WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Effect of Biogas Production Effluent on Oxidative Stress, Antioxidant Enzymes and Behavioural Characteristics of African Catfish (*Clarias Gariepinus*)

Ogbole Faith Ajiebabhio[1*] and Ebisintei Precious[2]

[1]Biochemistry Unit, Department of Chemical Sciences, University of Africa, Toru – Orua, Bayelsa State, Nigeria.
Department of Biological Sciences, University of Africa Toru – Orua, Bayelsa State, Nigeria.

ABSTRACT

The disposal of biogas production effluent is a major problem worldwide, thus, it is recycled for agricultural purposes. However its toxicity profile has not been fully established. The aim of the present study was to investigate the effect of a 10-day hydraulic retention time biogas production effluent (10-HRTE) collected from a cow dung biodigester on oxidative stress, antioxidant enzymes and physical characteristics of African catfish (*Clarias gariepinus*). Lipid peroxidation was measured in terms of nmol of malondialdehyde /mg protein, while the activities of superoxide dismutase and catalase were expressed as units/mg protein. Twelve *Clarias gariepinus* (weight and length, 120±3 g and 30±1 cm respectively) were purchased, acclimatized to laboratory conditions for four hours and randomly grouped into two groups of six animals each. Group I (control) was placed in 40 litres of freshwater for six hours and group II (exposed) was placed in 40 litres of a 10-HRTE for six hours. After six hours, the catfish in the control group were found swimming freely, while the catfish in the exposed group were found gasping at the surface and either moving sluggishly or still. Hepatic lipid peroxidation was found to be significantly ($P < 0.05$) higher in the exposed group compared with control (control: 5 ± 0.42 versus exposed: 20 ± 2.11). Compared with control a significant ($P < 0.05$) reduction in the activities of superoxide dismutase (control: 15 ± 0.25 versus exposed: 1 ± 1.01) and catalase (control: 35 ± 0.25 versus exposed: 2 ± 1.01) was found. The present study demonstrated that catfish (*Clarias gariepinus*) exposed to cow dung effluent generated under a short period displayed increased oxidative stress, a reduction in antioxidant enzyme activities and altered behaviours that indicate oxygen depletion in the water. Further study using effluent generated under a longer hydraulic retention time is recommended.

Keywords: Biogas, effluent, malondialdehyde, catalase, superoxide dismutase, gasping at the surface, sluggish movement

1. Introduction

Biogas production effluent (BPE) is the waste water produced during biogas production from a biodigester [1]. BPE disposal is a major problem worldwide thus, the recycling of BPE has served as an alternative to effluent disposal [2]. Effluent from biogas production was reported to have been effectively used as a nutrient source for the cultivation of the protein-rich and edible microalgae Spirulina (*Arthrospira platensis*) [1]. Another study reported the use of BPE for crop irrigation instead of water [2]. Biodigester effluent has also been previously used for the cultivation of tomato (*Solanum lycopersicum*) crops [3] and rice [4]. Also, in a previous study, biogas effluent from cow dung biogas plant was utilized as the only feed fed to a monosex (all male), monoculture of the Tilapia fish *Oreochromis mossambicus*, in a 0.002 ha ponds with no supplementary feed given to the fishes grown in biogas plant effluent. The study found that *Oreochromis mossambicus* fed with biogas-plant effluent attained a maximum weight gain of 0.67 g per fish per day [5]. Another study found a higher growth rate and net fish yield after biodigester effluent was applied over 120 days, to a pond stocked with Tilapia (*Oreochromis niloticus*) [6].

Despite the availability of evidence based data on the effectiveness of biodigester effluent as organic fertilizer for growing crops and rearing animals [1-6], the safety of biogas effluent to plants and animals is yet to be fully established. A previous study that investigated the presence of pathogenic organisms in the effluent from cow dung biodigester found that *Klebsiella, Staphylococcus, Acinetobacter, Edwardsiella, and Alcaligenes* species were all present in the biodigester effluent from cow dung [7]. Some of these micro-organisms have also been previously reported in the drinking water from Sagbama River [8], a location where high urinalysis related infection have been previously reported [9].

