

PAPER • OPEN ACCESS

Potential and phylogenetic of superior bacterial isolates in biogas sludge from anaerobic digestion of palm oil mill effluent

To cite this article: N E Mustamu *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **913** 012065

View the [article online](#) for updates and enhancements.

You may also like

- [Mapping the Conditions for Hydrodynamic Instability on Steady-State Accretion Models of Protoplanetary Disks](#)
Thomas Pfeil and Hubert Klahr
- [A 42 mV startup ring oscillator using gain-enhanced self-bias inverters for extremely low voltage energy harvesting](#)
Ryo Matsuzuka, Tatsuya Terada, Kaori Matsumoto et al.
- [Establishment and Application Demonstration of Standards for Agricultural Biogas Slurry Returning to Field in Citrus Orchard](#)
Tao Lan, Diwen Xiong, Qiao Chu et al.



The Electrochemical Society
Advancing solid state & electrochemical science & technology

242nd ECS Meeting

Oct 9 – 13, 2022 • Atlanta, GA, US

Abstract submission deadline: **April 8, 2022**

Connect. Engage. Champion. Empower. Accelerate.

MOVE SCIENCE FORWARD



Submit your abstract



Potential and phylogenetic of superior bacterial isolates in biogas sludge from anaerobic digestion of palm oil mill effluent

N E Mustamu¹, Z Nasution^{2*}, Irvan³, and M Sembiring²

¹ Doctoral Program of Agricultural Sciences, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Sumatera Utara, Indonesia

² Program Study of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Sumatera Utara, Indonesia

³ Program Study of Chemical Engineering, Faculty of Engineering, Universitas Sumatera Utara, Medan 20155, Sumatera Utara, Indonesia

* Corresponding author: zulnasution@usu.ac.id

Abstract. The study obtains potential and identifies Superior Bacterial Isolates (SBI), i.e., nitrogen-fixing and phosphate solubilizing from biogas sludge. The potential test was conducted using a Completely Randomized Design (CRD) within three replications, and the means were determined by ANOVA and DMRT at $P < 0.05$. The molecular identification of SBI is used by the PCR-16S rRNA sequencing method. This study was conducted from January to August 2020. The result found in the N3 and P7 from biogas sludge was more potential in the availability of total-N and available-P compared to other isolates. It also was identified as similar to *Bacillus paramycoides* and *Bacillus cereus*, respectively. This information can be used as a reference that biogas sludge can be used to support soil fertility.

1. Introduction

The palm oil industry produces several types of waste in the process, solid and liquid waste. The most important liquid waste from the palm oil industry was Palm Oil Mill Effluent (POME). The oil palm fresh fruit bunches per tonne can produce around 0.7 to 0.8 m³ of POME waste [1]. POME processing can be done using the anaerobic fermentation method, and this method is considered more effective in terms of cost and conversion into valuable products such as biogas [2]. Adela et al. [3] said that the lowest part of the first tank that received the incoming effluent showed the highest microbial population was 1.16×10^6 CFU/ml, thus facilitating the anaerobic digestion of POME for biogas generation. Mustamu and Triyanto [4] reported that the biogas sludge has macro and micronutrients along with the nitrogen-fixing and phosphate solubilizing that have the potential for availability of nitrogen and phosphate. The characteristics of biogas sludge from palm oil waste also reported by Tepsour et al. [5]; Choorit and Wisarnwan [6]; Alvionita et al. [7] such as C/N was 8; nitrogen of 0.14%, carbon by 1.12%, NH₃-N ranged of 91 to 112 mg.l⁻¹, pH ranged by 6.8 to 8.3; and the highest bacterial populations was 7.21×10^7 cells/ml, and the lowest was 3.15×10^7 cells/ml.

The diversity of bacterial populations such as nitrogen-fixing and phosphate solubilizing in biogas sludge has the opportunity to increase soil fertility. Sharma et al. [8] described the *Bacillus* genera such as *B. circulans*, *B. cereus*, *B. fusiformis*, *B. pumilus*, *B. megaterium*, *B. mycoides*, *B. coagulans*, *B. chitinolyticus*, *B. subtilis* had been reported as phosphate solubilizing. Ambrosini et al. [9] said that *Bacillus cereus* showed the highest nitrogenase activity among 42 different strains of *Bacillus spp.* Lim



et al. [10] 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*. Thus, it is necessary to the potential test in increasing the availability of nitrogen and phosphate along with identifying microbes in biogas sludge from anaerobic digestion of POME, especially one of the palm oil mills in Labuhanbatu District, Indonesia, which has never been recognized molecularly. The study aimed to obtain the potential and identify superior bacterial isolates (nitrogen-fixing and phosphate solubilizing) from the biogas sludge.

