



# PRELIMINARY ASSESSMENT OF BIO-FERTILIZER POTENTIAL OF LIQUID DIGESTATE FROM NCEE PILOT ANAEROBIC BIOGAS PLANT AT THE UNIVERSITY OF BENIN

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#### **ABSTRACT**

The study was carried out to determine the suitability of liquid digestate from a pilot biogas plant of the National Centre for Energy and Environment (NCEE) University of Benin, for land application. Digestate from the anaerobic digestion of cattle rumen was characterized, specifically for heavy metal content, presence of microbacteria, nutrient profile and rhizobacterial potentials. All replicate samples of the digestate contained the metals Mn, Cu, Zn, Pb, Cd, Cr, Fe, and Ni which ranged from 12.60-16.00, 30.00-38.00, 50.00-65.00, 0.20-0.60, 0.00, 0.60-0.86, 177.00-194.00, and 1.20 -1.80 mg/kg, respectively. However, their concentrations were lower than the specified standard limits. Total phosphorus and total nitrogen concentrations were very high and ranges from 1032 to 1409 mg/kg, and 960.06-3237.62 respectively. The presence of heavy metals in the biogas digestate may have been influenced by the grasses that were regularly consumed along with various essential elements to promote optimal nutrient supply and optimum growth rates. The bacterial isolates in the processed digestates were identified to be Bacillus subtilis, Enterobacter aerogenes, Klebsiella spp., Salmonella enterica, Escherichia coli and Clostridium difficle. The isolates in the processed digestates were further assessed for their rhizobacterial potentials and intriguingly. The study found that they could have beneficial effects on plants in that they could fix atmospheric nitrogen, solubilize and produce ammonia and Bacillus subtilis was also discovered to produce the plant growth hormone.

**Keywords:** Anaerobic, Digestate, Heavy, Metals and Microbial

#### INTRODUCTION

The population of Nigeria and indeed economic development have increased the demand for food and its consumption, leading to a considerable expansion of the agricultural needs of the country. More so, coupled with the negative effects of climate change on agricultural operations and crop quality, the food security of the country faces greater threat. Moreover, this has implications for national development since the agricultural sector is one of the mainstay of the economy, contributing 22.35% of overall GDP in real terms in Q1 2021, (National Bureau of Statistics, 2021) and employs 70 percent of the labour force in the country (Anderson *et al.*, 2017).

Unfortunately, the intensive use of mineral fertilizers has adverse impact on soil, surface and ground water resources. Furthermore, their residues in food above desirable safe level of exposure has negative effects on human and animal health. Similarly, chemical fertilizer saturation in agriculture has been linked to reduced soil absorption capacities with only 30–50% of fertilizers applied being retained in soils therefore leading to intensification of fertilizers usage in the future (Wu, *et al.*, 2021). In most regions, Qiao *et al.* (2025) reported





that about 20–70% of the fertilizer is lost to the environment as dissolved nutrients, greenhouse gases, and other pollutants. Other studies have reported that over dependence on inorganic chemical fertilizers has led to decline in soil quality, eutrophication and heavy metals pollution (Owamah *et al.*, 2014; Zhu *et al.*, 2023).

In contrast, the application of organic amendments and the consequential reduction in mineral fertilizers are considered more environmentally-friendly and economically feasible approaches that may enhance the development of more sustainable agriculture and contribute to a circular economy through the exploitation of organic byproducts (Ning *et al.*, 2017). One recent technology that has shown prospect in both clean, affordable energy with long term implication for food security is anaerobic digestion (AD) of organic waste. Digestate from organic waste is considered as an important by-product of anaerobic digestion from organic waste, which includes manure, crop residues, urban solid waste, sewage sludge, etc (Tsapekos *et al.*, 2021; Gross *et al.*, 2021). In addition, within the framework of the Sustainable Development goals (SDG), AD is a significant pillar towards guaranteeing cheap and sustainable energy (SDG 7), while also contributing to additional objectives (SDGs 1, and 2) all of centered on poverty eradication and Zero hunger.