Oxidative stress represents an imbalance between the antioxidants and free radicals in an organism, with the free radicals overwhelming the antioxidants [10]. Antioxidants refers to the variety of defense mechanisms used to counter the destructive effects of free radicals in a living organism. A free radical is a molecule with one or more unpaired electron in its outer shell. In other words it is a type of unstable molecule that contains oxygen. In order to achieve stability, free radicals abstract electron from macromolecules such as lipids in biological membranes resulting in lipid peroxidation. This destroys the stable architecture of biological membranes [11, 12]. Thus oxidative stress is a common measure for evaluating the effect of exposure to environmental

stress on the physiology of an organism [10]. Conditions such as exposure to toxicants has been previously reported to elevate the incidence of oxidative stress and reduced antioxidant activities in fish [12, 13]. However whether the exposure of fish to biogas plant effluent will produce the same effect is not known. Studies on the toxicity profile of biogas-plant based effluent on fish which would inform its safe and proper application for the growing of crops and animals is limited in literature.

The aim of the present study therefore was to investigate the effect of a 7-day hydraulic retention time biodigester effluent on oxidative stress and physical characteristics of African catfish (*Clarias gariepinus*).

2. Materials and methods

2.1 Animals and Study Design

Twelve *Clarias gariepinus* (weight and length, 120±3 g and 30±1 cm respectively) were purchased from Sagbama. Sagbama is a Local Government Area in Bayelsa State, Nigeria [14]. Bayelsa State is a core State in Niger Delta Nigeria located on latitude 5.152239 and longitude 6.192479. The State lies between the well-watered oil-palm freshwater bushy swamps and salt-water creeks and mangrove swamps [15]. Catfish (*Clarias gariepinus*) production is a major occupation in Bayelsa State [16]. After purchase, the fish were acclimatized to laboratory conditions for four hours and randomly grouped into two groups of six animals each. Group I (control) was placed in 40 litres of freshwater for six hours and group II (exposed) was placed in 40 litres of a 10-day hydraulic retention time biogas production effluent (10-HRTE) for six hours [17].

2.2 Biodigester effluent

Forty litres of biodigester effluent was collected from a biodigester containing a mixture of cow dung and water (ratio 1:1) on the tenth day of its hydraulic retention time. The biodigester was operated under mesophilic conditions and was located in the University of Africa Toru – Orua, Bayelsa State, Nigeria [18]. Visual examination for any altered behaviour in catfish after exposure to the 10-day HRTE was carried out.

2.3 Preparation of fish liver homogenate

The liver of each fish was excised, chopped into bits and homogenized in ice-cold potassium phosphate buffer (10 mM, pH 7.4; 1: 4 w/v). A Potter-elvegin homogenizer fitted with a Teflon pestle with pulse-on time, 15 sec for every 30 sec was used for homogenization. The liver homogenate was centrifuged using a cold centrifuge at 9000 rpm for 10 min at 4^{o}C to obtain the post-mitochondrial fraction (PMF). The supernatant (PMF) was decanted, stored under 4^{o}C and used to evaluate lipid peroxidation, catalase and superoxide dismutase activities [19, 20].

2.4 Determination of Total Protein (TP) Concentration

This was carried out using the manufacturer`s protocol for Randox Total Protein Kit based on the Biuret method, with bovine serum albumin as the protein standard [21, 22]. The principle was based on the reaction of cupric ions, in an alkaline medium with protein peptide bonds resulting in the formation of a blue coloured complex, which exhibited maximum absorbance between wavelengths of 530-570 nm. Briefly, 1 ml of reagent R1 (consisting of 100 mmol/l of sodium hydroxide, 16 mmol/l of sodium-potassium tartrate, 15 mmol/l of potassium iodide and 6 mmol/l of copper II sulphate) was added `to 0.02 ml of the liver homogenate. The mixture was incubated at 25^{o}C and absorbance was measured against the reagent blank at a wavelength of 546 nm. Total protein concentration was calculated using the formular below.

$$\text{Total protein concentration (mg/dl)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

2.5 Evaluation of lipid peroxidation

Lipid peroxidation was carried out according to the method of Ohkawa *et al*., [23] as previously described [24]. Briefly, under acidic condition, malondialdehyde (MDA) produced from the peroxidation of the fatty acids in the lipids on biological membranes reacted with the chromogenic reagent, 2-thiobarbituric acid to yield a pink coloured complex called thiobarbituric acid reactive substance (TBARS, fluorescent product) which had a maximum absorbance at 532 nm. Exactly 0.4 ml of liver homogenate was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30% trichloroacetic acid (TCA) has been added. Trichloroacetic acid was used for the precipitation of proteins and nucleic acid. Then 0.5 ml of 0.75% TBA was added and the mixture was placed in a water bath for 45 minutes at 80^{o}C. The mixture was then cooled on ice and centrifuged at 3000 *g.* The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. The MDA level was calculated in nmol/mg protein with the formular below and computed using a molar extinction coefficient (E_{532nm}) of 1.56 x 10^5 $M^{-1}cm^{-1}$ and a path length of cuvette of 1 cm.

