__Original Research Article__

GC-MS ANALYSIS OF BIOGAS FROM PINEAPPLE PEELS AND TOXICOLOGICAL EVALUATION OF GENERATED EFFLUENT

ABSTRACT

Aim: To determine the composition of gases in the biogas produced from pineapple peels and to evaluate the effect of effluent from pineapple peel biodigester (EPB) on theliver functions of Catfish (*Clariasgariepinus).*

Methodology: Pineapple peels were grounded, fed into an anaerobicbiodigester and emitted biogas was collected forGC-MS analysis. Twenty Catfish (*Clariasgariepinus)* were grouped into four: Group I: Control (placed in fresh water for 2 days); Group II: (placed in undiluted EPB for six hours), Group III: (placed in undiluted EPB for 2 days), Group IV: (placed in 1:10 dilution of EPB for 2 days). The serum activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) in catfish were determined using standard methods.

Result: Theidentified gases (percentage per volume)were: hydrogen (0.42%), methane (94.02%), carbon monoxide (0.36%), carbon dioxide (2.03%), hydrogen sulphide (0.89%), water (0.13%), nitrogen (1.52) and oxygen (0.63). Aside from methane, the rest gases were impurities. Emission of biogas was observed under 24 hours. All the fish in group III died before the twelfth hour. Compared with control, similar results for serum activities of AST, ALT and ALP were found in group IV. On the contrary, a significant increase ($P < 0.05$) in the activities of serum AST, ALT, and ALP was found in group II. In conclusion, biogas with a high percentage per volume of methane gas (94.02%) with negligible impurities was produced from pineapple peels in the present study. The present study also found that undiluted effluent from pineapple peelsanaerobic biodigester was toxic to the liver of catfish, while a 1:10 dilution of the effluent was nontoxic to the liver of catfish. Therefore, the disposal of pineapple peels by converting it to biogas is highly recommended, however effluents generated should be diluted before disposal into the environment.

Comment [A1]: The physical and chemical characters of effluent ofter biomethanation is more relevent, since effluent is tested for toxicological properties.

Keywords: [Biogas, GC-MS*,* methane*,* pineapple peels*,* effluents, AST, ALT, ALP)

1. INTRODUCTION

Pineapple (*Ananascomosus*) is the third most economically important tropical fruit in the world and the top ten producers of pineapple are Thailand, the Philippines, China, Brazil, India, Nigeria, Costa Rica, Mexico, Indonesia and Kenya [1]. Pineapple is mainly used in juice and jam industries [1] and ready to eat slices of the core of pineapple fruit are commonly sold along Nigerian road sides and markets, where it is usually sold as snack after peeling off the back, slicing it and tying it in a nylon or fruit pack[2]. The processing of pineapple into its marketable products generates huge peels and leaves as wastes which poses a great deal of environmental pollution [3]. Pineapple peel wastes mainly consist of cellulose, hemicellulose, sucrose, fructose, glucose and other nutrients which could be fermented through anaerobic digestion for renewable energy sources and additional income to farmers [4].

Anaerobic digestion which occurs in the absence of oxygen, converts organic matter into biogas with methane gas production acting as a most important and critical step in the digestion / degradation process [5]. The microbial communities that inhabit the large intestine of humans and the specialized fore stomachs of some herbivores naturally carry out anaerobic degradation. The nonmethanogenic microorganisms first ferment biodegradable substances into short chain volatile fatty acids, hydrogen gas (H_2) and carbon dioxide (CO_2), while the methanogenic microorganisms use the H_2 to reduce CO_2 to methane gas (CH_4), an inflammable and combustible gas [5]. Co-digestion of plant sources with livestock waste and sewage sludgehas been reported to enhance biogas production with the livestock waste and sewage sludge serving as a conventional source of methanogens [6]. Anaerobic digestion technology is a reliable and simple technology and the biogas produced from anaerobic digestion can be used for cooking, lightening homes, industrials water heating and for running combustion engine [7]. Also the technology can be operated from individuals systems to large production systems and it is a neutral and ecofriendly bioenergy for environment [5, 6, 7].