Moreover, in view of threats of global warming and associated climate change, biofertilizer from AD digestate is best considered sustainable for food production which has the potential to reduce dependence on inorganic fertilizers. For example, studies have shown that digestate from organic wastes contains latent cells of effective microorganisms that assist the absorption of nutrients by crop plants when applied via seed or soil by their interactions in the rhizosphere (Di Maria et al., 2017; Yasar et al., 2017). Digestate has been reported to increases the content of organic compound in the soil and, at the same time, reduces the rate of its transformation in comparison to non-digested input materials (Chen et al., 2012; Lopedota et al. 2013). However, the application of digestate for bio-fertilizer can sometimes cause nitrate and phosphate pollution of groundwater (Piccoli et al., 2021; O'Connor et al., 2022). Other studies have reported that the use of digestates involves a high risk of soil and plant contamination with heavy metals and toxic compounds (Kupper et al., 2014; Nkoa 2014; Montusiewicz et al., 2020). Digestates also contain a high microbial load and therefore require treatment prior to the application to soil (Gómez-Brandón et al., 2016).

However, for sustainable use of digestate as alternative for inorganic fertilizers, there is need for profiling and/or detailed investigation of constituents of digestate samples, including physico-chemical parameters, heavy metals, nutrients, microbial loads etc, in order to ensure compliances with environmental safe limits. It against this background that the present study was carried out to determine the suitability of liquid digestate from an ongoing pilot biogas project of the National Centre for Energy and Environment, University for possible use as biofertilizer.

#### MATERIALS AND METHODS

#### **Sample Collection and Chemical Analyses**

Digestate was obtained from mesophilic semi-continuous anaerobic digester (35 ± 1°C) located at the National Centre for Energy and Environment, University of Benin. Samples were stored at 4°C until their physicochemical, microbiological and ecotoxicological characterization. Digestate characterization parameters; Potassium (k) total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), Nitrate, Total Nitrogen (TN), Total Ammonium Nitrogen and metals (Mn, Cu, Zn, Pb, Cd, Cr, Fe, Ni) were measured following protocols established by APHA (2005). All laboratory analysis was carried out at Air Quality/Gas Geochemistry/ Diamond ENERGYTECH Company Limited, Warri, Delta State.





### **Digestate Characterization**

To understand the impact of digestate disposal a key aspect is its composition, especially in terms of polluting substances. The characterization of the digestate determined the content of heavy metals load, organic matter content and plant-available nutrients concentrations, as well as possible harmful properties, such as pathogens. Raw digestate withdrawn from anaerobic digester is a liquid to thick slurry that contains a significant quantity of solids. Liquid fraction after solid-liquid separation contain 90-95% of total mass of digestate in most liquid anaerobic digestion plants (Sheets *et al.*, 2015). The heavy metals analyzed were cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), Zinc (Zn), nickel (Ni), Iron (Fe) and zinc (Zn), which were selected based on the certification rules established by the Swedish Waste Management Association (Sverige, 2020). The heavy metals in the digestate samples were measured according to standardized methods. Cadmium, chromium, and lead were analyzed by inductively coupled plasma mass spectrometry (ICP)-MS, copper, nickel and zinc by ICP-atomic emission spectroscopy (AES), and mercury by atomic fluorescence spectroscopy (AFS). All analyses were performed according to ISO 11466/EN13346 and APHA 2005 (APHA, 2005).

#### Microbiological Analysis

The pour plate method was used for microbial isolation and enumeration of viable microorganisms present in the digestate. From the dilutions of each sample, 0.1 ml aliquot was transferred aseptically into freshly prepared nutrient agar plates and spread evenly on the medium in duplicates. The inoculated plates were incubated at 37°C for 24 hours after which, plates were examined for growth. Microbial characterization and identification was done on the basis of cultural appearance of colony, morphology, differential and selective media and by conventional biochemical tests (Cheesebrough, 2005). Gram staining and conventional biochemical test as urease, indole, citrate utilization, coagulase, oxidase and sugar fermentation tests were carried out to determine differential and selective media.