$$\text{MDA (nmol/mg protein)} = \frac{\text{Absorbance x vol. of mixture (ml)}}{E_{532nm}\ (M^{-1}cm^{-1}) \text{ x vol. of sample (ml) x path length of cuvette (cm) x total protein (mg/dl)}}$$

Molarity (M) = nmol/l, total protein (mg/dl), was converted to mg/l for the calculation of MDA

2.6 Determination of catalase activity

Catalase activity was determined by the method of Aebi [25] as previously described [20, 26]. Briefly, one hundred microliter of liver homogenate was mixed with 100 μl of absolute alcohol and incubated for 30 min in an ice bath to break down any catalase-hydrogen peroxide complex and to release active catalase. After 30 min the tubes were removed from ice and placed at room temperature, then 10 μl of Triton X-100 was added to lyse the cell. Two hundred microliter of phosphate buffer was added to a cuvette and mixed with 50 μl of the liver homogenate and 250 μl of 0.066 M H_2O_2 in phosphate buffer and the initial absorbance reading for hydrogen peroxide was immediately taken at 240 nm in a spectrophotometer. The decomposition of H_2O_2 was measured by monitoring the decrease in H_2O_2 absorbance for 30 s. A control was set up without liver homogenate. A molar absorption coefficient of 43.6 M^{-1} cm^{-1} was used to determine the initial and final concentration of hydrogen peroxide. The final concentration of hydrogen peroxide was subtracted from the initial concentration of hydrogen peroxide to obtain the concentration of hydrogen peroxide decomposed. Catalase activity was calculated using the formular below.

$$\text{Catalase activity } (\mu\text{mol of } H_2O_2 \text{ decomposed/min/ mg protein}) = \frac{\text{Concentration } H_2O_2 \text{ decomposed } (\mu\text{mol/l})}{\text{Concentration of protein (mg/l)}}$$

One unit of catalase activity is equal to the amount of catalase needed to decompose 1 μmoles of hydrogen peroxide per minute per mg of protein at 25°C.and was expressed as units per mg of protein.

2.7 Evaluation of Superoxide dismutase activity

Superoxide dismutase activity was determined according to the method of Misra and Fridovich [27] as previously described [10]. The principle of reaction for the evaluation of superoxide dismutase is based on the ability of the superoxide dismutase to inhibit the auto-oxidation of epinephrine measured by a spectrophotometer at an absorbance of 480 nm. Briefly, 0.02 mL of liver homogenates was added to 0.03 mL of epinephrine, 0.02 mL of water, and 2.95 mL of 0.05 M sodium carbonate buffer (pH 10.2). The Enzyme activity was calculated by estimating the change in absorbance at 480 nm for 5 min.

2.8 Statistical data analysis

SPSS version 24 was used for statistical analysis. Statistically significant mean differences between the two groups were evaluated using independent t test and were expressed as mean ± standard error of mean. The level of significance was set at $P < 0.05$ [28, 29].

3. Results

As shown in Table 1, compared with the catfish in the control group which were found to be swimming freely at the end of the expereimental period, the catfish exposed to a 10-HRTE where found to be gasping at the surface and either moving sluggishly or staying still.

Table 1 Behavioural characteristics of catfish (*Clarias gariepinus)* af*ter exposure to a 10-HRTE*

	Mobility	Sign of oxygen depletion
Control	swimming freely	none
Exposed	stayed still or moved sluggishly	gasping for air (lifting head above water)

3.2 Hepatic lipid peroxidation

As shown in Figure 1, compared with control, hepatic lipid peroxidation (measured in terms of MDA concentration) was significantly ($P < 0.05$) higher in the exposed group (control: 5 ± 0.42 versus exposed: 20 ± 2.11).