The production of biogas from pineapple peels has been reported in previous studies. In one study, biogas production from pineapple waste was optimized to yield 1.98m^3 biogas when the temperature was set at 30 ^{0}C, pH, 6.0 and mixing ratio of pineapple and livestock wastes set at 62.5% [8]. In another study, single batch loading was found to be more effective than fed batch loading and biogas was produced from the pineapple peels after 20 days with 48% methane at an optimum pH of 7, carbon to nitrogen ratio of 20:1 and an organic loading rate of 1 kg of waste/m^3/day [9]. Several other studies on the optimization of biogas production from pineapple wastes are available in literature [10, 11, 12], however, data on the level of gaseous impurities present in biogas generated from pineapple peels is limited in literature. This gap in research is what the present study intends to fill. Undesirable level of gaseous impurities in the biogas mixtures is hazardous to human health and corrodes operational equipment [13].

The use of biogas effluent as a rich source of nutrients for cultivation has been previously reported. However, a high concentration of heavy metal has been reported in the biomass grown on effluent-based mediums [14].Waste water / effluents contain contaminants which can be taken up by plants into the food chain or can enter the aquatic environment and damage tissues and organs of aquatic animals especially the liver [15]. Damage to liver cells and functionsor abnormal levels of liver enzymes and metabolites are often used to evaluate toxicity [16, 17]. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream has been previously reported [18]. Given that different sources of effluents generate different contaminants [14, 15, 18], thus evaluation of the biosafety of effluents before using it for cultivation or before discharging it into aquatic bodies is important.In most rural communities, freshwater bodies serve as drinking water sources [19] and pollution of such fresh water bodiescan lead to diseases [20]. Therefore, **the aim of the present study was to determine the composition of gaseous impurities and the percentage of methane in the biogas produced from pineapple peels. Evaluation of the effect of effluent from pineapple peel biodigesteron the activities of liver enzymes in catfish (*clariasgariepinus)*was also carried out.**

Comment [A2]: Two diffesrent objectives
1.Evaluation of biogas for its composition produced from pine apple
2. Impact of effluent on fish
It is essential to address the effluent characters to complement second objective

2. MATERIAL AND METHODS

2.1Anaerobic biodigestion

Pineapple peels were collected from Toru – Orua, Sagbama, Tombia and Swali marketsin Bayelsa State. The peels were weighed (Figure 1), grinded into fine consistency using a grinding machine, mixed with cow dung andwater and fed into an anaerobic biodigester. The biodigester was custom built from a PVC tank with three ports created; one for loading pineapple waste into the biodigester (fed batch operation mode), the other for effluent discharge from the biodigester and the third for the passage of emitted biogas into the gas storage tank[9].

Comment [A3]: Provide details of pine apple and cattle dung ratio, total solids concentration in slurry, loading rate, retention time, volume of digester and chemical composition of substrates used.

2.2 Gas chromatography-mass spectrophotometry (GC-MS) analysis of the biogas generated

GC-MS analysis of the biogas generated from the pineapple peels was carried out as previously described [21].

Comment [A4]: Give the procedure in brief, solvents, column, detector used and the components estimated.

Figure 1. A photograph of pineapple peels on a weighing scale

2.3 Toxicological evaluation of effluent discharged from pineapple peel biodigester

Twenty Catfish (*Clariasgariepinus)* weighing 500 ± 10 g were grouped into four groups of five fish each: Group 1: Control (catfish placed in fresh water for 2 days); Group 11: (catfish placed in undiluted EPB for six hours), Group III: (catfish placed in undiluted EPB for 2 days), Group IV: (catfish placed in 1:10 dilution of EPB for 2 days).**By the end of the duration,** the activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) in the liver of the fish in each experimental group were determined using Randox kits and the manufacturer's protocol was followed as previously described [22].

Comment [A5]: Provide the details of effluent for the purpose of comparison of digestion efficiency, organic matter input to biogas output, BOD and COD of effluent used in the study. Without this information, this study is incomplete, supplementary table may be included.

