Pikovskaya (PVK) medium (Pikovskaya, 1948) for the phosphate solubilizing bacteria isolates. The culture medium was incubated for 2 to 3 d at 37°C. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates was indicated by a halo zone around the microbial colonies on the PVK medium. Seven nitrogen-fixing and seven phosphate-solubilizing isolates were found to produce total N and available P. The isolates that showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.* (2021a; 2021b).

The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. The procedure for processing biogas sludge was the following: the palm oil mill removed the palm oil mill effluent (POME) waste from the second pond which has been mixed with oil and then separated at an optimal temperature of 35°C. Liquid waste was then pumped into the receiver tank of 10 m³ volume and filtered on a fiber tank screen to separate solid waste such as fiber and other materials. Liquid waste from the receiver tank was further pumped into the tower tank. Then, it was distributed evenly to the fixed tank at a temperature of 35 to 37°C and a flow rate of 20 to 30 m³/h. Finally, the biogas sludge was taken from the fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at

one week after planting. Biogas sludge samples of 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was the dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m³ ha⁻¹ equal to 126,000 L ha⁻¹ (Sutarta *et al.*, 2000), then converted to soil weight per polybag (Eq. 1). Each replicate was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop growth rate (CGR).

$$\begin{aligned} \text{Biogas sludge} &= \frac{\text{Dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \quad (1) \\ &= \frac{126,000 \text{ L ha}^{-1}}{2,000,000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 0.63 \text{ ml} \end{aligned}$$

Plant growth variables and data analysis

The growth variables were assessed by measuring the growth of upland rice (plant height, and total fresh and dry weights), contents and uptake of N and P in the aerial

TABLE 2. Chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L ⁻¹)	Spectrophotometry	4547.8
Biological oxygen demand (mg L ⁻¹)	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L ⁻¹)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml ⁻¹)	Plate count	29.4×10 ⁵
Total phosphate solubilizing bacteria (CFU ml ⁻¹)	Plate count	7.0×10 ⁴

Laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute) (2009).

parts, and crop growth rate (CGR). The plant height was measured from the root apex to the tip of leaves using a measuring tape, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was obtained after plant drying in an oven (VS-1202D3, Vision Scientific Co., Korea) at 60°C for 48 h and weighed using analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl method, and the P content was recorded using the destruction method through dry ashing (Bertramson, 1942). The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$\text{Nutrient uptake} = \frac{\text{nutrient content in the shoots}}{\text{total dry weight}} \times \text{total dry weight} \quad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} \times \frac{W_2 - W_1}{t_2 - t_1} \quad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t₁ = first sampling;

t₂ = second sampling.

The parameters of the second phase of the study were analyzed by an ANOVA. If the treatment had a significant effect, the Duncan's multiple range test was applied at $P < 0.05$ using SPSS v.20 software (IBM, 2011).

