

Bioavailability of Nutrients for Improved Methane gas Production by Addition of Chelating Ligands

Nwokem N. C.^{1,*}, Gimba C.E.¹, Ndukwe G.I.¹. And Ella E.E.²

¹ Department of Chemistry, Ahmadu Bello University, Samaru-Zaria, Kaduna State, Nigeria.

² Department of Microbiology, Ahmadu Bello University, Samaru-Zaria, Kaduna State, Nigeria.

*Corresponding author: nsidibe19@gmail.com

Abstract: Our irreplaceable fossil fuels are fast diminishing. Therefore, an important precautionary measure will be to develop renewable energy sources. Biogas is renewable and an environmentally friendly alternative energy. This research work focused on addition of chelating ligands in order to improve methane gas yield, and decrease concentration of Volatile Fatty Acid produced within digester systems. The experimental analysis carried out involved the use of Flame Atomic Absorption Spectrophotometer, Biogas5000 analyser and other classical analytical tools. The concentration of trace metals presents namely Iron, Cobalt, and Nickel ranged from 0.001-0.050 mg/L. On addition of chelating ligands, Ethylene diamine-N,N-diacetic acid, Nitrilotri-acetic acid and Diethylenetriamine-pentaacetic acid, an increase in the Methane yield was recorded which ranged from 2-15%. The Volatile Fatty Acid concentration was generally low with values for Ethylene diamine-N,N-diacetic acid, Nitrilotri-acetic acid and Diethylenetriamine-pentaacetic acid given as 476.05 mg/L, 671.96 mg/L, and 737.63 mg/L respectively; with pH ranging from 6-7.5. The improvement in methane gas yield was due to the increased bioavailability of essential nutrients on addition of chelating ligands which invariably promoted the growth and stability of the methane producing bacteria thereby, improving methane production. The Volatile Fatty Acid values which were generally lower than 2000 mg/L are implicated in the high methane gas yield observed.

Keywords: Methane, Nitrilotri-acetic acid, Ethylene diamine-N,N-diacetic acid, Diethylenetriamine-pentaacetic acid, Biogas5000.

I. Introduction

Reliance on fossil fuels importation from foreign countries creates a status of dependency and insecurity for most countries around the world. Also, the sources of fossil fuels are fast diminishing causing a lot of apprehension in our world today. The world's dependence on the declining reserves of fossil fuels poses not only environmental problems but also geopolitical ones (Angela *et al.*, 2012). Therefore, an important precautionary measure will be to develop alternative renewable energy sources; taking into consideration the increased level of energy consumption and mitigation of environmental pollution.

The emergence of biogas is currently engaging public interest as supplement to oil base to serve as transportation or automobile fuels and also sources of heat and electricity generation supplies. Biogas is renewable and an environmentally friendly alternative energy as compared to non-renewable fossil fuels because it burns with minimal release of Carbon (IV) oxide and water (Teodorita *et al.*, 2008). It is obtained through the process of anaerobic degradation of waste (Callander and Barford, 1983) and consists of methane (CH₄), Carbon (IV) oxide (CO₂) and Hydrogen sulphide (H₂S). This process of anaerobic degradation is carried out by a variety of micro-organisms, which are greatly influenced by some critical parameters; these conditions include: temperature, pH-value and available nutrients. Thus, it becomes imperative that the optimum conditions of operation for the anaerobic microorganisms be provided which will in turn improve the percentage yield of CH₄ which is the main constituent of biogas of fuel value.

The use of additives is fast becoming the focus of research efforts into the optimization of CH₄ gas produced from smaller digesters. Uemura (2010), observed that the addition of minerals [Nickel (Ni), Cobalt (Co) and Iron (Fe)] in batch mesophilic digesters, improved the digesters performance and CH₄ gas yield. More so, the additives helped to maintain favourable conditions such as pH and promotion of methanogenesis within the digester for rapid biogas production (Hanaki *et al.*, 1994). The focus of this research is to use chelating ligands[Ethylene diamine-N,N-diacetic acid (EDDA), Nitrilotri-acetic acid (NTA) and Diethylenetriamine-pentaacetic acid (DTPA)] to improve CH₄ gas potential by enhancing the microorganism population through the creation of nutrient rich environment favourable for the growth of methanogenesis from Cow manure substrate (CM).

II. Materials And Methods

2.1 Reagents

The EDDA, NTA and DTPA chelating ligands used were purchased from Sigma Aldrich; while the Cow manure waste sample (substrate) was obtained from the Anguwan-Yusi, Hanwa N/Extension, Zaria, Kaduna. All reagents used were of analytical grade.

2.2 Preparation of 1000 μM Chelating Ligand Stock Solutions

Appropriate weights of each of the salts were weighed out into a 1000cm³ volumetric flask containing 500cm³ of deionized water. The mixtures were each stirred continuously with a glass rod till a solution was obtained which was then diluted to the mark with deionized water.