2.4 Statistical analysis

SPSS version 24 was used for data analysis. Values were expressed as mean ± standard error of mean and percentages. One Way Analysis of Variance (ANOVA) was used to determine significant differences between groups and the level of significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Qualitative identification of the gas components in the biogas from pineapple peels

Shown in Figure 2 is the **qualitative identification of the gas components in the biogas from pineapple peels** as indicated by the peaks of their retention time in GC-MS using hexanoic acid as the carrier gas. Eight gases were identified in the biogas generated from pineapple peels, they were hydrogen, methane, carbon monoxide, carbon dioxide, hydrogen sulphide, water, nitrogen gas and oxygen. Aside from methane which is the gas of interest, the rest gases were impurities. In a previous study in California, methane, carbon dioxide, nitrogen, and oxygen were the gas components found in the biogas produced from feedstock [23]. In another study, methane gas, carbon dioxide gas, hydrogen sulphide gas, and oxygen gas were the components of the biogas produced from cafeteria food, vegetables, fruit and cattle manure [24].This shows that different raw materials for biogas production generates different impurities and emphasizes the need to always identify the composition of impurities in biogas.

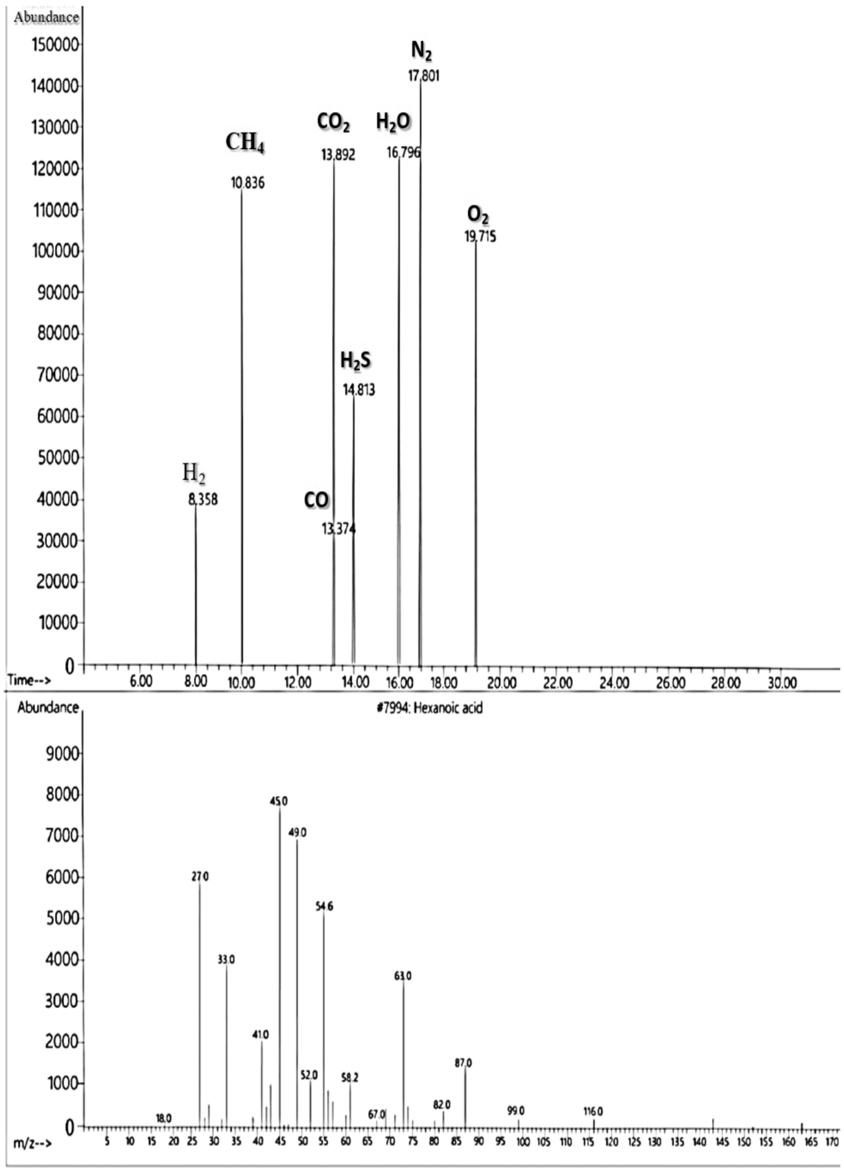

Figure 2 GC-MS chromatogram of gases in the biogas produced from pineapple peels

3.2**Quantitative determinationof the gas components in the biogas from pineapple peels**