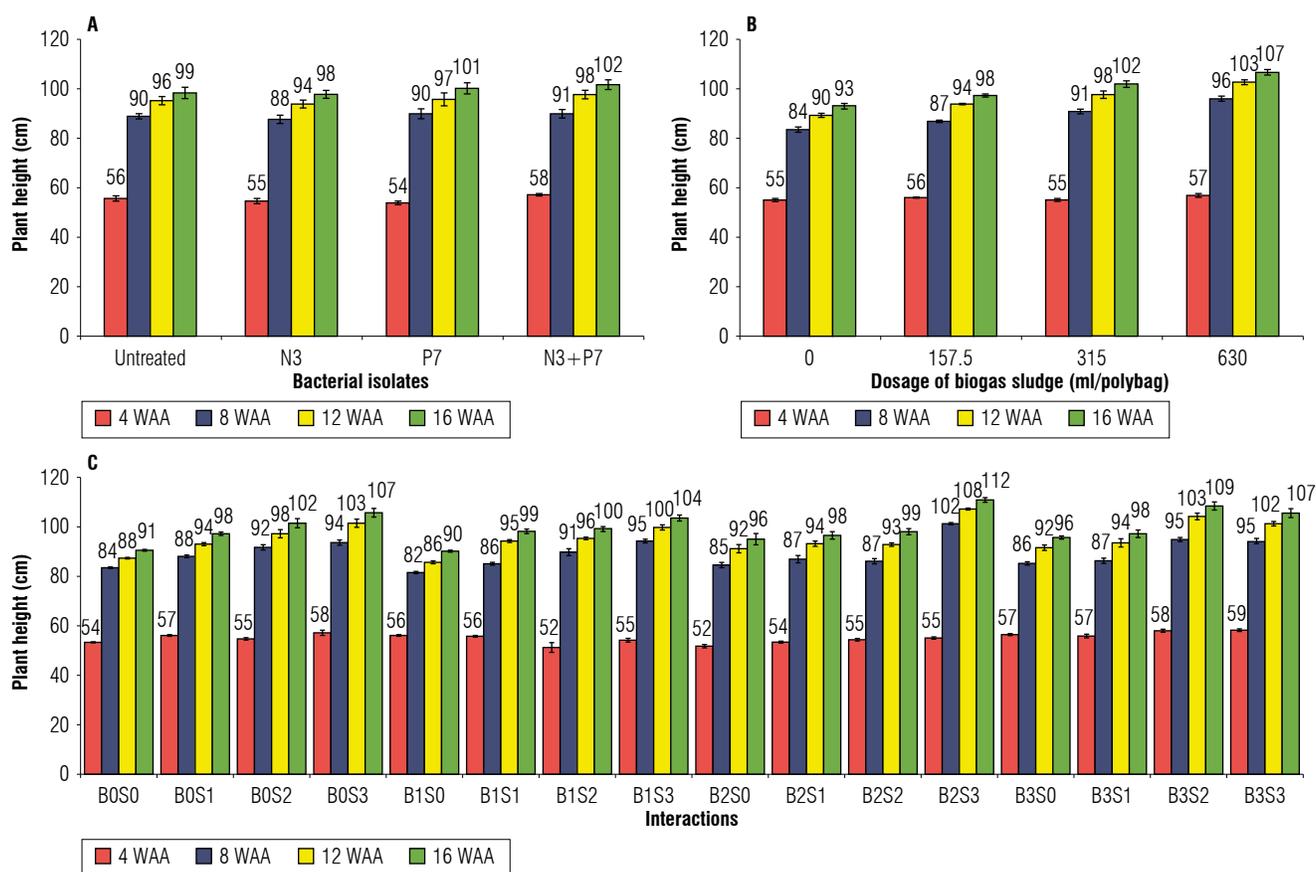


FIGURE 1. A) Effect of superior bacterial isolates, B) dosage of biogas sludge, and C) their interactions on plant height of upland rice at 4, 8, 12, and 16 weeks after application (WAA). Values followed by a different letter in the graph significantly differed according to the Duncan test at $P < 0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3 = isolates N3+P7).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1A-C). A significant increase in plant height of upland rice was observed with higher doses of biogas sludge of 630 ml/polybag at 8, 12,

and 16 WAA, with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest increase in plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total fresh weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96
B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAA (Tab. 4).

A significant increase in the total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice with 20.84% and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12-16 WAA but did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				
B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice 4, 8, 12, and 16 weeks after the application (WAA).

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
4-8 WAA					
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
8-12 WAA					
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
12-16 WAA					
B0	0.327 fgh	0.298 gh	0.246 h	0.403 b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882 a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400 a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866 a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12-16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12-16 WAA. The interaction of B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 times greater than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Contents of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the content of N and P in the upland rice (Fig. 2A-C). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) increased P and N in the plant tissue of upland rice by 33.33% and 4.53%, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissues of upland rice with values 1.63% higher than those of

the control. However, all isolates (B1-B3) showed a similar level of P in the plant tissues of upland rice compared to the control.

Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased the uptake of N and P. The superior bacterial isolates significantly increased the uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the uptake of N and P in the upland rice (Fig. 2D-F).

A significant increase in the uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11% and 65.20%, respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased the uptake of N in the upland rice with the highest increase with B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest uptake of P in the upland rice of 19.93% compared to the control.