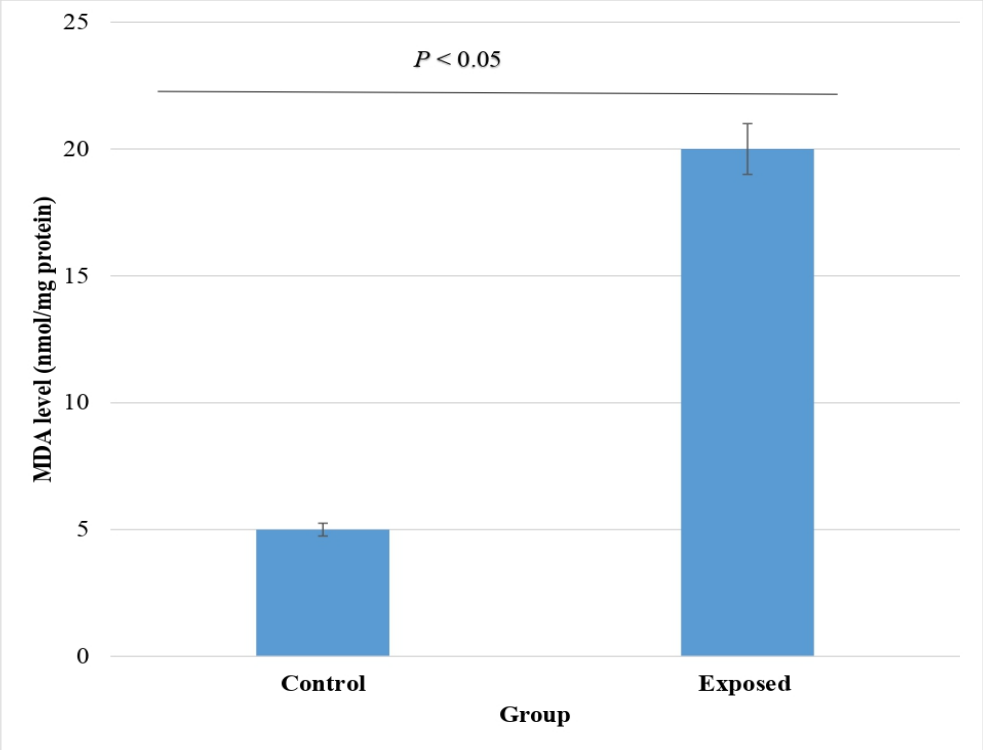

Figure 1 Hepatic malondialdehyde concentration of catfish (*Clarias gariepinus*) exposed to a 10-HRTE. Result presented as mean ± standard error of mean; MDA = malondialdehyde.

3.3 Superoxide dismutase activity

Figure 2 showed that the activity of hepatic superoxide dismutase was significantly lower ($P < 0.05$) in the exposed group compared with control (control: 15 ± 0.25 versus exposed: 1 ± 1.01).

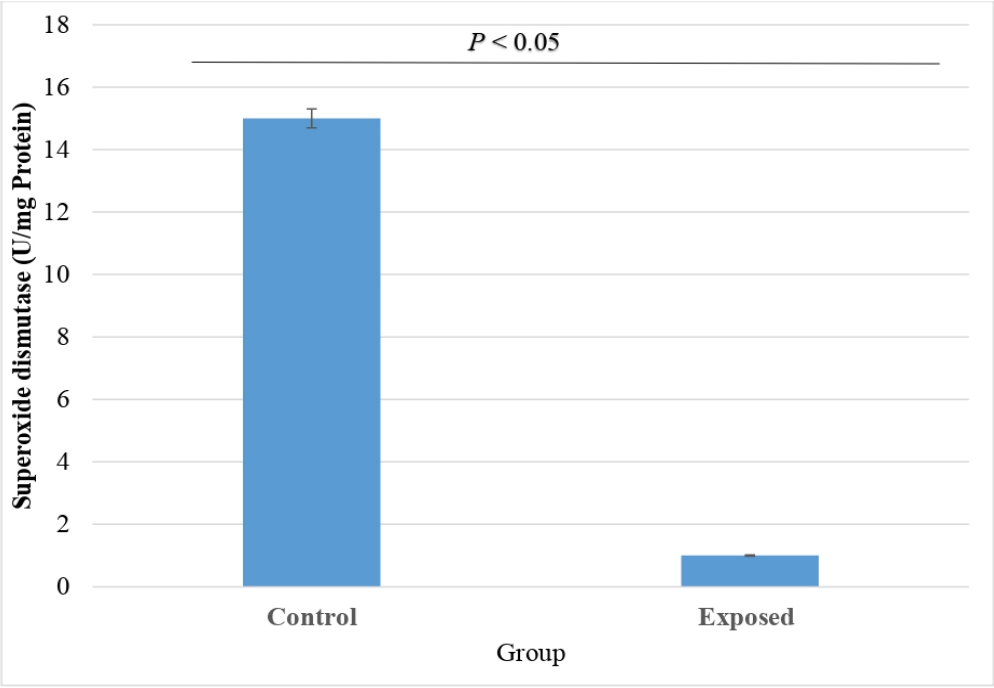

Figure 2 Hepatic superoxide dismutase activity of catfish (*Clarias gariepinus*) exposed to a 10-HRTE. Result presented as mean ± standard error of mean.