2.3 Preparation of Chelating Ligand Standard Solutions

Standard solution of 10 μM , 50 μM and 100 μM for the chelating ligand were made from the stock solution and stored in sterilized amber-coloured bottles.

2.4 Waste Sample Preparation

The waste sample was air-dried for 72 hours and ground to smaller particles sizes of about 30.0 nm with a mortar and pestle. The sample was then properly labelled and stored in an air-tight plastic container until when needed.

III. Laboratory Analysis

3.1 Trace Metal Determination

The concentrations of Fe, Co and Ni in the CM sample were determined by flame atomic absorption spectrophotometer (AAS) version AA240FS.

3.2 Biogas Sample Analysis

Figure 1 shows the experimental setup for the biogas production. The systems were operated at mesophilic temperature. Two litres Pyrex digester bottles were used. Two hundred grammes of the dry waste samples were each loaded into the digesters and 1.5 litres of deionized water was added. The digesters were sealed with a rubber bung having two bore holes to exclude air from getting into the digesters. One hole was used for temperature determination, while the other was connected to delivery tubing which was used to collect and measure the volume of biogas produced under water through the downward displacement of water method. The digesters were subjected to periodic agitation to ensure thorough mixing of the contents while maintaining intimate contact between the micro-organisms and the substrate to enhance the complete digestion of the substrate. The composition of the biogas produced was equally monitored using a biogas analyzer (Biogas 5000, UK) on a daily basis.



Figure 1: Biogas production Setup

3.2 VFA Analysis

Before analysis, the samples from each digester were filtered through a 0.45µm membrane filter. The filtered 20 cm³ samples were transferred into a 100 cm³ Pyrex beaker. The initial pHs of the filtrates was determined using the pH meter (Jenway1000, UK). The samples were titrated slowly with 0.05 M sulphuric acid until pH of 5.0 was reached. The added volume of titrant was recorded. More acid was slowly added until pH of 4.3 was reached. The total volume of the added titrant was again recorded. The latter step was repeated until pH of 4.0 was reached; the volume of the added titrant was again recorded. The concentration of VFA was then calculated using the formula:

$$S_a = \frac{131340 \cdot M \cdot V_{A_{5-4, \text{meas}}}}{V_s} - 3.08 \cdot \text{Alk}_{\text{meas}} - 25$$

Where S_a = concentration of VFA

M = Molarity

V_{A_{5-4, meas}} = volume of acid (cm³) required to titrate a sample from pH 5.0 to pH 4.0.

V_s = volume of sample (cm³)

Alk_{meas} = measured alkalinity

$$[\text{Alk}_{\text{meas}}] = \frac{V_{A_{4.3 \text{ meas}}} \cdot M \cdot 1000}{V_s}$$

3.3 Analysis of Data

Data collected at the end of the study were analysed using statistical package for social sciences (SPSS) version 15.0 (SPSS Inc. Chicago, IL, USA).

IV. Results and Discussion

4.1 Effect of Trace Metals on CH₄ gas Yield

One of the factors which determine the performance of an anaerobic digester system is the concentration of the trace metals Fe, Co, and Ni; which are particularly important to the rapid growth of bacteria (Mosey *et al.*, 1997). The amount of trace metals present in the digester is presented in Figure 2. High concentrations of Cobalt and Nickel usually have an inhibitory effect on the anaerobic digester system. Thus, the presence of these metal ions within the minimum acceptable range is necessary to enhance CH₄ gas production. Results from Figure 2 show the presence of these metal ions at the acceptable range (0.001-0.050 mg/L) for samples taken out of the CM digester system. Since these metals are present at sufficient and balanced nutrient levels, thus, explains the reason for the high CH₄ gas yield obtained from the system.

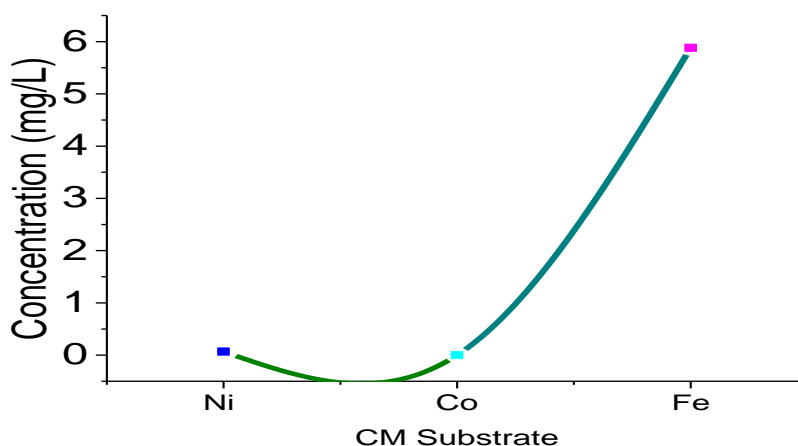


Figure 2: Results Showing the Concentration of Trace Metals in CM-Substrate

4.2 Effect of Addition of Chelating Ligands

In a bid to improve the performance of the CM digester, the following ligands were added prior to the commencement of the anaerobic digestion process: EDDA, NTA, and DTPA. Figure 3 shows that on addition of these chelating ligands, an increase which ranged from 2-15% in the CH₄ gas produced was recorded. The NTA chelating ligand performed best with a 15% improvement in the CH₄ gas yield (72.2% CH₄ produced) over the control which recorded a CH₄ gas yield of 64.4%. EDDA chelating ligand showed 12% increase in CH₄

production (70.5% CH₄ gas yield) and then DTPA showed 2% improvement in CH₄ production (65.6% CH₄ gas yield) compared to the control.