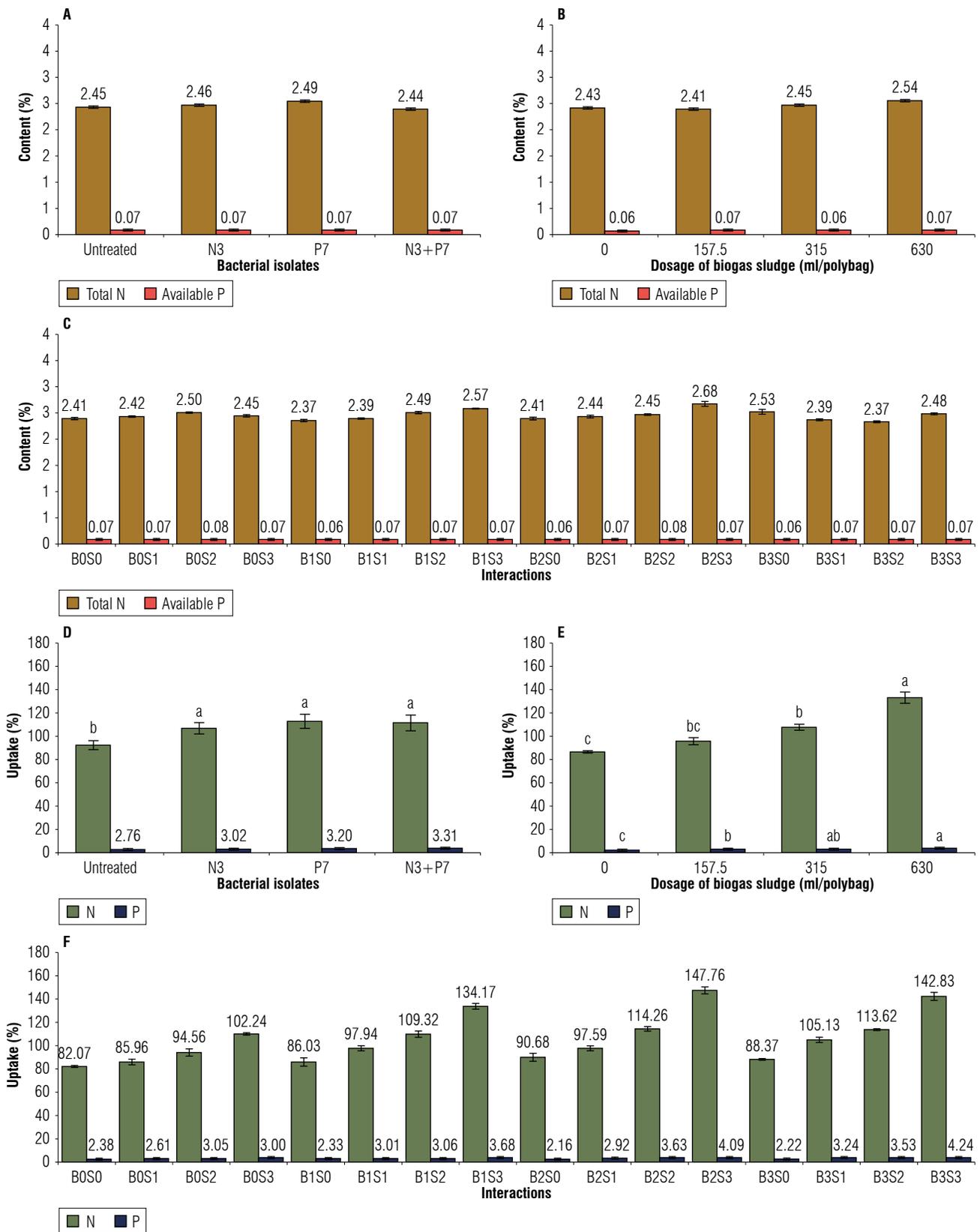


FIGURE 2. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content (A-C) and uptake of N and P (D-F) of upland rice. Values followed by different letters significantly differed according to the Duncan test at $P < 0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of nitrogen and the crop growth rate of upland rice in an Ultisol at 12-16 WAA but did not have a significant effect on plant height, total fresh weight, total dry weight, content of N and P in leaves, uptake of phosphorus by the plants, and crop growth rate of upland rice from 4 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) increased the uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, with the crop growth rate of upland rice at 12-16 WAA increased by 2.57, 2.81, and 2.39 times, respectively, due to the selected superior bacterial isolates (N3, P7, N3+P7) compared to the control. The results indicate that a single P7 bacterial isolate increased the nitrogen uptake and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by the isolate P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids, such as lactic, oxalic, acetic, and citric, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*. This was due to the production of organic acids and other substances, including citric, tartaric, and oxalic acids, that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellin and IAA can increase plant growth under stressful conditions. Kang *et al.* (2014) indicated that the plant growth-promoting bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp., *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and