3.4 Catalase activity

Figure 3 shows that compared with control a significant ($P < 0.05$) reduction in the activity of catalase was found (control: 35 ± 0.25 versus exposed: 2 ± 1.01).

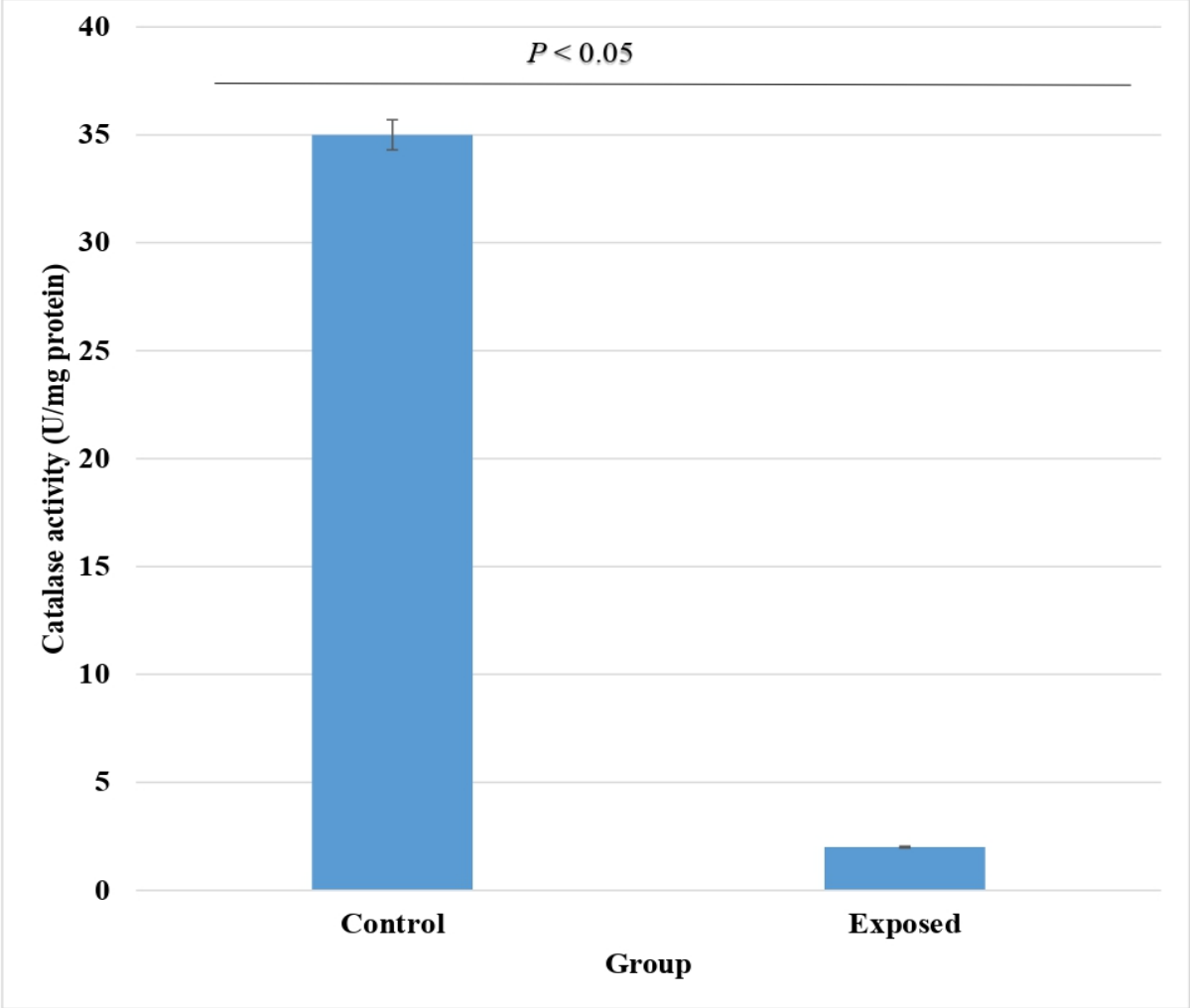

Figure 3 Hepatic catalase activity of catfish (*Clarias gariepinus*) exposed to a 10-HRTE. Result presented as mean ± standard error of mean.