Results for the**quantitative determinationof the gas components in the biogas generated from pineapple peels as detected by GC-MS**is presented in Figure 3. Methane had the highest percentage by volumecomposition.The percentage of methane found in the biogas from pineapple peels in the present study was higher than reports from previous studies [10, 25]. Although biogas impurities(hydrogen, methane, carbon monoxide, carbon dioxide, hydrogen sulphide, water, nitrogen gas and oxygen) found in the present study were in negligible amount, however, their removal is necessary in order to obtain pure biomethane. The presence of oxygen in the biogas might lead to explosion, while the presence of hydrogen sulphide in the biogas might lead to corrosion of operating equipment [14]. Further studies on the removal of these impurities from biogas generated from pineapple peels is suggested. The emission of biogas from the biodigester was observed under 24 hours. This duration is shorter than findings from previous studies [26, 27, 28]. Theshort duration under which biogas emission was detected, coupled with the high percentage of methane produced, make pineapple peels an ideal raw material for biogas.

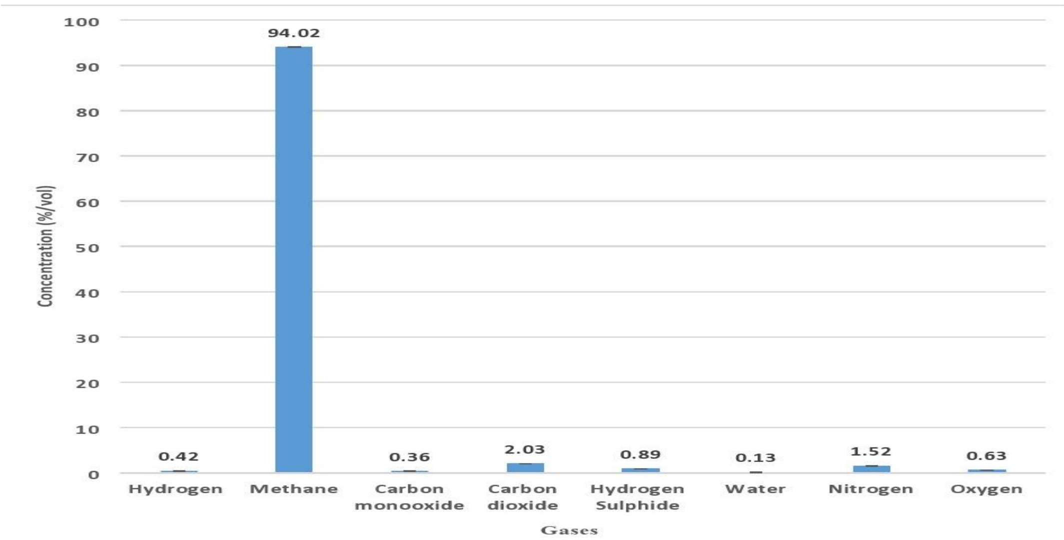

Figure 3 Percentage per volume of the gas components in the biogas from pineapple peels

Comment [A6]: Give possible reasons for high concentration of methane, it is more of an unusual findings, and presence of oxygen in the gas mixture, any experimental error ?

3.3 Effect of pineapple peel biodigester effluent on the activities of serum AST, ALT and ALP in catfish (*Clariasgariepinus*)

Results for the effect of pineapple peel biodigester effluent on the activities of serum AST, ALT and ALP in catfish (*Clariasgariepinus*) are presented in Figure 4, 5 and 6. Compared with control, a significant increase in the activities of serum AST, ALT and ALP was found in Group II **catfish (*Clariasgariepinus*) exposed to undiluted effluent.**On the contrary, the activities of serum AST, ALT and ALP in **catfish (*Clariasgariepinus*) exposed to 1:10 dilution of effluents (group IV) were similar to that of control** (P< 0.05). Compared with control, alterations in the serum activities of ALP, AST and ALT in catfish exposed to effluents from pineapple peels biodigester were found in the present study. All the fish in group III died before the twelfth hour. Previous studies have reported alteration in the activities of these enzymes in Tilapia fish exposed to industrial effluents [29] and in juveniles and adults of *Clariasgariepinus* reared in earthen ponds [22]. Abnormal liver blood tests such AST, ALT and ALP test indicate damage to the liver [30]. Thus the unusually high activities of these liver enzymes in the serum suggest damage to the liver of catfish due to overwhelming contaminants in the effluent which was beyond the detoxifying capacity of the liver. The damage would have enabled the leakage of these enzymes out of the liver into the blood. Given that the **composition** and nutrient **content** of biogas, digestate and effluent depends on the raw materials (feedstock) added to the biodigester [23].Toxicological evaluation of effluents from different raw materials (feedstock) is recommended.