2. Materials and methods

2.1. Study area

The isolate potential was tested at the Laboratory of Soil Biology, Faculty of Agriculture, Universitas Sumatera Utara, Medan. The analysis of total-N and available-P was conducted at the Analytical Laboratory of PT. Socfin Indonesia, Medan. Molecular identification of bacterial isolates was conducted at the Genetics Laboratory of PT. Genetics Science Indonesia, Banten. Soil sampling for the potential test was conducted in Simalingkar, Medan, Indonesia. The study was conducted from January to August 2020.

2.2. Selection of superior bacterial isolates

The biogas sludge derived the isolates used from the digester tank at the palm oil mill of PT. Nubika Jaya, Pinang City, Labuhanbatu District. A total of 1 ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water and homogenized. It put a total of 1 ml of the suspension from the dilution into 9 ml of distilled water. The dilution was made to 10^{-5} . A total of 0.1 ml of the suspension from the last dilution was spread over the James Nitrogen Free Malat Bromothymol Blue (JNFB) medium for the Nitrogen-Fixing Bacteria (NFB) isolates test. While the suspension Phosphate Solubilizing Bacteria (PSB) isolates, the test was spread over Pikovskaya (PVK) medium. The culture medium was incubated for 2 to 3 days at room temperature.

Bacteria isolates were purified and cultured in Nutrient Broth (NB) medium for two days, then inoculated one ml of isolate into an Erlenmeyer flask containing 100 g of ultisols sterilized and incubated for 14 days. The cultures were harvested by centrifugation at 100 rpm. The NFB isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of PSB isolates is indicated by a halo zone around the microbial colonies on the PVK medium. At the end of incubation, the total-N was determined by the Kjeldahl method and the available-P using the Bray-II method.

The result of the potential test was found in seven nitrogen-fixing and seven phosphate solubilizing isolates to produce total-N and available-P. The potential test was conducted in a Completely Randomized Design (CRD) within three replications and analyzed using ANOVA, followed by DMRT at $P < 0.05$.

2.3. Molecular identification of bacterial isolates

The bacteria with superior ability to produce the highest total-N and available-P were selected for each bacterial isolate, N3 and P7 isolated and identified by PCR-16S rRNA sequencing method. Identification using DNA markers in the 16S rRNA gene. 16S rRNA was amplified by the Polymerase Chain Reaction (PCR) technique using universal primers in the 27R and 1492R primers. Amplicons were visualized by electrophoresis technique on 0.8% agarose gel. The visualized DNA bands measure around 1300-1400 base pairs (Figure 1). The PCR product is continued to the sequence stage to obtain the nucleotide sequence of the target bacteria.

Sequences of N3 and P7 bacterial isolates were aligned with the bacterial sequence data through the Basic Local Alignment Tool (BLAST). The BLAST results showed that the genus of the species homologous to the two isolates was *Bacillus* and then formed phylogenetically by involving 31 nucleotide sequences from the National Center for Biotechnology Information (NCBI) (Table 1) using the MEGA 5 software [11].

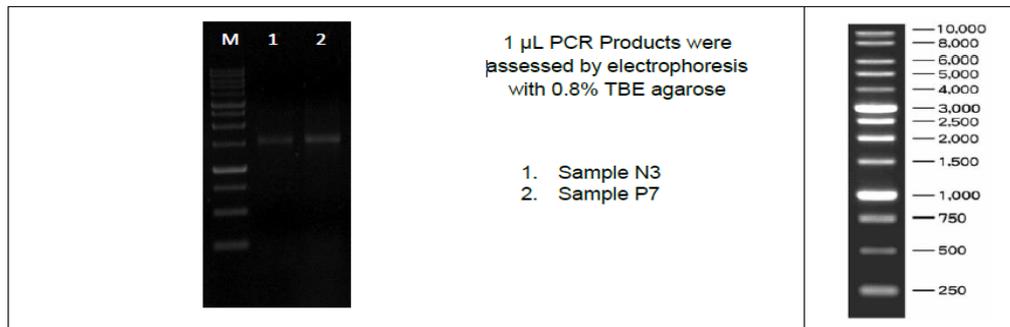


Figure 1. DNA band electrophoresis of nitrogen-fixing (N3) and phosphate solubilizing isolates (P7).

