

## 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 the liver functions of Catfish (*Clarias gariepinus*).

**Methodology:** Pineapple peels were grounded, fed into an anaerobic biodigester and emitted biogas was collected for GC-MS analysis. Twenty Catfish (*Clarias gariepinus*) 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:** The identified 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 peels anaerobic 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.

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

## 1. INTRODUCTION

Pineapple (*Ananas comosus*) 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 sludge has 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<sup>3</sup> biogas when the temperature was set at 30 °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<sup>3</sup>/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 functions or 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 bodies can 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 biodigester on the activities of liver enzymes in catfish (*clarias gariepinus*) was also carried out.**

## 2. MATERIAL AND METHODS

### 2.1 Anaerobic biodigestion

Pineapple peels were collected from Toru – Orua, Sagbama, Tombia and Swali markets as well as from road side pineapple sellers in Bayelsa State at no cost. The peels were weighed (Figure 1), grinded into fine consistency using the grinding machine available in the University and mixed with cow dung and water. The mixture was 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].

### 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].



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

### **2.3 Toxicological evaluation of effluent discharged from pineapple peel biodigester**

Twenty Catfish (*Clarias gariepinus*) weighing  $500 \pm 10$  g were grouped into four groups of five fish each: Group 1: Control (catfish placed in fresh water for 2 days); Group II: (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].

### **2.4 Statistical analysis**

SPSS version 24 was used for data analysis. Values were expressed as mean  $\pm$  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.