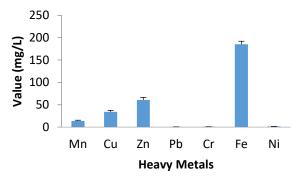
## RESULTS AND DISCUSSION

#### **Heavy metals content**

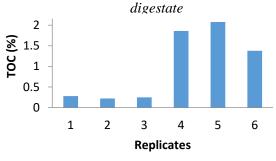
Due to the methane content of the biogas, anaerobic digestion is becoming a more common process for the treatment of organic waste and the production of renewable energy. The waste that is left over after treatment is produced by this biological process as digestate, which has a high fertilizing potential. Due to the different organic wastes used as feedstock, the various plant configurations and the employed post-treatment processes, the digestate composition is highly variable. The agronomic characterization of digestate is crucial in order to optimize digestate spreading on agricultural soils by optimizing the fertilizer dose and thereby reducing the environmental effects associated with digestate application (Zennaro et al., 2022). Table 1 lists the final concentrations of heavy metals in the digestate. The results are contrasted with the limit values specified in Turkish, Swedish, and SPCR regulations. In reality, there is no specific law that governs the use of agricultural biogas digestate on land. However, it is crucial to control the maximum heavy metal content in digestate from cattle rumen treatment plants used for land application. As a result, these laws (Turkish, Swedish, and SPCR) were compared in this work. Mn, Cu, Zn, Pb, Cd, Cr, Fe, and Ni concentrations in the digestate ranges from 12.60 to 16.00, 30.00 to 38.00, 50.00 to 65.00, 0.20 to 0.60, 0.00, 0.60-0.86, 177.00 to 194.00 and 1.20 to 1.80 mg/kg, respectively. All of these concentrations fell below the regulated maximums (Figure 1 and Table 1).







**Figure 1**: Levels of heavy metals in



**Figure 2**: *TOC concentration in cattle rumen digestate* 

Table 1: Physiochemical properties of digestate

NI4	3.51 3.5	-	3.5 000			
Ni	1.20-1.80	0.60	1.50±0.22	50	400	30
Fe	177-194	17.00	$185.17 \pm 6.85$			
Cr	0.60-0.86	0.26	$0.78\pm0.09$	100	120	100
Cd	0.00	0.00	0.00	1	40	1
Pb	0.20-0.60	0.40	$0.43\pm0.19$	100	1200	50
Zn	50.00-65.00	15.00	60.67±5.79	800	4000	1500
Cu	30.00-38.00	8.00	34.33±3.01	600	1750	1000
Mn	12.60-16.00	3.40	13.87±1.55			
Heavy Metals	Min-Max	Range	Mean±SD	2020)	Standard	Standard
				SPCR(Sverige	Turkish	Norway

Nutrient	Min-Max	Range	Mean±SD
K (cmol/kg)	1.31-4.46	3.15	2.34±1.13
TOC (%)	0.22-2.08	1.86	1.012±0.86
TK (mg/kg)	588.90-978.90	390	724.10±187.19
TP (mg kg-1)	1032-1409	377	1231.00±181.01
Total Ammonium			
Nitrogen(mg/kg)	957-3237	2280	2143.42±1189.88
Nitrate	2.37-2.76	0.39	2.58±0.19
Nitrite	0.27-0.66	0.39	$0.47 \pm 0.19$
TN (Mg/kg)	960.06-3237.62	2277.6	2145.83±1189.22

#### **Nutrient profile**

The digestate can be used as a plant fertilizer directly or after further processing, contributing to the closing of global energy and nutrient cycles (Alburquerque *et al.*, 2012; Möller and Müller, 2012). According to Jain *et al.* (2019), digestate could replace 5–7% of the inorganic macro nutrient fertilizer currently used globally if the potential of biogas industry were fully utilized. Digestate is generally spread directly onto fields, like manure (Odhner *et* 

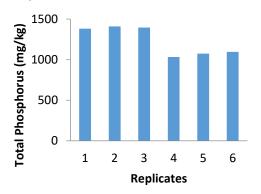




al., 2015). However, due to the high share of plant-available macro- and micro-nutrients, the nitrogen (N) content in a digestate from cattle rumen feedstock may typically be small of the dry weight, digestate also has the potential to replace mineral fertilizers in protected horticulture.