Enterobacter sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus*, *Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased the plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and crop growth rate of upland rice at 8-12 WAA. However, it did not have a significant effect on the content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, uptake of nitrogen and phosphorus, and also crop growth rate of upland rice in an Ultisol with a higher dose of biogas sludge of 630 ml/polybag was seen at the end of this study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of biogas sludge of 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is due to the chemical characteristics of the biogas sludge such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and the biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ml⁻¹) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml⁻¹) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the uptake of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020), who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml⁻¹, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml⁻¹) was significantly higher than nitrogen-fixing bacteria (0.5-1.4 CFU ml⁻¹), showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported an increase in concentrations of NH₄⁺-N from 45% to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice in Ultisols at 12-16 WAA but did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of

upland rice compared to other interactions and was 5.76 times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml⁻¹), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml⁻¹) (Tab. 2) that could improve soil quality and increase the CGR. This result is supported by Urrea *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014) and Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages for the plants such as availability of nutrients balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and nutrient availability for microorganisms to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge + biofertilizer (36 ml + 10 L ha⁻¹) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice in Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice in Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice in Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, and developed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of the results and the other research outputs. All authors conducted the critical review of the manuscript.

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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de tierras altas

Novilda Elizabeth Mustamu¹, Zulkifli Nasution^{2*}, Irvan³, and Mariani Sembiring²

ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice grown in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P < 0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction between N3 and biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination with biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes del arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizadoras de fosfato (P7), B3 = combinación de aislamientos (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P < 0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20.77% y la tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81 veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación con lodos de biogás de 630 ml/polybag tiene el potencial de aumentar la TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo.

Introduction

Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and plant yield (Adela *et al.*, 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L⁻¹, total P of 110 mg L⁻¹, total K of 1.9 mg L⁻¹ (Lubis *et al.*, 2014), C/N 8; 0.14% N,

1.12% C (Tepsour *et al.*, 2019), and N-NH₃ of 91-112 mg L⁻¹ (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3, with the highest bacterial population of 7.21×10⁷ cells ml⁻¹ and the lowest of 3.15×10⁷ cells ml⁻¹ (Alvionita *et al.*, 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

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The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al.* (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility and plant yield, and reducing the use of chemical fertilizers. Sharma *et al.* (2013) described different *Bacillus* species, such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B. mycoides*, *B. coagulans*, *B. chitinolyticus*, and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al.* (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al.* (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e.*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Thermotogae*.

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, including Ultisols. According to the Pusat Penelitian Tanah dan Agroklimat (Center for Soil and Agro-climate Research) (2000), the area in Indonesia covered by Ultisols is 45.8 million ha or 24% of the total area of the country. Furthermore, according to the Ministry of Agriculture, the area dedicated to rice cultivation in Indonesia is 15,712,025 ha with a yield of 81,148,617 t in 2017, and the contribution of upland rice yield is 4.66% (Kementerian Pertanian, 2017). The yield contribution of upland rice is classified as low and, therefore, it is necessary to find options to increase it. Therefore, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate and, thus, the growth response of upland rice in Ultisols. This study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N, 98°38.898' E, and altitude of 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature was 27.4°C, the average air humidity was 82%, and the average rainfall was 228.5 mm per month.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants was the Ultisol soil from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, collected at a depth of 0 to 20 cm. One hundred g of soil sample were taken and analyzed for chemical characteristics of pH H₂O, organic C by Walkley-Black, available P by Bray-II, total N using the Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. To prevent heat from the sterilization process, the soil was incubated for 1 d and then placed into a 10 kg polybag (18 cm × 18 cm). A basic NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc. (Medan, Indonesia) at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) of the inbred variety Inpago-8 from the Indonesian Agency for Agricultural Research and Development were soaked in water for 24 h, followed by the application of the fungicide Propineb (70%) for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisol soil samples after sterilization at 100°C.

