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 Mustamu[1], Zulkifli Nasution[2*], Irvan[3], and Mariani Sembiring[2]

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 $N\text{-}NH_3$ 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 7.21×10^7 cells ml^{-1} and the lowest of 3.15×10^7 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.

Received for publication: May 24, 2021. Accepted for publication: December 20, 2021. Doi: 10.15446/agron.colomb.v39n3.97583

[1] Doctoral Program in Agricultural Sciences, Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara (Indonesia).
[2] Program of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara (Indonesia).
[3] Program of Chemical Engineering, Faculty of Engineering, Universitas Sumatera Utara, Sumatera Utara (Indonesia).
[*] Corresponding author: zulnasution@usu.ac.id

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 H_2O, 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_2O)	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 (H_2O) = 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 m^3 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 m^3/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 m^3 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).

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

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

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):

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

$$\text{CGR} = \frac{\Delta W}{\Delta t} \times \frac{\text{W2-W1}}{\text{t2-t1}} \quad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t_1 = first sampling;

t_2 = 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).

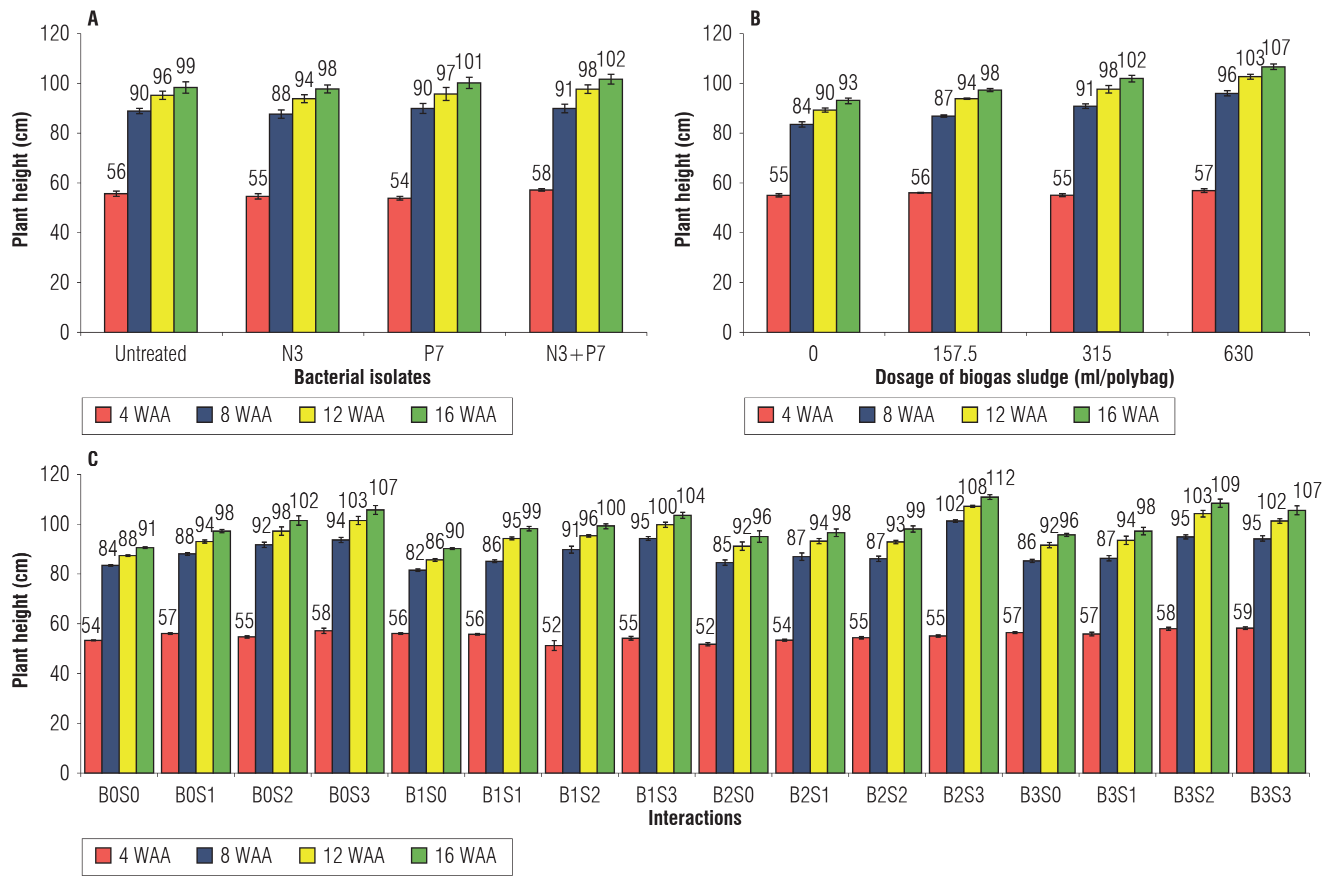

FIGURE 1. A) Effect of superior bacterial isolates, B) dosage of biogas sludge, and C) their interactions on plant height of upland rice at 4, 8, 12, and 16 weeks after application (WAA). Values followed by a different letter in the graph significantly differed according to the Duncan test at P<0.05. 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.