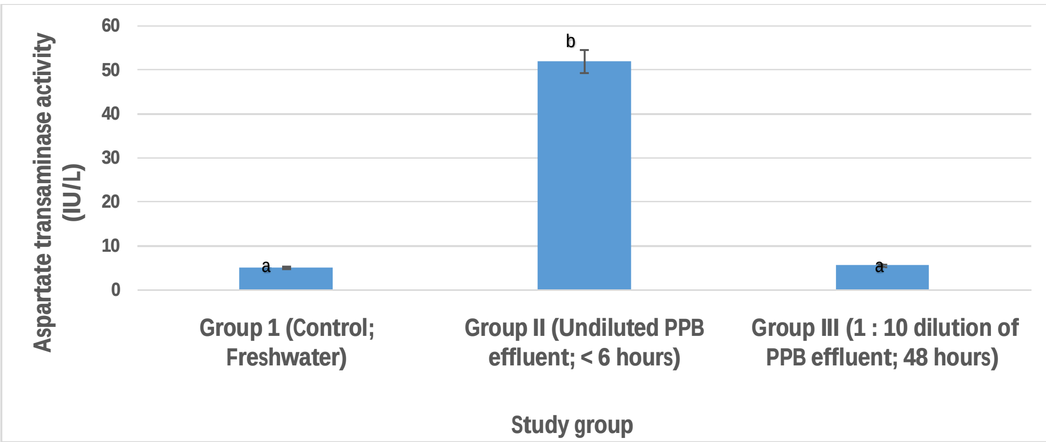

Figure 4Activities of serum AST in catfish (*Clariasgariepinus*) exposed to effluent from pineapple peel biodigester

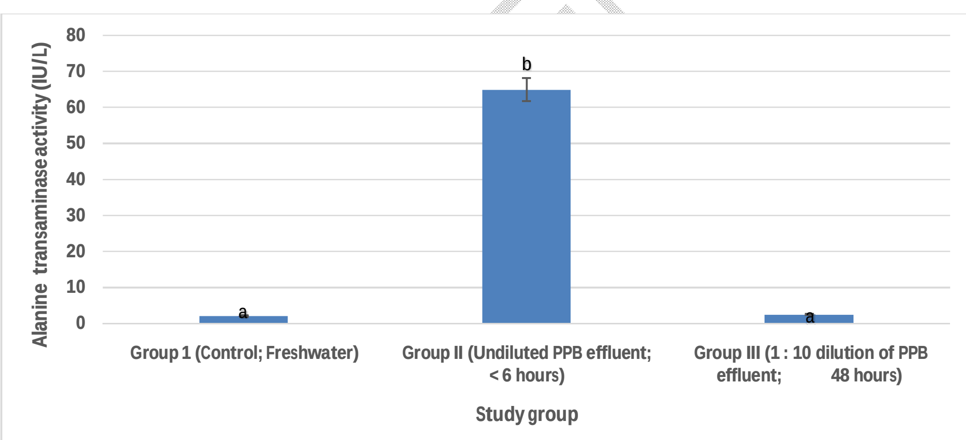

Figure 5 Activities of serum alanine amino transaminase (ALT) in catfish (*Clariasgariepinus*) exposed to effluent from pineapple peel biodigester