Due to the high content of nutrients, mainly nitrogen, this product can be successfully used as a fertilizer. This study compared the digestate sample total nitrogen ( $N_{tot}$ ) and its selected fractions from five other replicates. The nitrogen fractions included N-NH<sub>4</sub>, N-NO<sub>3</sub>, and Norganic. Samples used for the research came from six (6) replicates in total. The results obtained showed that digestate from the fixed dome is a nitrogen-rich fertilizer. The content of  $N_{tot}$  in the tested samples ranges from 960.06mg/kg to 3237.62mg/kg. The N-NH<sub>4</sub> content in the analyzed material ranges from 957 to 3237 mg/kg. The ammoniacal nitrogen (N-NH<sub>4</sub>) content in the analyzed material ranges from 957 to 3237 mg/kg (Table 1).

The lowest content of N-NH<sub>4</sub> was found from the digestate solid fraction for biogas replicate 6 and the highest was found in replicate 2. Total organic carbon (TOC) in the six (6) samples replicates varied from 0.22-2.08 (%), respectively. High TOC was observed in cattle rumen digestate in replicates 4, 5 and 6. However, TOC in replicates 1-3 digesters were still very low compared to TOC of cattle rumen digestate from replicates 4-6 (Fig. 2). The primary nutrients in manure are phosphorous and nitrogen, therefore the effect of manure processing on the biogeochemical P cycle may be pertinent. Studies have shown that, solid fractions of cattle rumen digestate show significantly higher concentrations of phosphorus. Total phosphorus (TP) concentration ranges from 1032 to 1409 mg/kg, while Total Nitrogen (TN) ranges from 960.06-3237.62. Replicates samples 1-3 had the highest compared to 3-6 (Figs. 3 and 4).



3500 Fotal Nitrogen (mg/kg) 3000 2500 2000 1500 1000 500 0 1 2 3 4 5 6 Replicates

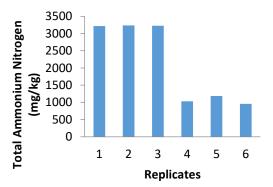
**Figure 3**: Total available phosphorus in cattle rumen digestate

**Figure 4**: Total available nitrogen in cattle rumen digestate

The lower C:N ratio in the digestates may be as a result of NH4+ accumulation during the AD process (Monlau *et al.*, 2015). The high ammonium concentration may help balance low nitrogen feedstock like lignocellulosic biomass (Fig 5).







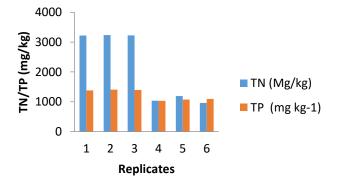


Figure 5: *Total ammonium nitrogen* in cattle rumen digestate

**Figure 6**: Total nitrogen and Total phosphorus present in digestate

Nitrogen (N) and NH4 + concentrations in the six (6) replicate digestate samples from cattle rumen is important to avoid excessiveness in case of land application where maximum N application is 150–250 kg N/ha/y according to the European Commission (Saveyn and Eder, 2014). Figure 5 shows TKN concentrations and its distribution into suspended particles, coarse colloids, fine colloids and dissolved matter (NH4 + and dissolved organic nitrogen) (Fig 6).

#### Microbial characterization digestate samples

The occurrence and levels of the eight microorganisms investigated in the anaerobic digestate samples from the selected replicates are shown in Table 2. All investigated microorganisms showed similar occurrence and levels at the six replicate samples. The suitability and usage of cattle rumen digestates produced after five weeks of anaerobic digestion as alternative sources of biofertilizer was assessed. The bacterial isolates in the processed digestates were identified to be *Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella spp.*, *Salmonella enterica*, *Escherichia coli* and *Clostridium difficle*. The isolates in the processed digestates were further assessed for their rhizobacterial potentials and intriguingly, it was discovered that they had beneficial effects on plants in that they could fix atmospheric nitrogen, solubilize and produce ammonia, and *Bacillus subtilis* was also discovered to produce the plant growth hormone (Indole-3-acetic acid) (Table 3).