Chemical characteristics	Method	Value	Category
Soil pH (H ₂ O)	Electrometry	4.80	Acid
Organic C (%)	Walkley-Black	0.44	Very low
Total N (%)	Kjeldahl	0.04	Very low
Available P (mg kg ⁻¹)	Spectrophotometry	870.25	Very high
CEC (meq 100 g ⁻¹)	Ammonium acetate pH 7	28.31	High
Base saturation (%)	Ammonium acetate pH 7	4.85	Very low
Exchangeable cations			
K (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.60	High
Ca (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.34	Very low
Mg (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.32	Very low
Na (meq 100 g ⁻¹)	Ammonium acetate pH 7	0.09	Very low
Al (%)	Ammonium acetate pH 7	0.02	Very low

Criteria for pH (H₂O) = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg⁻¹ (very high); cation exchange capacity (CEC) = 25-40 meq 100 g⁻¹ (high); base saturation <20% (very low); exchangeable K = 0.60-1.00 meq 100 g⁻¹ (high); exchangeable Ca <2 meq 100 g⁻¹ (very low); exchangeable Mg <0.4 meq 100 g⁻¹ (very low); exchangeable Na <0.1 meq 100 g⁻¹ (very low); exchangeable Al <5% (very low) (Balai Penelitian Tanah (Indonesia Soil Research Institute), 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

One ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water, homogenized and then diluted to 10⁻⁵. A total of 0.1 ml of the suspension from the last dilution was spread over James nitrogen free malat bromothymol blue

(JNFB) medium (Kirchhof *et al.*, 1997) for the nitrogen-fixing bacterial isolates test and Pikovskaya (PVK) medium (Pikovskaya, 1948) for the phosphate solubilizing bacteria isolates. The culture medium was incubated for 2 to 3 d at 37°C. The nitrogen-fixing bacterial isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacterial isolates was indicated by a halo zone around the microbial colonies on the PVK medium. Seven nitrogen-fixing and seven phosphate-solubilizing isolates were found to produce total N and available P. The isolates that showed the highest phosphate and nitrogen increasing abilities were selected, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3), which were confirmed by Mustamu *et al.* (2021a; 2021b).

The biogas sludge was collected from Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia. The procedure for processing biogas sludge was the following: the palm oil mill removed the palm oil mill effluent (POME) waste from the second pond which has been mixed with oil and then separated at an optimal temperature of 35°C. Liquid waste was then pumped into the receiver tank of 10 m³ volume and filtered on a fiber tank screen to separate solid waste such as fiber and other materials. Liquid waste from the receiver tank was further pumped into the tower tank. Then, it was distributed evenly to the fixed tank at a temperature of 35 to 37°C and a flow rate of 20 to 30 m³/h. Finally, the biogas sludge was taken from the fixed tank. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at

one week after planting. Biogas sludge samples of 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was the dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m³ ha⁻¹ equal to 126,000 L ha⁻¹ (Sutarta *et al.*, 2000), then converted to soil weight per polybag (Eq. 1). Each replicate was harvested at 4, 8, and 12 weeks after application (WAA) for determination of the crop growth rate (CGR).

$$\begin{aligned} \text{Biogas sludge} &= \frac{\text{Dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \quad (1) \\ &= \frac{126,000 \text{ L ha}^{-1}}{2,000,000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 630 \text{ ml} \end{aligned}$$

Plant growth variables and data analysis

The growth variables were assessed by measuring the growth of upland rice (plant height, and total fresh and dry weights), contents and uptake of N and P in the aerial

TABLE 2. Chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L ⁻¹)	Spectrophotometry	4547.8
Biological oxygen demand (mg L ⁻¹)	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L ⁻¹)	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml ⁻¹)	Plate count	29.4×10 ⁵
Total phosphate solubilizing bacteria (CFU ml ⁻¹)	Plate count	7.0×10 ⁴

Laboratory analysis based on the Balai Penelitian Tanah (Indonesia Soil Research Institute) (2009).

parts, and crop growth rate (CGR). The plant height was measured from the root apex to the tip of leaves using a measuring tape, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots + shoots) was obtained after plant drying in an oven (VS-1202D3, Vision Scientific Co., Korea) at 60°C for 48 h and weighed using analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl method, and the P content was recorded using the destruction method through dry ashing (Bertramson, 1942). The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.*, 1997):

$$\text{Nutrient uptake} = \frac{\text{nutrient content in the shoots}}{\text{total dry weight}} \times \text{total dry weight} \quad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} \times \frac{W_2 - W_1}{t_2 - t_1} \quad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t₁ = first sampling;

t₂ = second sampling.