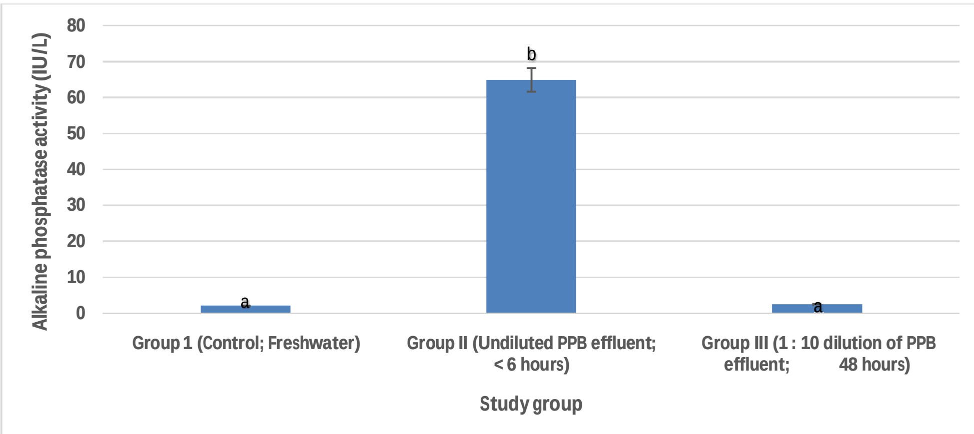

Figure 6 Activities of serum alkaline phosphatase (ALP)in catfish (*Clariasgariepinus*) exposed to effluent from pineapple peel biodigester

4. CONCLUSION

The present research clearly showed that biogas produced from pineapple peels contained 94.02% of methane gas and a mixture of gaseous impurities in negligible concentrations. The present study also demonstrated that undiluted effluent from pineapple peel biodigesterdamages the liver of catfish. However a 1:10 dilution of effluent from pineapple peels biodigesterwas non-toxic to the liver of catfish. Thus, the use of pineapple peels for rapid production of biogas with negligible impurities is recommended. Dilution of effluent before discharging it into the environment is also recommended.

REFERENCES

Comment [A7]: Follow the format of the journal.

1. Li D. Jing M. Dai X. Chen Z. Ma C. Chen J. Current status of pineapple breeding, industrial development, and genetics in China. *Euphytica* 2022;218:85 (2022). https://doi.org/10.1007/s10681-022-03030-y

2.Okoronkwo FI.Disegha GC. (2020). Bacteriological Quality Evaluation of Ready-to-Eat Pineapple Fruit Slices Sold in Markets around Port Harcourt and it's Environ in Rivers State, Nigeria. *JASEM.* 2022;24(8).

3.AiliHAF.Hamzah MH. Che MH. Jamali NS. Siajam SI. Ismail MH. Recent Updates on the Conversion of Pineapple Waste (*Ananascomosus*) to Value-Added Products, Future Perspectives and Challenges. *Agronomy* **2021**;*11*.

4.Unnikrishnan G. Vijayarghavan R. Anaerobic Digestion of Pineapple Waste for Biogas Production and Application of Slurry as Liquid Fertilizer Carrier for Phosphate Solubilizers. *Indian J. Agric. Res.* 2021;5777:1-8.

5.Getabalew M. Alemneh T. Bzuneh E. Review on methanogenesis and its role. *World J. Agri& Soil Sci* 6(2): 2020.

6.González R. Peña DC. Gómez X. Anaerobic Co-Digestion of Wastes: Reviewing Current Status and Approaches for Enhancing Biogas Production. *Appl. Sci.* **2022**, *12*:8884.

7. Bello SK. Alamu OS Design and construction of a domestic biogas digester. *JET* 2016;6(2):8-15.

8.Otieno EO. Kiplimo R. Mutwiwa U. Optimization of anaerobic digestion parameters for biogas production from pineapple wastes co-digested with livestock wastes. *Heliyon* 2023;9(3).

9. Chulalaksananukul S. Sinbuathong N. Chulalaksananukul W. Bioconversion of Pineapple Solid Waste under Anaerobic Condition through Biogas Production. *KKU Res. J.* 2012;17(5):734-742.

10.Rani DS.Nand K. Ensilage of pineapple processing waste for methane generation. *Waste Manag*. 2004;24(5):523-8.