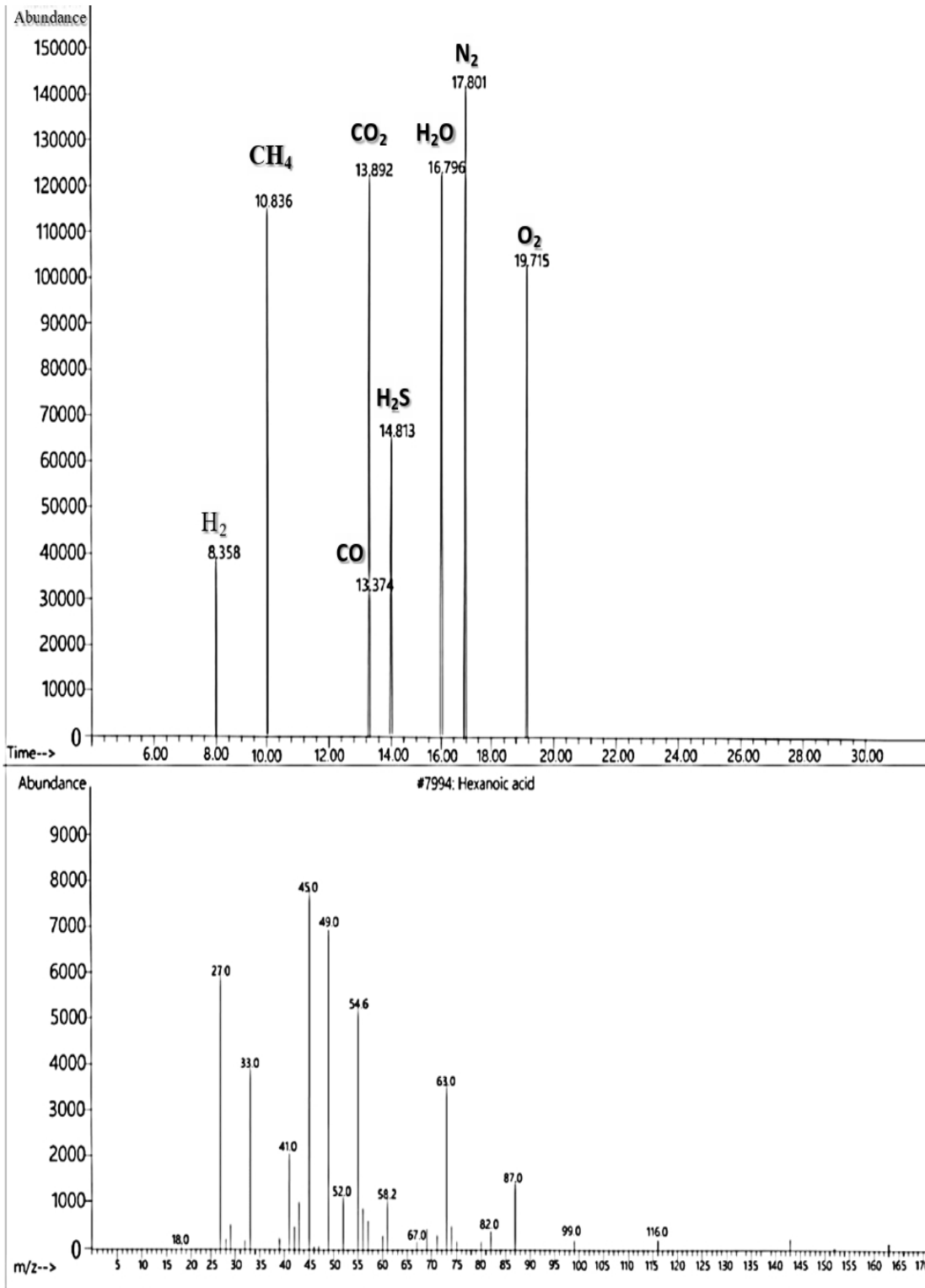


Figure 2 GC-MS chromatogram of gases in the biogas produced from pineapple peels

### 3.2 Quantitative determination of the gas components in the biogas from pineapple peels

Results for the quantitative determination of 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 volume composition. 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]. The short duration under which biogas emission was detected, coupled with the high percentage of methane produced, make pineapple peels an ideal raw material for biogas. **The yield of methane at 94% in the present study was very high. About 60% CH<sub>4</sub> and about 40% CO<sub>2</sub> is the yield that has been reported in previous studies [23, 29, 30]. The high methane yield and low CO<sub>2</sub> found in the present study might be due to the optimisation of the biogas production process. The optimisation employed in the present study involved grinding the pineapple peels before feeding into the biodigester. The grinding process made the substrate (pineapple peels) readily available to micro-organisms for digestion [31]. Another reason for the high yield of methane found in the present study might be due to the low level of methanogens inhibitors in the pineapple peels itself. Methanogens are very sensitive to toxic compounds and are inhibited by long chain fatty acids and high level of ammonia [30, 32]. Pineapple peel is not a rich source of long chain fatty acid and ammonia. Although a low level of ammonia (200mg/l) and a carbon: nitrogen ratio of 30: 1 are needed as nutrient for microbial activities, however, high level of ammonia is toxic to methanogens [30]. Also, unfavourable pH and temperature enhance inhibition of methanogens by ammonia and can result in 30% loss in biogas yield [32, 33]. High levels of ammonia (up to 1.77 – 14 g/l) can reduce biogas yield by 50% [32, 34]. This might be responsible for the low biogas and methane yield reported in previous studies [23, 29, 30]. Thus careful selection of substrate is required for optimal biogas production. Pineapple peels mainly consist of cellulose, hemicellulose, sucrose, fructose, glucose [4]. Methanogens are able to use hydrogen to reduce carbon dioxide to methane more effectively in the absence of inhibitors [32, 34, 35, 36, 37].**