3. Results and discussion

3.1. Potential of superior bacterial isolates

The types of superior bacterial isolates (nitrogen-fixing and phosphate solubilizing) from biogas sludge significantly increased total-N and available-P (Figure 2). It was found that the N3 and P7 bacterial isolates from biogas sludge had the highest total-N and available-P abilities by 62.56% and 36.21%, respectively, compared to the untreated.

Table 1. The accession number of nucleotides of the genus *Bacillus*

No	Accession number	Bacteria strains
1	AB049195.1	<i>Bacillus</i> sp. NAF001
2	AB271757.1	<i>Geobacillus stearothermophilus</i>
3	ACMX01000133.1	<i>Bacillus pseudomycooides</i> DSM 12442
4	ACNF01000156.1	<i>Bacillus thuringiensis</i> serovar ATCC 10792
5	AF290553.1	<i>Bacillus anthracis</i> strain Vollum
6	AJ276351.1	<i>Bacillus subtilis</i> strain DSM10
7	AJ320493.1	<i>Paenibacillus polymyxa</i> strain DSM 36T
8	AJ419629.1	<i>Bacillus luciferensis</i> strain LMG 18422
9	AJ781029.2	<i>Bacillus herbersteinensis</i> strain D-15a
10	DQ374637.1	<i>Bacillus acidiceler</i> strain CBD 119
11	FJ416489.1	<i>Bacillus gaemokensis</i> strain BL3-6
12	FJ416490.1	<i>Bacillus manliponensis</i> strain BL4-6
13	FN995265.1	<i>Bacillus kochii</i> strain WCC 4582T
14	HM460884.1	<i>Bacillus zhanjiangensis</i> strain JSM 099021
15	HQ433453.3	<i>Alteribacillus bidgolensis</i> strain P4B
16	JN885201.1	<i>Bacillus bingmayongensis</i> strain FJAT-13831
17	KJ733017.1	<i>Bacillus solisilvae</i> strain NEAU-cbsb5
18	MK183820.1	<i>Bacillus paramycooides</i> strain MCCC 1A04098
19	MK184150.1	<i>Bacillus albus</i> strain MCCC 1A02146
20	NR028865.1	<i>Solibacillus silvestris</i> strain HR3-23
21	NR043403.1	<i>Bacillus thuringiensis</i> strain IAM 12077

22	NR074540.1	<i>Bacillus cereus</i> ATCC 14579 (rrnA)
23	NR074914.1	<i>Bacillus cytotoxicus</i> strain NVH 391-98
24	NR114582.1	<i>Bacillus cereus</i> ATCC 14579
25	NR152692.1	<i>Bacillus wiedmannii</i> strain FSL W8-0169
26	NR157729.1	<i>Bacillus albus</i> strain MCCC 1A02146
27	NR157735.1	<i>Bacillus proteolyticus</i> strain MCCC 1A00365
28	NR170494.1	<i>Bacillus fungorum</i> strain 17-SMS-01
29	X76437.1	<i>Bacillus cohnii</i> DSM 6307 T
30	X76443.2	<i>Bacillus horikoshii</i> DSM 8719
31	X76447.1	<i>Bacillus halmapalus</i> DSM 8723