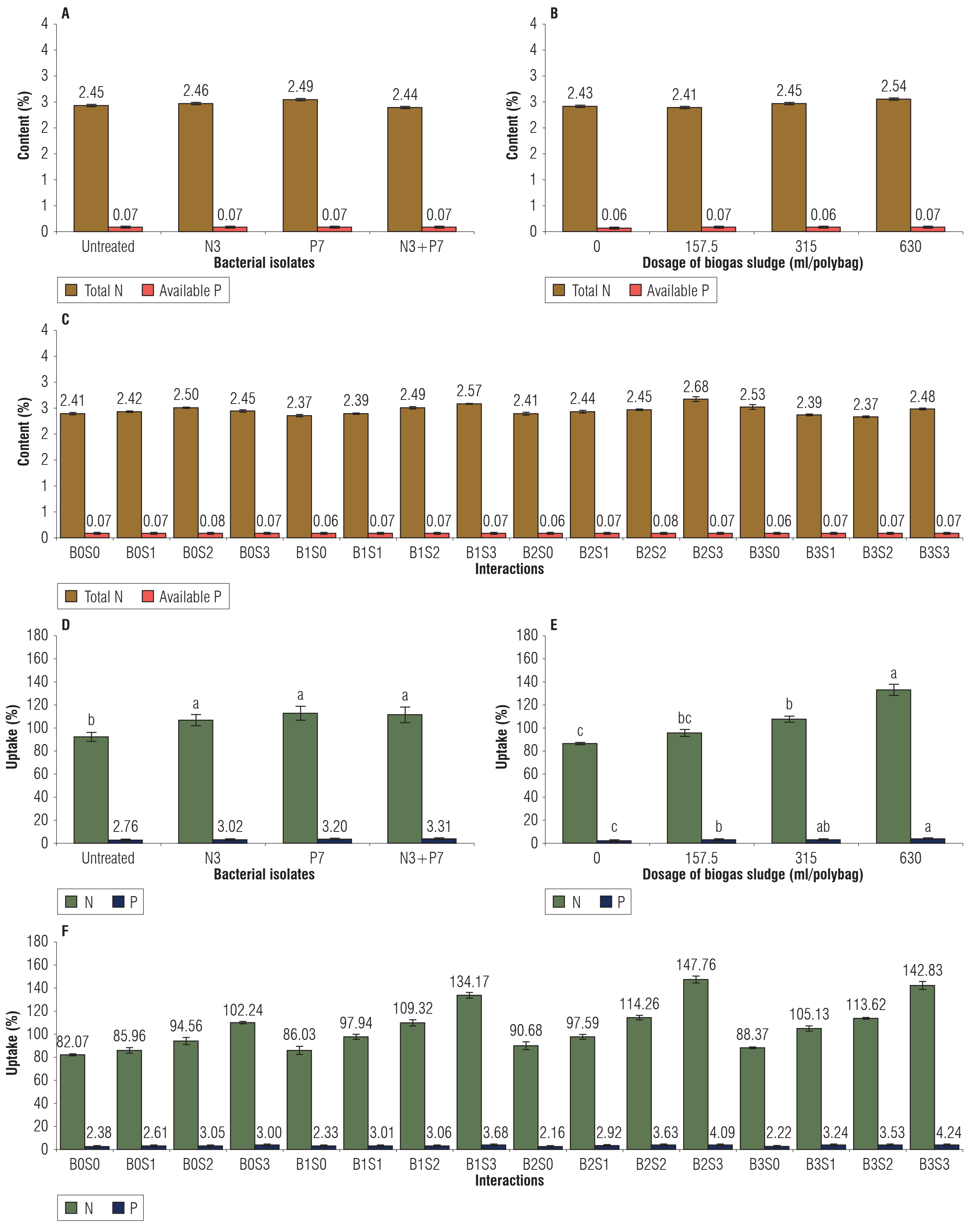

FIGURE 2. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content (A-C) and uptake of N and P (D-F) of upland rice. Values followed by different letters significantly differed according to the Duncan test at $P<0.05$. Vertical bars indicate the standard 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 NH_4^+-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-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.