4. Discussion

The present study investigated the effect of a 10-day hydraulic retention time effluent (10-HRTE) from cow dung biodigester on the behavioural characteristics of catfish (*Clarias gariepinus*)**,** oxidative stress, and antioxidant enzyme activities in the liver. The present study found that compared with control, catfish exposed to a 10-HRTE were found gasping at the surface and stayed still most of the time or moved sluggishly. A previous study reported that gasping of fish at the surface of water or sluggish movement indicates a reduction in the concentration of dissolved oxygen (hypoxia) and if left unchecked could eventually cause the fish to die with the larger fish dying before the smaller fish [30]. A previous study reported that reduction in the concentration of dissolved oxygen (hypoxia) may be due to environmental stress such as the presence of pollutants in water which interferes with healthy gill function [31]. Findings of the present study is similar to findings of a previous study where fish living downstream from a wastewater treatment plant were found to show behaviours that were different from their normal behaviors and these altered behaviours made them vulnerable to predators [32]. Other studies have showed that in an oxygen depleted environment, most fresh water fish reduce their swimming skills due to the high metabolic cost of aerobically propelled swimming and the physiological stress of hypoxia [33, 34].

In addition, the present study found a high level of oxidative stress, measured in terms of lipid peroxidation in the group of fish exposed to a 10-HRTE. Elevated oxidative stress is generally regarded as a pathological condition and one of the most common physiological response of animals to environmental stress. [11, 33, 35]. Findings of the present study is similar to the findings of a previous study where increase in hepatic lipid peroxidation was found in fishes exposed to hydrogen sulfide [36]. Findings of the present study is also similar to the findings of a previous study where the effect of battery-manufacturing effluent on endogenous lipid peroxidation in freshwater fish was investigated and an increase in lipid peroxidation in the fish

exposed to battery manufacturing effluent was found [37]. In a previous report where mass mortality of millions of fish was observed, oxidative stress was reported as one of the possible causes of such fish kills [30].

Superoxide dismutases and catalase are part of the antioxidant enzyme defense system against oxygen toxicity. Superoxide dismutase is an enzyme that converts superoxide anion (O_2^-), a type of free radical to hydrogen peroxide (H_2O_2) [11], while catalase is a hydrogen peroxide scavenger [38]. Superoxide anion and hydrogen peroxide are types of reactive oxygen species with hydrogen peroxide serving as a moderately reactive oxygen species compared with superoxide anion [11]. A previous study showed that exposure to contaminants induces the production of reactive oxygen species [38] which attack DNA as well as proteins and lipids on biological membranes leading to lipid peroxidation [11]. Thus high level of lipid peroxidation found in the present study indicates high level of reactive oxygen species.

In addition, the present study found that compared with control, a reduction in the activities superoxide dismutase and catalase were found. Findings of the present study corroborates the findings of a previous study where a reduction in hepatic superoxide dismutase activity in freshwater tilapia fish, (Oreochromis niloticus) was found on exposure to contaminated Monjolinho River [11]. Findings of the present study is also similar to the findings of another previous study where a decrease in catalase activity in fish exposed to battery manufacturing effluent was found [37].

Given that studies on the health impart of biogas-effluent on the physiology of fish is limited in literature, it is important that more scientific investigations are carried out so that sluggish movement of fish and gasping of fish as well increase in oxidative stress and reduced antioxidant activities will be avoided in fish fed with biogas-effluent. It is also important that the populace are sensitized on consequence of feeding fish with effluent produced under a very short duration.

5. Conclusion

The present study showed that catfish (*Clarias gariepinus*) exposed to cow dung effluent generated under a 10-day hydraulic retention time displayed increased oxidative stress, a reduction in antioxidant enzyme activities and two major physical behaviours that indicate oxygen depletion in water (gasping at the surface and sluggish movement). This suggests that effluent generated under a short hydraulic retention time should be subjected to proper treatment before been used as fish feed. Further study using effluent generated under a longer hydraulic retention time is recommended.