Table 2: Results of microbes in cattle rumen (CR) digestate

Week 0	Week 1	Week 2	Week 4	Week 5
E. coli	E. coli	E. coli	E. coli	E. coli
Pseudomonas aeruginosa	Klebsiella spp.	Klebsiella spp.	Klebsiella spp.	Klebsiella spp.
Klebsiella spp.	Enterobacter	Enterobacter	Enterobacter	Enterobacter
Enterobacter	Salmonella enterica	Salmonella enterica	Salmonella enterica	Salmonella enterica
Salmonella enterica	Bacillus subtilis	Bacillus subtilis	Bacillus subtilis	Bacillus subtilis
Bacillus subtilis	Bacillus cereus	Bacillus cereus	Bacillus cereus	Bacillus cereus
Bacillus cereus	Clostrdium spp.	Clostrdium spp.	Clostrdium spp.	Clostrdium spp.
	Clostridium difficle	Clostridium difficle	Clostridium difficle	Clostridium difficle

Table 3: Bacterial isolates from the digestates and their plant growth promoting characteristics

	Plant growth promoting properties				
<b>Bacteria Identity</b>	N <sub>2</sub>	NH <sub>3</sub>	IAA	PSB	
Bacillus sp.	+	+	+	+	
Enterobacter	+	-	-	+	
Salmonella	+	+	-	+	
E. coli	+	+	-	+	

Key:  $N_2$  = Nitrogen fixation capacity,  $NH_3$  = ammonia production, IAA = Indole Acetic Acid production, PSB = Phosphate Solubilization Capacity

#### CONCLUSION AND RECOMMENDATIONS

The findings suggested that agricultural applications could make use of the biogas digestate produced by the pilot anaerobic digestion of cattle rumen project. In terms of heavy metals content, this study indicated that the biogas digestate from cattle rumen could be utilized for agricultural activities. Since such agricultural residues have no commercial use (they are discarded as waste) and the cattle rumen has to be managed properly in an environmentally friendly manner, the end products of anaerobic digestion process, such as the digestate, can be used in rural areas for agricultural purposes. In addition to the heavy metals content, the pathogenic bacteria and mineral nitrogen (N) contents of the biogas digestate should also be taken into account when evaluating the potential use of the biogas digestate for soil applications.

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#### **REFERENCES**

Akhiar, A. (2017). Characterization of liquid fraction of digestates after solid-liquid separation from anaerobic co-digestion plants. *Chemical and Process Engineering*. Université Montpellier, 2017. English. ffNNT: MONTS004ff.