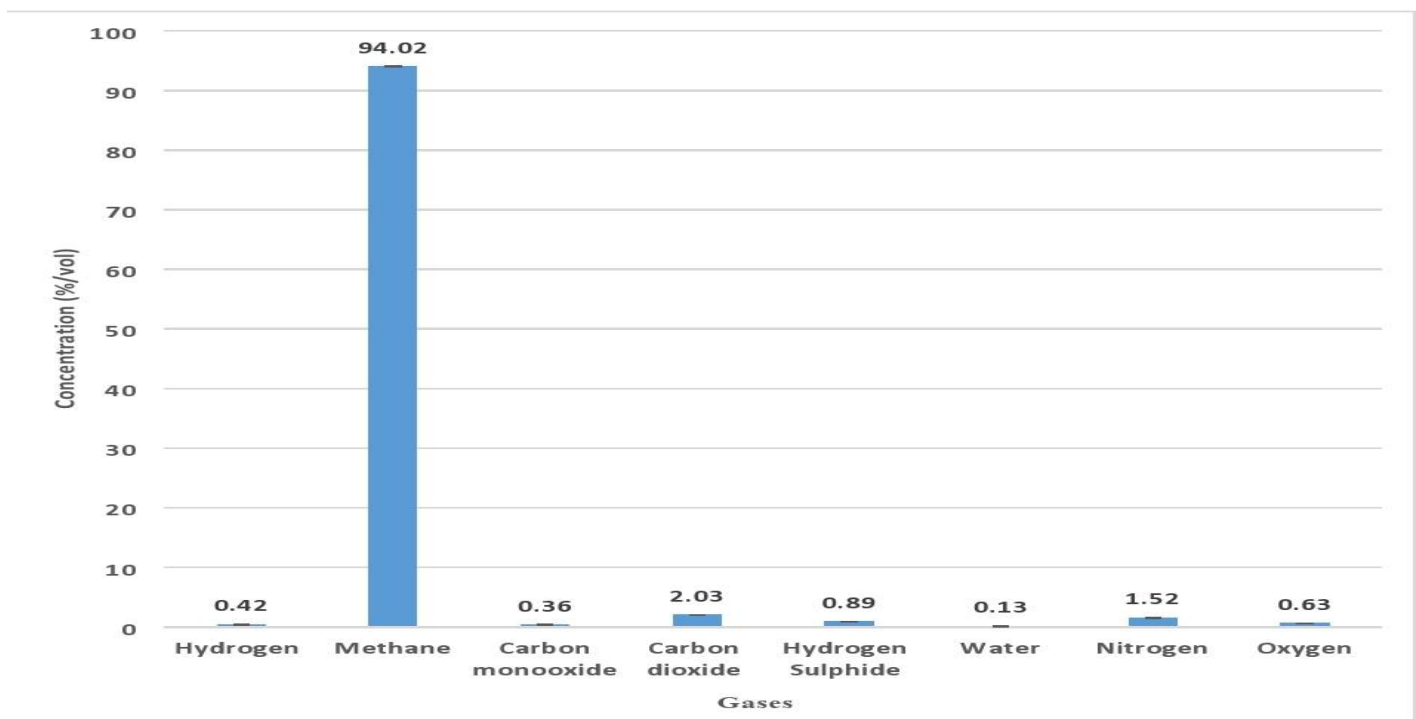


Figure 3 Percentage per volume of the gas components in the biogas from pineapple peels

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

Results for the effect of pineapple peel biodigester effluent on the activities of serum AST, ALT and ALP in catfish (*Clarias gariepinus*) 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 (*Clarias gariepinus*) exposed to undiluted effluent. On the contrary, the activities of serum AST, ALT and ALP in catfish (*Clarias gariepinus*) exposed to 1:10 dilution of effluents (group IV) were similar to that of control ( $P < 0.05$ ). All the fish in group III died before the twelfth hour. Previous studies have reported alterations in the activities of these enzymes in Tilapia fish exposed to industrial effluents [38] and in juveniles and adults of *Clarias gariepinus* reared in earthen ponds [22]. Abnormal liver blood tests such AST, ALT and ALP test indicate damage to the liver [39]. 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.