Reference

[1] Hultberg M, Lind O, Birgersson G, Asp H. (2017). Use of the effluent from biogas production for cultivation of Spirulina. *Bioprocess Biosyst Eng*. 40(4):625-631.

[2] Tak HI, Bakhtiyar Y, Ahmad F, and Inam A. (2014). Effluent Quality Parameters for Safe use in Agriculture. InTech: In book: Water Quality, Soil and Managing Irrigation of Crops. DOI: 10.5772/31557.

[3] Hooton E, Ni Y, Wang C. (2019). Is biodigester effluent a suitable replacement for commercial fertilizers? Assessing the efficacy of liquid biogas digestate for cultivation of tomato (*Solanum lycopersicum*) crops in Barbados. *Journal of Sustainable Tropical Agriculture* McGill University, Montreal, Canada University of the West Indies, Cave Hill, Barbados. https://www.mcgill.ca/bits/files/bits/biodigest_and_ tomato_production_final_report.pdf. Accessed: August 2024.

[4] Minamikawa K, Khanh HC, Hosen Y, Nam TS, Chiem NH. (2019). Variable-timing, fixed-rate application of cattle biogas effluent to rice using a leaf color chart: microcosm experiments in Vietnam. *Soil Sci. Plant Nutr*. 66(1), 225–234.

[5] Balasubramanian PR, Bai RK (1996). Biogas plant-effluent as an organic fertilizer in monosex, monoculture of fish (*Oreochromis mossambicus*). *Bioresour Technol*. 55(2):119-124.

[6] Thy S, Preston TR. (2003) Effluent from biodigesters with different retention times for primary production and feed of Tilapia (*Oreochromis niloticus*). *LRRD*. 15(64). Accessed: August 8, 2024 http://www.lrrd.org/lrrd15/9/sant159.htm

[7] Ogunkeyede AO, Okorhi-Damisa FB, Efemena T and Adeniyi S. (2020). Identication and morphology of pathogens in liquid effluent from a Cow dung biodigester. *IBSPR*, 8(3):51-57.

[8] Ogbole FA**,** Oyelana O. (2020). Health Risk Assessment of the Drinking Water from Sagbama River, Bayelsa State, Niger Delta, Nigeria. *IJSER*, 11(9):1455 – 1460.

[9] Ogbole FA. Urinalysis for dehydration, kidney injury and urinary tract infection assessment in rural Riverside, Bayelsa State, Nigeria. *IJISRT*. 6(12):805 – 810.

[10] Ogunwole GA, Abiya SE, Amaeze NH, Eze CT. (2021). Antioxidant markers in gills, liver and muscle tissue of the African Sharptooth Catfish (*Clarias gariepinus*) exposed to subchronic levels of Ibuprofen and Dibutyl phthalate, *Sci Afri.* 12:e00816, doi.org/10.1016/j.sciaf.2021.e00816.

[11] Carvalho CS, Bernusso, VA, de Araújo HSS, Espíndola GLG, Fernandes, MN. (2012). Biomarker responses as indication of contaminant effects in *Oreochromis niloticus*. *Chemosphere* 89:60–69.

[12] Tkachenko H, Natalia K, Joanna G, Anastasiia A. (2014). Tissue-specific responses of oxidative stress biomarkers and antioxidant defenses in rainbow trout *Oncorhynchus mykiss* during a vaccination against furunculosis. *Fish Physiol. Biochem*. 40:1289-1300.

[13] Lushchak VI, Bagnyukova TV. (2006). Effects of different environmental oxygen levels on free radical processes in fish. *Comp Biochem Physiol B Biochem Mol Biol*. 144(3):283-9.

[14] Ogbole FA, Igwe CU, Onuoha HC, Nzebude CP. (2023). Characterization of ABO / Rhesus antigen polymorphism associated with malaria in a malaria hotspot in Bayelsa State, Niger Delta, Nigeria. *IJBRR*. 32(7): 42-52.

[15] Ogbole FA, Igwe CU, Onuoha HC, Nzebude CP. (2023). Evaluating the Prevalence of Malaria Parasite Infection among Adults in Wetlands Using Nested PCR and High Resolution Melting Analysis. *AJBGMB*. 14(4):53–63.

[16] Okpukpara BC, Morgan CN (2015). Socio-economic analysis of catfish (*Clarias gariepinus*) production in Bayelsa State, Nigeria. *JASR*. 15(1):36-45.