The parameters of the second phase of the study were analyzed by an ANOVA. If the treatment had a significant effect, the Duncan's multiple range test was applied at $P < 0.05$ using SPSS v.20 software (IBM, 2011).

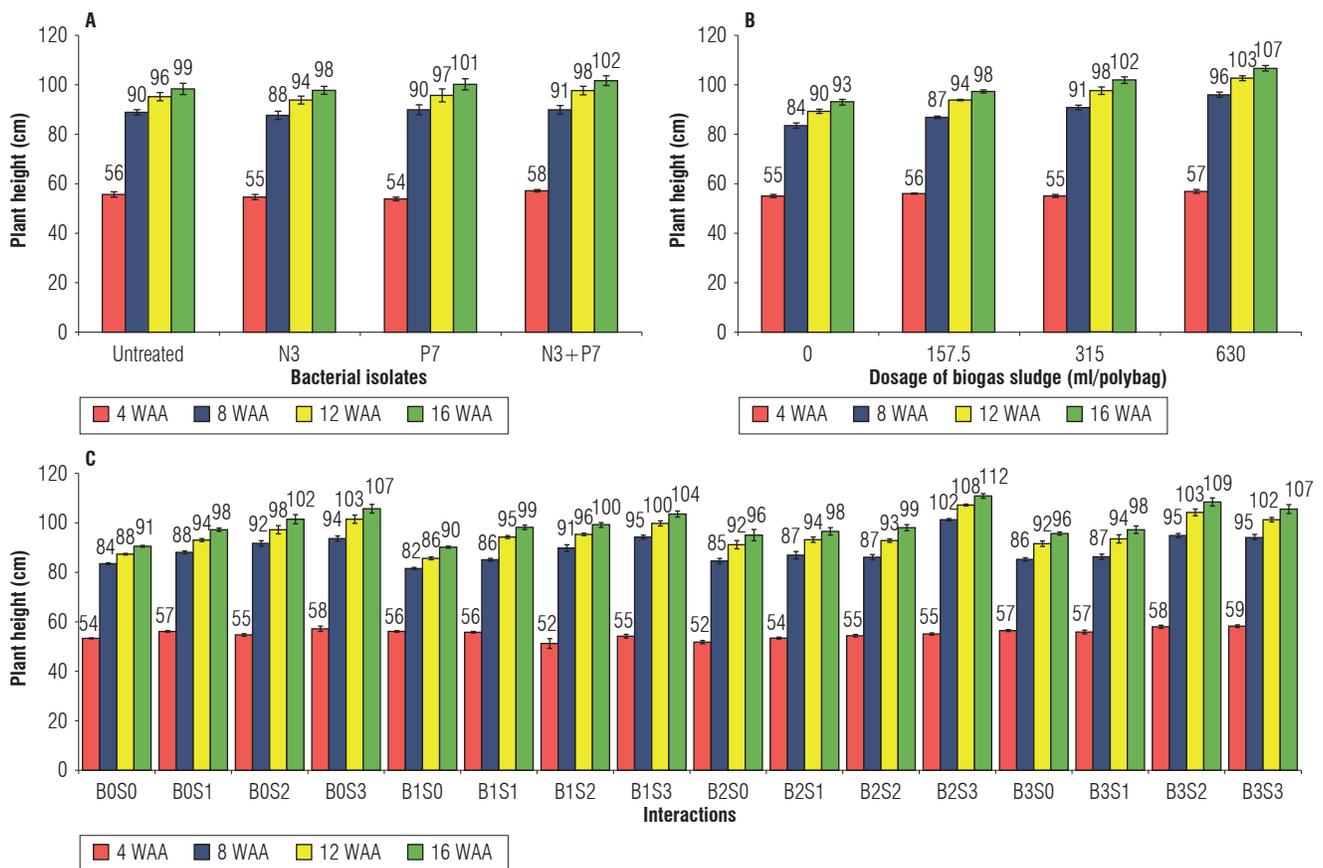


FIGURE 1. A) Effect of superior bacterial isolates, B) dosage of biogas sludge, and C) their interactions on plant height of upland rice at 4, 8, 12, and 16 weeks after application (WAA). Values followed by a different letter in the graph significantly differed according to the Duncan test at $P < 0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7; B3 = isolates N3+P7).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1A-C). A significant increase in plant height of upland rice was observed with higher doses of biogas sludge of 630 ml/polybag at 8, 12,

and 16 WAA, with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest increase in plant height of upland rice by 2.94% and 22.06%, respectively, compared to the control.