- Anderson, J., Marita, C., Musiime, D. and Thiam, M (2017). *National Survey and Segmentation of Smallholder Households in Nigeria*. Understanding Their Demand for Financial, Agricultural, and Digital Solutions. CGAP Working Paper p. 1-89
- British Standards Institution [BSI] (2014). Specification for Whole Digestate, Separated Liquor and Separated Fibre Derived from the Anaerobic Digestion of Source-segregated Biodegradable Materials. London.
- Chen, S., Chen, B. and Song D (2012). Life-cycle energy production and emissions mitigation by comprehensive biogas—digestate utilization, *Bioresource Technology*, **114**:357-364, https://doi.org/10.1016/j.biortech.2012.03.084.
- Di Maria, F., Barratta, M., Bianconi, F., Placidi, P. and Passeri, D. (2017). Solid anaerobic digestion batch with liquid digestate recirculation and wet anaerobic digestion of organic waste: Comparison of system performances and identification of microbial guilds. *Waste Management*. **59**:172-180. doi: 10.1016/j.wasman.2016.10.039. PMID: 27816470.
- Edith, K., Francis, K., Martin, K. and Felix, K. (2019). Characterization of Digestates from Anaerobic Co-Digestion of Manioc Effluent, Human Urine and Cow Dung. *Journal of Water Resource and Protection*, **11**(6):777-788. doi: 10.4236/jwarp.2019.116047.
- Gómez-Brandón, M., Fernández-Delgado M. J., Zangerle, M. and Insam, H. (2016). Effects of digestate on soil chemical and microbiological properties: A comparative study with compost and vermicompost, *Journal of Hazardous Materials*, **302**:267-274, https://doi.org/10.1016/j.jhazmat.2015.09.067.
- Goswami, D., Thakker, J. N. and Dhandhukia, P. C. (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Food and Agriculture* **2(1)**:1-9.
- Govindasamy, V., Senthilkumar, M., Magheshwaran, V., Kumur, U., Bose, P., Sharma, V. and Annapurna, K. (2011). *Bacillus* and *Paenibacillus* spp.: Potential PGPR for susitaniable agriculture. In: D. K. Maheshwari (Editor), *Plant growth and health promoting bacteria* 333-364. Berlin, Springer-Verlag.
- Gross, T., Breitenmoser, L., Kumar, S., Ehrensperger, A., Wintgens, T. and Hugi, C. (2021). Anaerobic digestion of biowaste in Indian municipalities: effects on energy, fertilizers, water and the local environment. *Resources, Conservation and Recycling*, **170**:105569, https://doi.org/10.1016/j.resconrec.2021.105569
- Huygens, D., Orveillon, G., Lugato, E., Tavazzi, S., Comero, S., Jones, A., Gawlik, B. and Saveyn, H. G. M. (2020). *Technical proposals for the safe use of processed manure above the threshold established for Nitrate Vulnerable Zones by the Nitrates Directive* (91/676/EEC), EUR 30363 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-21539-4, doi: 10.2760/373351, JRC121636.
- Jin, W., Xu, X. and Yang, F. (2018). Application of Rumen Microorganisms for Enhancing Biogas Production of Corn Straw and Livestock Manure in a Pilot-Scale Anaerobic Digestion System: Performance and Microbial Community Analysis. *Energies*, **11**(4):920. https://doi.org/10.3390/en11040920.
- Jubaedah, D., Sasanti, A. D., Marsi and Mukhlas, M. A. (2020). Application of organic fertilizer on swamps pond for catfish (*Pangasius sp.*) culture IOP Conf. Ser.: *Earth and Environmental Science* **521** (2020): 012002.
- Kadam, R., Khanthong, K., Jang, H., Lee, J. and Park, J. (2022). Occurrence, Fate, and Implications of Heavy Metals during Anaerobic Digestion: A Review. *Energies*. **15**(22):8618. https://doi.org/10.3390/en15228618.





- Kupper, T., Bürge, D., Bachmann, H. J, Güsewell, S. and Mayer, J. (2014). Heavy metals in source-separated compost and digestates. *Waste Management* **34**(5):867-74. doi: 10.1016/j.wasman.2014.02.007. Epub 2014 Mar 7. PMID: 24613591.
- Lopedota, O., Leogrande, R., Fiore, A., Debiase G. and Montemurro, F. (2013). Yield and Soil Responses of Melon Grown with Different Organic Fertilizers, *Journal of Plant Nutrition*, **36**(3) 415-428, Doi: 10.1080/01904167.2012.748062
- Möller, K. (2009). Influence of different manuring systems with and without biogas digestion on soil organic matter and nitrogen inputs, flows and budgets in organic cropping systems. *Nutrient Cycling in Agroecosystems* **84**, 179–202 https://doi.org/10.1007/s10705-008-9236-5.
- Montusiewicz, A., Szaja, A., Musielewicz, I., Cydzik-Kwiatkowska, A. and Lebiocka, M. (2020). Effect of bioaugmentation on digestate metal concentrations in anaerobic digestion of sewage sludge. PLoS ONE **15**(7): e0235508. https://doi.org/10.1371/journal.pone.0235508
- National Bureau of Statistics [NBS] (2021): Nigerian Gross Domestic Product Report (Q1 2021), Central Business District Abuja, Nigeria
- Ning, C. C., Gao, P. D., Wang, B. Q., Lin, W. P., Jiang, N. H., and Cai, K. Z. (2017). Impacts of chemical fertilizer reduction and organic amendments supplementation on soil nutrient enzyme activity and heavy metal content. *Journal of Integrated. Agriculture* **16**:1819–1831. doi: 10.1016/S2095-3119(16)614-764
- Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. *Agronomy for Sustainable Development*, **34**(2):473-492. doi:10.1007/s13593-013-0196-z.
- O'Connor, J., Mickan, B. S., Rinklebe, J., Song, H., Siddique, K. H. M., Wang, H., Kirkham, M. B. and Bolan, N. S. (2022): Environmental implications, potential value, and future of food-waste anaerobic digestate management: A review. *Journal of Environmental Management* **15**:318:115519. doi: 10.1016/j.jenvman.115519. Epub 2022 Jun 15. PMID: 35716555.
- Owamah, H. I., Dahunsi, S. O, Oranusi, U. S. and Alfa, M. I., (2014). Fertilizer and sanitary quality of digestate biofertilizer from the co-digestion of food waste and human excreta, *Waste Management*, **34**(4):747-752, ISSN 0956-053X, https://doi.org/10.1016/j.wasman.2014.01.017.
- Parra-Orobio, B. A., Rotavisky-Sinisterra, M. P. and Pérez-Vidal, A. (2021). Physicochemical, microbiological characterization and phytotoxicity of digestates produced on single-stage and two-stage anaerobic digestion of food waste. *Sustain Environ Res* **31**:11 https://doi.org/10.1186/s42834-021-00085-9.
- Piccoli, I., Virga, G., Maucieri, C. and Borin, M. (2021). Digestate Liquid Fraction Treatment with Filters Filled with Recovery Materials. *Water*. **13**(1):21. https://doi.org/10.3390/w13010021
- Qiao, C., Liu, L., Hu, S., Compton, J. E., Greaver, T. L. and Li, Q., (2015). How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input," *Global Change Biology*, **21**(3):1249–1257
- Reyes, G. E. and Cortés, J. D. (2017). Intensidad en el uso de fertilizantes en América Latina y el Caribe (2006-2012). *Bioagro*, **29**(1):45-52.
- Sheets, J. P., Yang, L., Ge, X., Wang, Z. and Li, Y. (2015). Beyond land application: emerging technologies for the treatment and reuse of anaerobically digested agricultural and food waste. *Waste Management*, **44**:94–115.