Literature cited

Adela, B. N., Muzzammil, N., Loh, S. K., & Choo, Y. M. (2014). Characteristics of palm oil mill effluent (POME) in an anaerobic biogas digester. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, *16*(1), 225–231.

Alvionita, F., Faizal, M., Komariah, L. N., & Said, M. (2019). Biogas production from palm oil mill effluent with indigenous bacteria. *International Journal on Advanced Science, Engineering and Information Technology*, *9*(6), 2060–2066. https://doi.org/10.18517/ijaseit.9.6.10462

Ambrosini, A., Stefanski, T., Lisboa, B. B., Beneduzi, A., Vargas, L. K., & Passaglia, L. M. P. (2016). Diazotrophic bacilli isolated from the sunflower rhizosphere and the potential of *Bacillus mycoides* B38V as biofertiliser. *Annals of Applied Biology*, *168*(1), 93–110. https://doi.org/10.1111/aab.12245

Balai Penelitian Tanah. (2009). *Petunjuk teknis 2: Analisis kimia tanah, tanaman, air, dan pupuk*. Kementerian Pertanian.

Bertramson, B. R. (1942). Phosphorus analysis of plant material. *Plant Physiology*, *17*(3), 447–454. https://doi.org/10.1104%2Fpp.17.3.447

Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, *13*, Article 66. https://doi.org/10.1186/1475-2859-13-66

Carvajal-Muñoz, J. S., & Carmona-Garcia, C. E. (2012). Benefits and limitations of biofertilization in agricultural practices. *Livestock Research for Rural Development*, *24*(3), Article 43.

Choorit, W., & Wisarnwan, P. (2007). Effect of temperature on the anaerobic digestion of palm oil mill effluent. *Electronic Journal of Biotechnology*, *10*(3), 376–385. https://doi.org/10.2225/vol10-issue3-fulltext-7

FAO. (1977). *FAO soils Bulletin 40 - China: recycling of organic wastes in agriculture*. Food and Agriculture Organization of the United Nations. https://www.fao.org/publications/card/en/c/34d03d32-bd9f-5d08-aa08-ed2499349eb1/

Ferrara, F. I. S., Oliveira, Z. M., Gonzales, H. H. S., Floh, E. I. S., & Barbosa, H. R. (2012). Endophytic and rhizospheric enterobacteria isolated from sugar cane have different potentials for producing plant growth-promoting substances. *Plant and Soil*, *353*, 409–417. https://doi.org/10.1007/s11104-011-1042-1

IBM. (2011). *IBM SPSS statistics for Windows version 20.0*. International Business Machines Corporation.

Kang, S. M., Radhakrishnan, R., You, Y. H., Joo, G. J., Lee, I. J., Lee, K. E., & Kim, J. H. (2014). Phosphate solubilizing *Bacillus megaterium* mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. *Indian Journal of Microbiology*, *54*, 427–433. https://doi.org/10.1007/s12088-014-0476-6

Kementerian Pertanian. (2017). *Produksi tanaman pangan di Indonesia*.

Khan, M. A., Asaf, S., Khan, A. L., Jan, R., Kang, S. M., Kim, K. M., & Lee, I. J. (2020). Thermotolerance effect of plant growth-promoting *Bacillus cereus* SA1 on soybean during heat stress. *BMC Microbiology*, *20*, Article 175. https://doi.org/10.1186/s12866-020-01822-7

Kirchhof, G., Reis, V. M., Baldani, J. I., Eckert, B., Döbereiner, J., & Hartmann, A. (1997). Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. In J. K. Ladha, F. J. de Brujin, & K. A. Malik (Eds.), *Opportunities for biological nitrogen fixation in rice and other non-legumes* (pp. 45–55). Springer. https://doi.org/10.1007/978-94-011-7113-7_6

Liaquat, R., Jamal, A., Tauseef, I., Qureshi, Z., Farooq, U., Imran, M., & Ali, M. I. (2017). Characterizing bacterial consortia from an anaerobic digester treating organic waste for biogas production. *Polish Journal of Environmental Studies*, *26*(2), 709–716. https://doi.org/10.15244/pjoes/59332

Lim, J. W., Ge, T., & Tong, Y. W. (2018). Monitoring of microbial communities in anaerobic digestion sludge for biogas optimisation. *Waste Management*, *71*, 334–341. https://doi.org/10.1016/j.wasman.2017.10.007

Lubis, F. S., Irvan, Anwar, D., Harahap, B. A., & Trisakti, B. (2014). Kajian awal pembuatan pupuk cair organik dari effluent pengolahan lanjut limbah cair pabrik kelapa sawit (LCPKS) skala pilot. *Jurnal Teknik Kimia USU*, *3*(1), 32–37. https://doi.org/10.32734/jtk.v3i1.1499