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total fresh weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96
B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31 ± 6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest increase in the total fresh weight of upland rice with 9.66% and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4-16 WAA (Tab. 4).

A significant increase in the total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest increase in the total dry weight of upland rice with 20.84% and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12-16 WAA but did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot + roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total dry weight (g) ± standard error			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				
B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$. CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice 4, 8, 12, and 16 weeks after the application (WAA).

Superior bacterial isolates (B)	Biogas sludge (S)				Average
	S0	S1	S2	S3	
4-8 WAA					
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%
8-12 WAA					
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
12-16 WAA					
B0	0.327 fgh	0.298 gh	0.246 h	0.403 b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882 a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400 a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866 a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by a different letter in the column significantly differed according to the Duncan test at $P < 0.05$; CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = isolates N3+P7).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12-16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12-16 WAA. The interaction of B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76 times greater than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Contents of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the content of N and P in the upland rice (Fig. 2A-C). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) increased P and N in the plant tissue of upland rice by 33.33% and 4.53%, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissues of upland rice with values 1.63% higher than those of

the control. However, all isolates (B1-B3) showed a similar level of P in the plant tissues of upland rice compared to the control.

Uptake of N and P in the upland rice

The effect of biogas sludge significantly increased the uptake of N and P. The superior bacterial isolates significantly increased the uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the uptake of N and P in the upland rice (Fig. 2D-F).

A significant increase in the uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11% and 65.20%, respectively, compared to the control. The bacterial isolates B1-B3 also significantly increased the uptake of N in the upland rice with the highest increase with B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest uptake of P in the upland rice of 19.93% compared to the control.