[17] Makaras T, Stankevičiūtė M, Šidagytė-Copilas E, Virbickas T, Razumienė J. (2021). Acclimation effect on fish behavioural characteristics: determination of appropriate acclimation period for different species. *J Fish Biol.* 99(2):502-512.

[18] Ogbole FA, Akemi CO. (2023) GC-MS analysis of biogas from pineapple peels and toxicological evaluation of generated effluent. *AJBGE*. 6(2):96-104.

[19] Ogbole FA, Crown OO, Olayeriju OS, Olaleye MT, Akindahunsi AA (2019). Antidiabetic effect of methanolic extract of Garcinia kola leaves on streptozotocin-induced diabetic rats. *GSJ*, 7(4):634 – 641.

[20] Sivrikaya A, Kolayli S, Kucuk M, Aliyazicioglu R (2009). In vitro effects of peroxynitrite treatment on fish liver catalase activity, *J. Enzyme Inhib Med Chem*. 24(2):432-436.

[21] Tietz NW. (1995). Clinical Guide to Laboratory Test. 3rd Edition. W. B. Saunders Company. Philadelphia. PA 518-519.

[22] Weichselbaum TE. (1946). Estimation of serum total protein by Biuret method. *Am. J. Clin Pathol.* 16:40-48.

[23] Ohkawa H, Ohishi N, Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem* 95(2):351-358.

[24] Ogbole FA, Crown OO, Olayeriju OS, Olaleye MT, Akindahunsi AA. (2019). Hepatoprotective and antidyslipidemic effect of methanolic extract of *Garcinia kola* leaves on Streptozotocin-induced diabetic rats. *IJEAST*. 4(4):1-5.

[25] Aebi H. (1984). Catalase in vitro. *Meth. Enzymol*. 105:121–126.

[26] Kiran TR, Aruna HK (2010). Antioxidant enzyme activities and markers of oxidative stress in the life cycle of earthworm, *Eudrilus eugeniae*. *Ital. J. Zool*. 77(2):144-148.

[27] Misra HP, Fridovich I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 247(10):3170-5.

[28] Ogbole FA, Moroyei BE. (2023). Upregulation of interleukin-2 among hypertensive subjects in Bayelsa State, Nigeria. *IJRR*. 10(6): 557-565.

[29] Ogbole FA, Harold BA (2023). Association of undiagnosed pre-diabetes and type-2 diabetes mellitus with interleukin-2 mRNA expression among adults in Bayelsa State, Nigeria. *IJRR* 10(5): 210-215.

[30] Kibria G. (2014). Global fish Kills: Causes and Consequences. https://www.researchgate.net/publication/261216309_Global_fish_Kills_Causes_and_Consequences Accessed: August, 2024

[31] Tokatlı C, Islam ARMT. (2023). Spatial–temporal distributions, probable health risks, and source identification of organic pollutants in surface waters of an extremely hypoxic river basin in Türkiye. *Environ Monit Assess* 195:435. https://doi.org/10.1007/s10661-023-11042-x

[32] McMaster University (2017). Fish exposed to treated wastewater have altered behavior. *ScienceDaily*. www.sciencedaily.com/releases/2017/12/171205092134.htm. Accessed: July 2024.

[33] Anushka BA, Mishra A. (2022). Effects of dissolved oxygen concentration on freshwater fish: A review. *IJFAS*. 10(4):113-127.

[34] Fu SJ, Brauner CJ, Cao ZD, Richards JG, Peng JL, Dhillon R, Wang Y. (2011). The effect of acclimation to hypoxia and sustained exercise on subsequent hypoxia tolerance and swimming performance in goldfish (*Carassius auratus*). *J. Exp. Biol*. 214:2080-2088.

[35] Tsikas D. (2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges, *Anal Biochem*. 524:13-30.

[36] Sreejai R, Jaya DS. (2010). Studies on the changes in lipid peroxidation and antioxidants in fishes exposed to hydrogen sulfide. *Toxicol Int.* 17(2):71-7.

[37] Ahmad A, Alib D. (2013). Effect of battery-manufacturing effluent on endogenous antioxidant in freshwater fish. *Chem Spec Bioavailab* 25(2):106-112.

[38] Liu, Fobang, Saavedra, Maria G., Champion, Julie A., Griendling, Kathy K., Ng, Nga L. (2020). Prominent Contribution of Hydrogen Peroxide to Intracellular Reactive Oxygen Species Generated upon Exposure to Naphthalene Secondary Organic Aerosols. *Environ Sci Technol* 7(3):171-177.