Meena, V. S., Bahaur, I., Maurya, B. R., Kumar, A., Meena, R. K., Meena, S. K., & Verma, J. P. (2016). Potassium-solubilizing microorganism in evergreen agriculture: an overview. In V. S. Meena, B. R. Maurya, J. P. Verma, & R. S. Meena (Eds.), *Potassium solubilizing microorganisms for sustainable agriculture* (pp. 1–20). Springer. https://doi.org/10.1007/978-81-322-2776-2_1

Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. *Engineering in Life Sciences*, *12*(3), 242–257. https://doi.org/10.1002/elsc.201100085

Mustamu, N. E., Nasution, Z., Irvan, & Sembiring, M. (2021a). Isolation of phosphate solubilizing bacteria from anaerobic digestion sludge of palm oil mill effluent on ultisols. *Plant Cell Biotechnology and Molecular Biology*, *22*(35–36), 220–230.

Mustamu, N. E., Nasution, Z., Irvan, & Sembiring, M. (2021b). Potential and phylogenetic of superior bacterial isolates in biogas sludge from anaerobic digestion of palm oil mill effluent. *IOP Conference Series: Earth and Environmental Science*, *913*, Article 012065.

Mustamu, N. E., & Triyanto, Y. (2020). Nature of chemical and biological sludge biogas liquid waste oil palm. *International Journal of Innovative Science and Research Technology*, *5*(2), 955–957.

Ndubuisi-Nnaji, U. U., Ofon, U. A., Ekponne, N. I., & Offiong, N. A. O. (2020). Improved biofertilizer properties of digestate from codigestion of brewer's spent grain and palm oil mill effluent by manure supplementation. *Sustainable Environment Research*, *30*, Article 14.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya*, *17*, 362–370.

Pusat Penelitian Tanah dan Agroklimat. (2000). *Atlas peta tanah Indonesia*. Jakarta Puslittanak.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, *2*, Article 587. https://doi.org/10.1186/2193-1801-2-587

Shon, T. K., Haryanto, T. A. D., & Yoshida, T. (1997). Dry matter production and utilization of solar energy in one year old *Bupleurum falcatum*. *Journal of the Faculty of Agriculture Kyushu University*, *41*(3–4), 133–139.

Siswanti, D. U., & Lestari, M. F. (2019). Growth rate and capsaicin level of curly red chili (*Capsicum annum* L.) on biofertilizer and biogas sludge application. *Jurnal Biodjati*, *4*(1), 126–137. https://doi.org/10.15575/biodjati.v4i1.4216

Suksong, W., Kongjan, P., Prasertsan, P., Imai, T., & O-Thong, S. (2016). Optimization and microbial community analysis for production of biogas from solid waste residues of palm oil mill industry by solid-state anaerobic digestion. *Bioresource Technology*, *214*, 166–174. https://doi.org/10.1016/j.biortech.2016.04.077

Sutarta, E. S., Winarna, P. L., & Sufianto, T. (2000, June 13–14). *Aplikasi limbah cair pabrik kelapa sawit pada perkebunan kelapa sawit* [Conference presentation]. Pertemuan Kelapa Sawit II, Medan, Indonesia.

Tepsour, M., Usmanbaha, N., Rattanaya, T., Jariyaboon, R., O-Thong, S., Prasertsan, P., & Kongjan, P. (2019). Biogas production from oil palm empty fruit bunches and palm oil decanter cake using solid-state anaerobic co-digestion. *Energies*, *12*(22), Article 4368. https://doi.org/10.3390/en12224368

Urra, J., Alkorta, I., Mijangos, I., Epelde, L., & Garbisu, C. (2019). Application of sewage sludge to agricultural soil increases the abundance of antibiotic resistance genes without altering the composition of prokaryotic communities. *Science of the Total Environment*, *647*, 1410–1420. https://doi.org/10.1016/j.scitotenv.2018.08.092

Youssef, M. A., & Eissa, M. A. (2017). Comparison between organic and inorganic nutrition for tomato. *Journal of Plant Nutrition*, *40*(13), 1900–1907. https://doi.org/10.1080/01904167.2016.1270309

Zhang, A. M., Zhao, G. Y., Gao, T. G., Wang, W., Li, J., Zhang, S. F., & Zhu, B. C. (2013). Solubilization of insoluble potassium and phosphate by *Paenibacillus kribensis* CX-7: a soil microorganism with biological control potential. *African Journal of Microbiology Research*, *7*(1), 41–47. https://doi.org/10.5897/AJMR12.1485