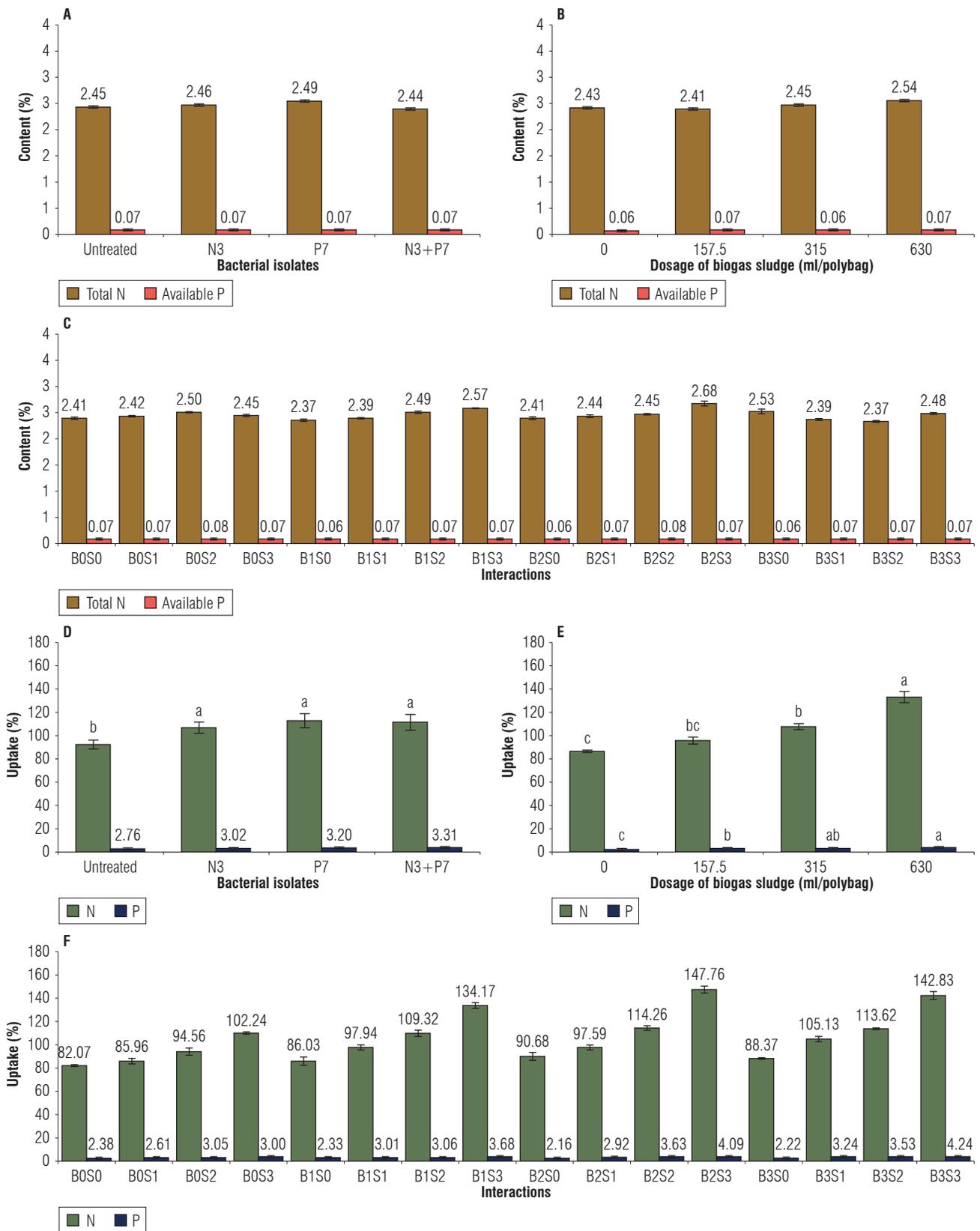


FIGURE 2. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content (A-C) and uptake of N and P (D-F) of upland rice. Values followed by different letters significantly differed according to the Duncan test at $P < 0.05$. Vertical bars indicate the standard error. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the uptake of nitrogen and the crop growth rate of upland rice in an Ultisol at 12-16 WAA but did not have a significant effect on plant height, total fresh weight, total dry weight, content of N and P in leaves, uptake of phosphorus by the plants, and crop growth rate of upland rice from 4 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) increased the uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, with the crop growth rate of upland rice at 12-16 WAA increased by 2.57, 2.81, and 2.39 times, respectively, due to the selected superior bacterial isolates (N3, P7, N3+P7) compared to the control. The results indicate that a single P7 bacterial isolate increased the nitrogen uptake and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by the isolate P7 that can increase the uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al.* (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids, such as lactic, oxalic, acetic, and citric, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al.* (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*. This was due to the production of organic acids and other substances, including citric, tartaric, and oxalic acids, that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al.* (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al.* (2012) reported that gibberellin and IAA can increase plant growth under stressful conditions. Kang *et al.* (2014) indicated that the plant growth-promoting bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al.* (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp., *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and

Enterobacter sp. Liaquat *et al.* (2017) also reported abundance of *Bacillus*, *Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased the plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), uptake (N and P), and crop growth rate of upland rice at 8-12 WAA. However, it did not have a significant effect on the content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, uptake of nitrogen and phosphorus, and also crop growth rate of upland rice in an Ultisol with a higher dose of biogas sludge of 630 ml/polybag was seen at the end of this study (16 WAA). In contrast, the total fresh weight increased along with the higher dose of biogas sludge of 315 ml/polybag and then decreased at the dose of 630 ml/polybag. This result is due to the chemical characteristics of the biogas sludge such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and the biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5 CFU ml⁻¹) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml⁻¹) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the uptake of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020), who determined the macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml⁻¹, respectively. Ndubuisi-Nnaji *et al.* (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml⁻¹) was significantly higher than nitrogen-fixing bacteria (0.5-1.4 CFU ml⁻¹), showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported an increase in concentrations of NH₄⁺-N from 45% to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice in Ultisols at 12-16 WAA but did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of

upland rice compared to other interactions and was 5.76 times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml⁻¹), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml⁻¹) (Tab. 2) that could improve soil quality and increase the CGR. This result is supported by Urra *et al.* (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al.* (2014) and Carvajal-Muñoz and Carmona-García (2012) showed that the application of a biofertilizer had advantages for the plants such as availability of nutrients balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and nutrient availability for microorganisms to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge + biofertilizer (36 ml + 10 L ha⁻¹) significantly increased the plant height, number of leaves, and capsaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice in Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice in Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice in Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, and developed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of the results and the other research outputs. All authors conducted the critical review of the manuscript.

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