11. NsubugaMD. KabengeI. KiggunduN. Wydra KD.**Comparative Study of Biogas Production from Jackfruit Waste, Banana Peels, and Pineapple Peels Co-Digested with Cow Dung.** *J. Sustain. Bioenergy Syst*. **2023;13(1).**

12.Wichitsathian B. Yimrattanabavorn J. Wonglertarak W.Enhancement of biogas production from pineapple waste by acid-alkaline pretreatment. *IOP Conf. Ser.: Earth Environ. Sci. 2020;* **471.**

13. Yang LL. Li **Y.** Biogas Cleaning and Upgrading Technologies. Accessed: March 26 2014. Available:

https://ohioline.osu.edu/factsheet/AEX-653.1-14.
14. Hultberg M. Lind O.Birgersson G. Asp H. Use of the effluent from biogas production for cultivation of Spirulina, *Bioprocess Biosyst Eng*. 2017;40(4): 625–631.
15. Popović NT. Čižmek L. Babić S. Strunjak-Perović I.Čož-Rakovac R. Fish liver damage related to the wastewater treatment plant effluents. *Environ SciPollut Res Int.* 2023;30(17):48739-48768.
16.Ogbole FA. Crown OO. Olayeriju OS. Olaleye MT. Akindahunsi AA.Hepatoprotective and antidyslipidemic effect of methanolic extract of *Garcinia kola* leaves on streptozotocin-induced diabetic rats. *IJEAST*. 2019, 4(4), 1-5.
17.Ogbole FA. Crown OO. Olayeriju OS. Olaleye MT.Akindahunsi AA. Antidiabetic effect of methanolic extract of *Garcinia kola* leaves on streptozotocin-induced diabetic rats. *GSJ* 2019;7(4):634 – 641.
18.Grabicova K. Grabic R. Fedorova G. Fick J. Cerveny D. Kolarova J. Turek J. Ziabek V. Randak T. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. *Water Res*. 2017;124(7).

19.Ogbole FA. Oyelana O. Health Risk Assessment of the Drinking Water from Sagbama River, Bayelsa State, Niger Delta, Nigeria.***Int. j. sci. eng. res***.2020;11(9).

20.Ogbole FA. Urinalysis for Dehydration, Kidney Injury and Urinary Tract Infection Assessment in Rural Riverside, Bayelsa State, Nigeria.*IJISRT* 2021;6(12):806 – 810.

21.Piechota G. Siloxanes in Biogas: Approaches of Sampling Procedure and GC-MS Method Determination. *Molecules* 2021;26(7):1953.

22. Achilike NM. Anyanwu PE. Enzymes activities in juveniles and adults of Clariasgariepinus reared in earthen ponds and concrete tanks. International Journal of Fisheries and aquatic studies. *IJFAS* 2019;7(2):258-262.

23. Li Y. Alaimo CP. Kim M. Kado NY. Peppers J. Xue J. Wan C. Green PG. Zhang R. Jenkins BM. Vogel CFA. Wuertz S. Young TM.Kleeman MJ. Composition and Toxicity of Biogas Produced from Different Feedstocks in California. *Environ Sci Technol*. 2019;53(19):11569-11579.
24. Rashed MMA. Shuichi T. Enhancement of Methane Concentration by Removing Contaminants from Biogas Mixtures Using Combined Method of Absorption and Adsorption. *Int. J. Chem. Eng*. 2017.

25. Rusína J. Chamrádováa K. Jastrzembskib T. Skřínskýc J. Explosion Characteristics of a Biogas/air Mixtures. *Chem. Eng. Trans*. 2022;90:271 – 276.

26. Sreekanth KM. Sahu D. Effect of iron oxide nanoparticle in bio digestion of a portable food-waste digester.***J. Chem. Pharm. Res***.2015;7(9):353-359.
27. Wichitsathian B. Yimrattanabavorn J. Wonglertarak W. Enhancement of biogas production from pineapple waste by acid-alkaline pretreatment. *IOP Conf. Ser.: Earth Environ. Sci.* 2020;471.

28Wellington A. Baraza LD. Mageto M. Orori KF. Energy evaluation and qualitative analysis of biogas produced from co-digesting kitchen waste and cow dung. *IJPS* 16(4): 1-13, 2017.

29. Nte ME. Hart A. Edun OM. AkinrotimiOA. Alterations in enzymes activities as a biomarker in black IjawTiliapia (Sarotherodonmelanotheron)exposed to industrial effluents. *CJBiolSci* 2011;4 (2):37 – 44.

30.Newsome PN. Cramb R. Davison SM.Dillon JF.Foulerton M.Godfrey EM. Hall R. Harrower U. Hudson M. Langford A. Mackie A. Mitchell-ThainR. Sennett K. SheronNC. Verne J. WalmsleyM. Yeoman A. Guidelines on the management of abnormal liver blood tests. *Gut* 2018;67:6–19.