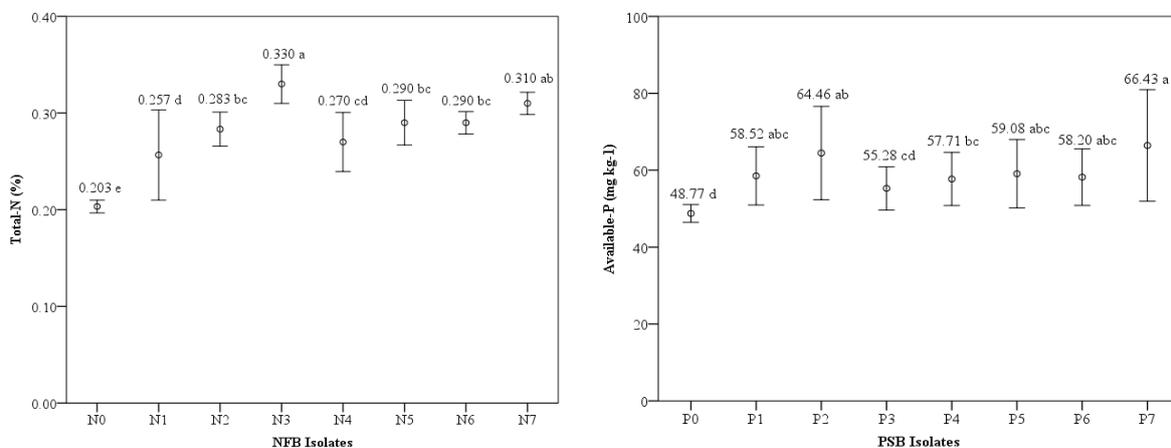


Figure 2. The potential test of Nitrogen-Fixing Bacteria (NFB) and Phosphate Solubilizing Bacteria (PSB) isolates from biogas sludge to increase the total-N and available-P of ultisols. Values followed by a different letter explain the significant difference in the DMRT at $P < 0.05$. The vertical line indicates the standard error (SE). Data PSB has been published by Mustamu et al. [12].

The results showed that bacterial isolates from biogas sludge could increase the total-N and available-P of ultisols with the highest increase in N3 and P7 isolates compared to other isolates. The nitrogen-fixing bacteria isolate (N3) contribution was higher than the phosphate solubilizing isolate (P7). This result was similar to Rodriguez-Gonzalez et al. [13]; nitrifying and denitrifying bacteria use nitrogenase enzymes to metabolize nitrogen to be available, and 54.42% are reported nitrogen-fixing bacteria. It was due to the P7 isolate as phosphate solubilizing bacteria produce organic acids characterized by a decrease in pH that the availability of phosphorus increases. Richardson and Simpson [14]; Khan et al. [15] said that phosphate solubilizing bacteria produce organic acids that solubilizing unavailable-P (PO_4^{3-}) into available-P (HPO_4^{2-} , H_2PO_4^-) and Perez et al. [16] reported a decrease in pH from 7 to 2 along with the acidification process. Mustamu et al. [12] also said that isolates of phosphate solubilizing bacteria (P7) from biogas sludge had the highest organic acids sequentially, namely lactic, oxalic, acetic, and citric acids.

3.2. Phylogenetic of superior bacterial isolates

The phylogenetic results of nitrogen-fixing (N3) and phosphate solubilizing bacteria isolates (P7) can be seen in Figure 3. It was identified the nitrogen-fixing bacteria isolate (N3) was nearby, similar to *Bacillus paramycooides* strain MCCC 1A04098 (MK183820 accession) and the phosphate solubilizing isolate (P7) to *Bacillus cereus* ATCC 14579 (NR114582 accession).