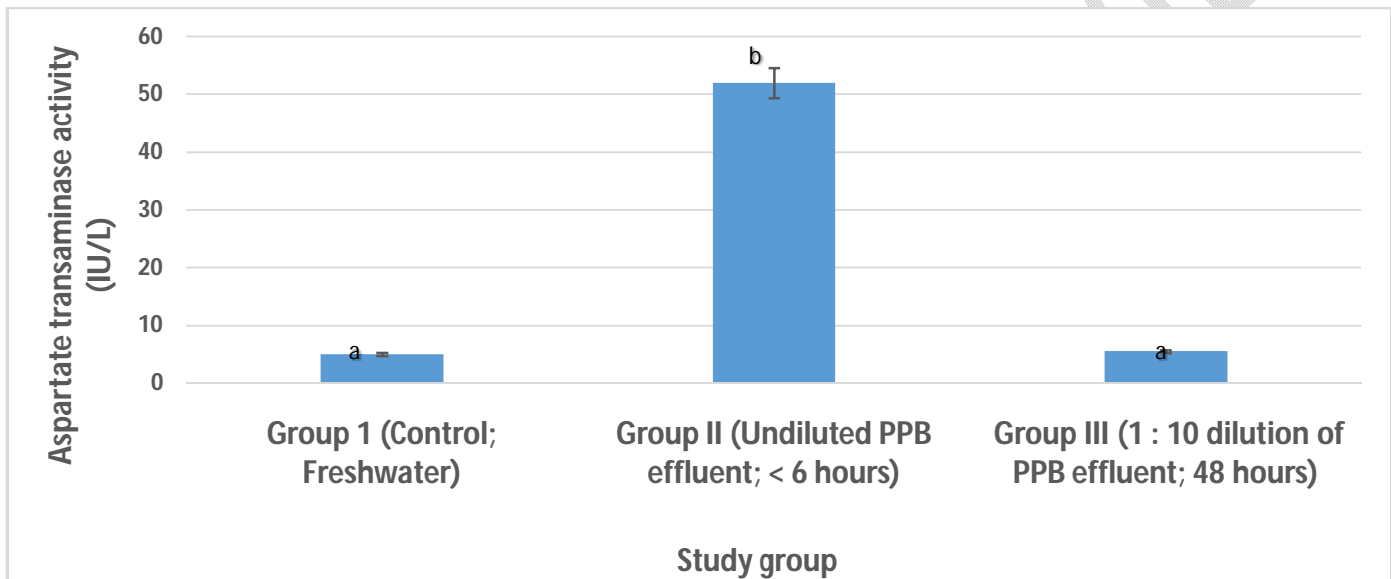
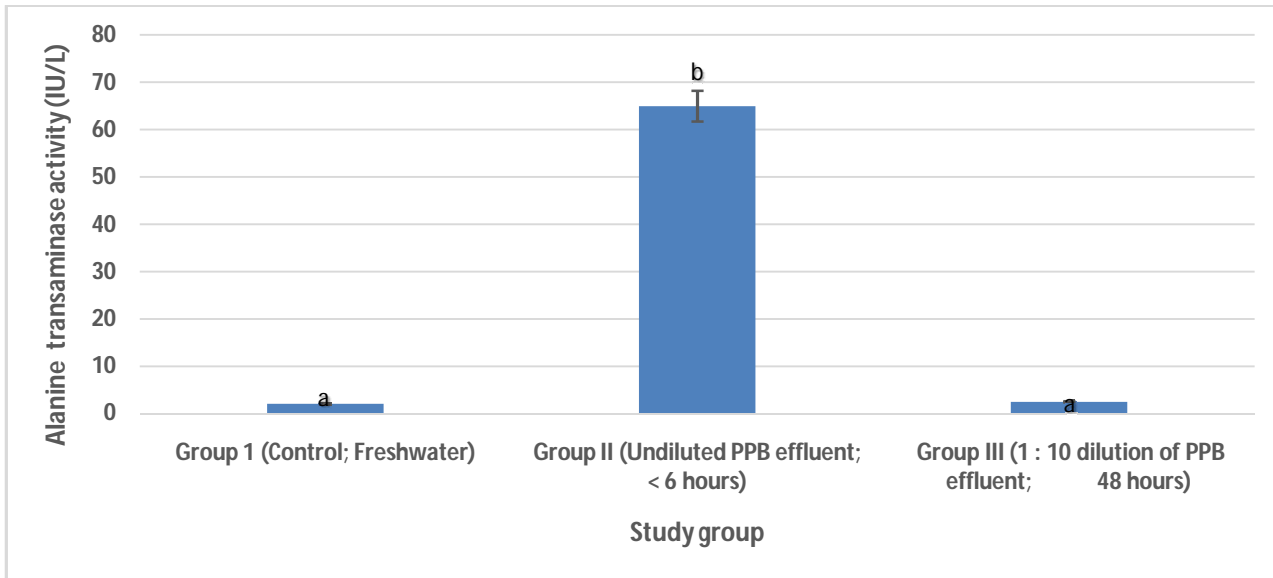
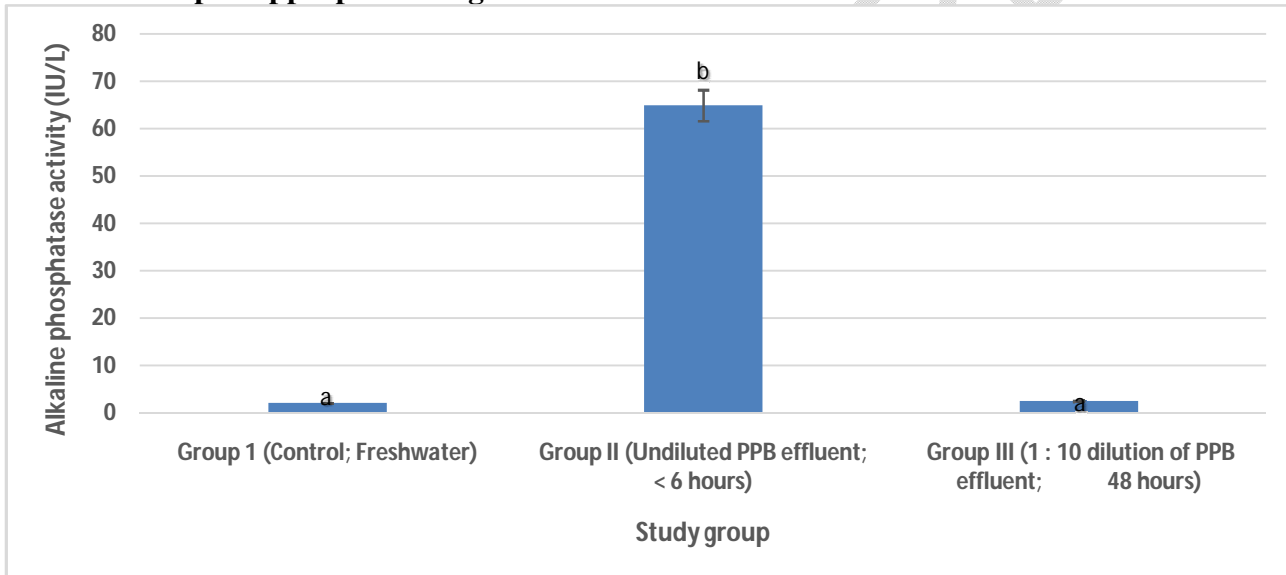


Figure 4 Activities of serum AST in catfish (*Clarias gariepinus*) exposed to effluent from pineapple peel biodigester



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



**Figure 6** Activities of serum alkaline phosphatase (ALP) in catfish (*Clarias gariepinus*) 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 biodigester damages the liver of catfish. However a 1:10 dilution of effluent from pineapple peels biodigester was 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.

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