- Siddikee, M. A., Chauhan, P. S., Anandham, R., Han, G. H. and Sa, T. (2010). Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. *Journal of Microbiology and Biotechnology* **20**: 1577-1584.
- Sirohi, G., Upadhyay, A., Srivastava, S. P. and Srivastava, S. (2015). PGPR mediated Zinc biofertilization of soil and its impact on growth and productivity of wheat. *Journal of Soil Science and Plant Nutrition*, **15**(1):115-132.
- Sverige, A. (2020). Certification rules for digestate from biodegradable waste by the quality assurance system of Avfall Sverige—The Swedish Waste Management Association. https://www.yumpu.com/en/document/view/42962058/swedish-waste-management-2010-avfall-sverige
- Tsapekos, P., Khoshnevisan, B., Alvarado-Morales, M., Zhu, X., Pan, J., Tian, H. and Angelidaki, I. (2021). Upcycling the anaerobic digestion streams in a bioeconomy approach: A review, *Renewable and Sustainable Energy Reviews*, **151**:111635, https://doi.org/10.1016/j.rser.2021.111635.
- Wu, S., Thapa, B., Rivera, C. and Yuan, Y. (2021) "Nitrate and nitrite fertilizer production from air and water by continuous flow liquid-phase plasma discharge," *Journal of Environmental Chemical Engineering*, **9**(2): 104761.
- Yasar, A., Rasheed, R., Tabinda, A. B., Tahir, A. and Sarwar, F. (2017). Life cycle assessment of a medium commercial scale biogas plant and nutritional assessment of effluent slurry. *Renewable and Sustainable Energy Reviews*, **67**(C):364-371.
- Zennaro, B., Marchand, P., Latrille, E., Thoisy, J. C., Houot, S., Girardin, C., Steyer, J. P., Béline, F., Charnier, C., Richard, C., Accarion, G. and Jimenez, J. (2022). Agronomic characterization of anaerobic digestates with near-infrared spectroscopy. *Journal of Environmental Management*. **317**:115393. doi: 10.1016/j.jenvman.2022.115393.
- Zhu, X., Ros, G. H., Xu, M., Cai, Z., Sun, N., Duan, Y. and de Vries, W. (2023). Long-term impacts of mineral and organic fertilizer inputs on nitrogen use efficiency for different cropping systems and site conditions in Southern China, *European Journal of Agronomy*, **146**: 2023, 126797 https://doi.org/10.1016/j.eja.2023.126797