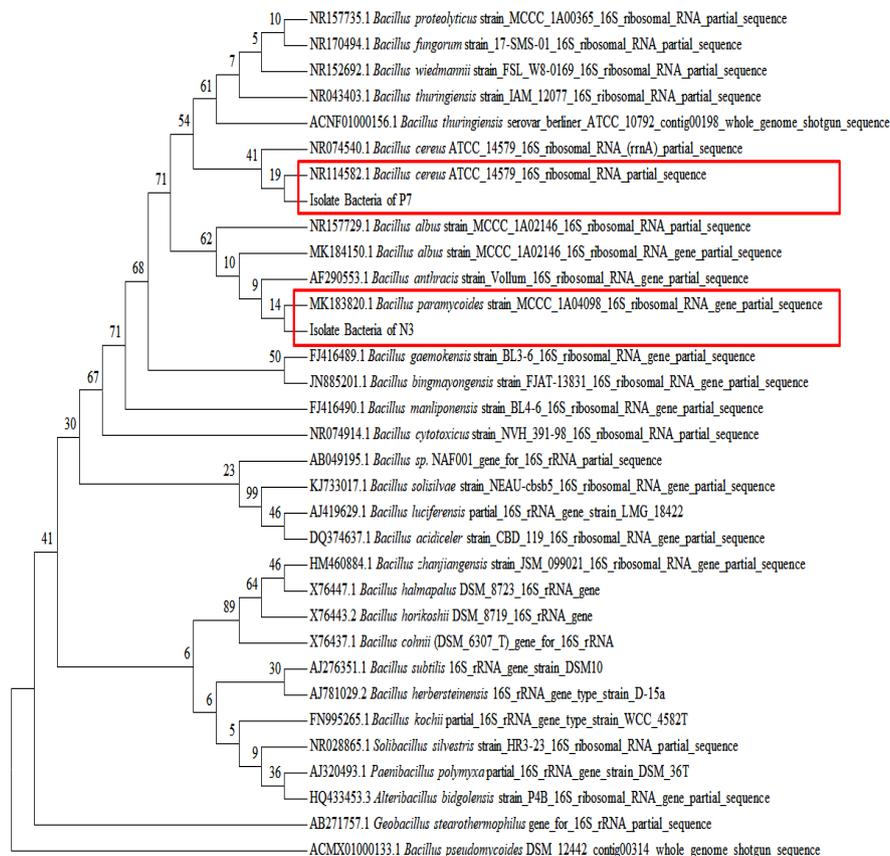


Figure 3. Phylogenetic tree of N3 and P7 bacteria isolates from biogas sludge with 31 nucleotides of the genus *Bacillus* using the neighbor-joining method with a bootstrap repeat value of 1000x with MEGA 5 software.

Both bacteria superior (N3 and P7) were identified as having the nearby similarity to the *Bacillus* genus, namely nitrogen-fixing bacteria (N3) with *Bacillus paramycooides* and phosphate solubilizing bacteria (P7) with *Bacillus cereus*. It was also reported by Ndubuisi-Nnaji et al. [17] that the anaerobic processing bioreactor contained a group of nitrogen-fixing bacteria (*Clostridium sp*, *Bacillus sp*, *Enterobacter sp*, *Clostridium sp*, *Salmonella sp*), phosphate solubilizing bacteria (*Clostridium sp*, *Bacillus sp*, *Staphylococcus sp*, *Lactobacillus sp*, *Salmonella sp*, *Enterobacter sp*, *Citrobacter sp*, *Clostridium sp*, *Pseudomonas sp*, *Micrococcus sp*). There are groups of bacteria, including nitrogen-fixing and phosphate solubilizing (*Clostridium sp*, *Bacillus sp*, *Enterobacter sp*). Safitri et al. [18] reported that the thermophilic bacteria strain D41A had 99.79% similarity to *Bacillus paramycooides*. Suksong et al. [19] said that bacteria of palm oil solid waste from 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. [20] also reported an abundance of *Bacillus*, *Clostridium*, and *Enterobacter spp* in an anaerobic digester of wastewater in producing biogas.

Conclusion

The N3 and P7 isolates from the biogas sludge had more potentials in the availability of total-N and available-P by 62.56% and 36.21%, respectively, compared to un-isolated. The nitrogen-fixing bacteria (N3) and phosphate solubilizing bacteria (P7) isolates from the biogas sludge were identified similar to *Bacillus paramycooides* and *Bacillus cereus*, respectively.

References

- [1] Shintawati, Hasanudin U and Haryanto A 2017 Characteristic of palm oil mill wastewater treatment using semicontinuous anaerobic cigar bioreactor. *Jurnal Teknik Pertanian Lampung* **6**(2) 81-88.
- [2] Poh P E, Yong W J and Chong M F 2010 Palm oil mill effluent (POME) characteristic in high crop season and the applicability of high-rate anaerobic bioreactors for the treatment of POME. *Ind. Eng. Chem. Res.* **49**(22) 11732-11740. <https://doi.org/10.1021/ie101486w>.
- [3] Adela B N, Muzzammil N, Loh S K and Choo Y M 2014 Characteristics of palm oil mill effluent (POME) in an anaerobic biogas digester. *Asian J. Microbiol. Biotechnol. Environ. Sci. Paper* **16**(1) 225-231.
- [4] Mustamu N E and Triyanto Y 2020 Nature of chemical and biological sludge biogas liquid waste oil palm. *Int. J. Innov. Sci. Res. Technol.* **5**(2) 955-957.
- [5] Tepsour M, Usmanbaha N, Rattanaya, T, Jariyaboon R, Prasertsan P and 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) 4368. <https://doi.org/10.3390/en12224368>.
- [6] Choorit W and Wisarnwan P 2007 Effect of temperature on the anaerobic digestion of palm oil mill effluent. *Electron. J. Biotechnol.* **10**(3) 376-385. <http://dx.doi.org/10.2225/vol10-issue3-fulltext-7>.
- [7] Alvionita F, Faizal M, Komariah L N and Said M 2019 Biogas production from palm oil mill effluent with indigenous bacteria. *Int. J. Adv. Sci. Eng. Inf. Technol.* **9**(6) 2060-2066. <http://dx.doi.org/10.18517/ijaseit.9.6.10462>.
- [8] Sharma S B, Sayyed R Z, Trivedi M H and Gobi T A 2013 Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, **2** 587. <https://doi.org/10.1186/2193-1801-2-587>.
- [9] Ambrosini A, Stefanski T, Lisboa B B, Beneduzi A, Vargas L K and Passaglia L M P 2016 Diazotrophic bacilli isolated from the sunflower rhizosphere and the potential of *Bacillus mycoides* B38V as biofertiliser. *Ann. Appl. Biol.* **168**(1) 93-110. <https://doi.org/10.1111/aab.12245>.
- [10] Lim J W, Ge T and Tong Y W 2018 Monitoring of microbial communities in anaerobic digestion sludge for biogas optimisation. *Waste Manage.* **71** 334-341. <https://doi.org/10.1016/j.wasman.2017.10.007>.
- [11] Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**(10) 2731-2739. <https://doi.org/10.1093/molbev/msr121>.
- [12] Mustamu N E, Nasution Z, Irvan and Sembiring M 2021 Isolation of phosphate solubilizing bacteria from anaerobic digestion sludge of palm oil mill effluent on ultisols. *Plant Cell Biotechnol. Mol. Biol.* **22**(35&36) 220-230.
- [13] Rodriguez-Gonzalez C, Ospina-Betancourth C and Sanabria J 2021 High resistance of a sludge enriched with nitrogen-fixing bacteria to ammonium salts and its potential as a biofertilizer. *Bioengineering* **8**(5) 55. <https://www.mdpi.com/2306-5354/8/5/55#>.
- [14] Richardson A E and Simpson R J 2011 Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiol.* **156**(3) 989-996. <https://doi.org/10.1104/pp.111.175448>.
- [15] Khan M S, Zaidi A, Ahemad M, Oves M and Wani P A 2010 Plant growth promotion by phosphate solubilizing fungi—current perspective. *Arch. Agron. Soil Sci.* **56**(1) 73-98. <https://doi.org/10.1080/03650340902806469>.
- [16] Perez E, Sulbaran M, Ball M M and Yarzabal L A 2007 Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biol. Biochem.* **39**(11) 2905-2914. <https://doi.org/10.1016/j.soilbio.2007.06.017>.
- [17] Ndubuisi-Nnaji U U, Ofon U A, Ekponne N I and 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. *Sustain. Environ. Res.* **30**(1) 1-11. <https://doi.org/10.1186/s42834-020-00056-6>.
- [18] Safitri R, Kusumawardhani D P, Annisa A, Partasmita R, Asharina S and Maskoen A M 2020 Characterization and identification of three thermophilic *Bacillus* strain isolated from Domas Crater,

Mt. Tangkuban Perahu, Indonesia. *Biodiversitas* **21**(8) 3444-3453. <https://doi.org/10.13057/biodiv/d210805>.

[19] Suksong W, Kongjan P, Prasertsan P, Imai T and Sompong O 2016 Optimization and microbial community analysis for production of biogas from solid waste residues of palm oil mill industry by solid-state anaerobic digestion. *Bioresour. Technol.* **214** 166-174. <https://doi.org/10.1016/j.biortech.2016.04.077>.

[20] Liaquat R, Jamal A, Tauseef I, Qureshi Z, Farooq U, Imran M and Ali M I 2017 Characterizing bacterial consortia from an anaerobic digester treating organic waste for biogas production. *Pol. J. Environ. Stud.* **26**(7) 709-716. <https://doi.org/10.15244/pjoes/59332>.