A partial list of chelating ligands and their log stability constants is given in Table 1. The uptake of these metals within the CM substrate depends on the metal speciation and bioavailability. The speciation is controlled by metal concentrations, digester operational conditions as well as chemical processes such as complexation (Mosey *et al.*, 1971; Pinheiro and van Leewen, 2001). It is also assumed that these metals are only available for microbial uptake when they are present as free metal ions and as metal complexes in the soluble form (Saito *et al.*, 2002; Worms *et al.*, 2006). Since most of these metals are present as metal sulphides within the digester systems (Rinzema and Lettinga, 1988); therefore the presence of organic and inorganic chelates will affect the metal bioavailability by the formation of metal chelates with high stability constants. Note that the larger the stability constant, the higher the proportion of the chelates that exists in solution and thus, the greater its availability to the methanogens (Marvin, 1989). Therefore, it is believed that stimulation of the anaerobic digestion process results from the degree of binding of Fe, Co and Ni to chelating ligands in the various substrates. This leads to an increase in the solubility of these essential inorganic nutrients which in turn promotes the growth and stability of the methanogenic population and thereby improving methane production.

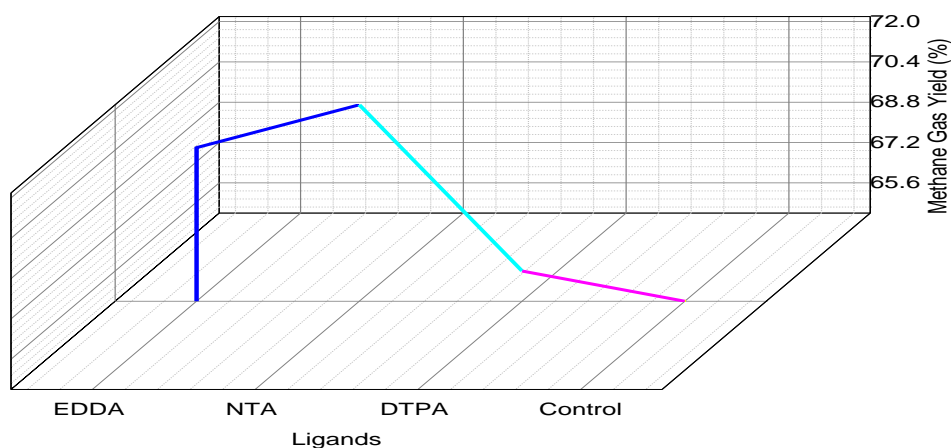


Figure 3: Results Showing the Effect of Chelating Ligands on CH₄ gas Yield

In addition, chelating ligands which form lipophilic complexes may permeate the plasma membrane of an organism (methanogen) and assist in the uptake of metal ions. This explains why the addition of the chelating ligand DTPA to the digester systems (which form stable complexes with Fe, Ni and Co) does not bring about a significant improvement in CH₄-production. DTPA is poorly absorbed into cells of methanogens which limits their capacity to chelate-out metals from inside the cells of the methanogens (Klaaseen, 2006). This is likely responsible for the not-so significant CH₄-production observed in some digester systems into which they were added.

Though NTA and EDDA form stable chelates with Fe, Co and Ni as is the case with DTPA, their complexes are lipophilic (Haitwig *et al.*, 1993). As a result, NTA and EDDA are able to permeate the membranes of the methanogens allowing them to reach the sites of metal storage and thus form stable complexes with these metal ions thereby leading to the significant improvement in CH₄-production observed within those digesters.

Table 1: Logarithm Stability Constants of Chelating Ligands Employed in This Study

Chelating Ligand	Fe ³⁺	Co ²⁺	Ni ²⁺
EDDA	8.63	11.25	13.6
NTA	15.87	10.38	11.54
DTPA	28.60	18.40	20.32

4.3 Effect of Addition of Chelating Ligands on VFA

The varying performance of the digester systems seen in Figure 3 can be attributed to the VFA concentrations of the systems. Various studies have tried to draw a correlation between the concentration of VFA and the health of the anaerobic digestion system (Hill and Holmberg, 1988). Therefore, a well working biogas digester is characterized by low levels of fermentation intermediates and VFA concentration of less than 2000 mg/L (Hill and Holmberg, 1988).

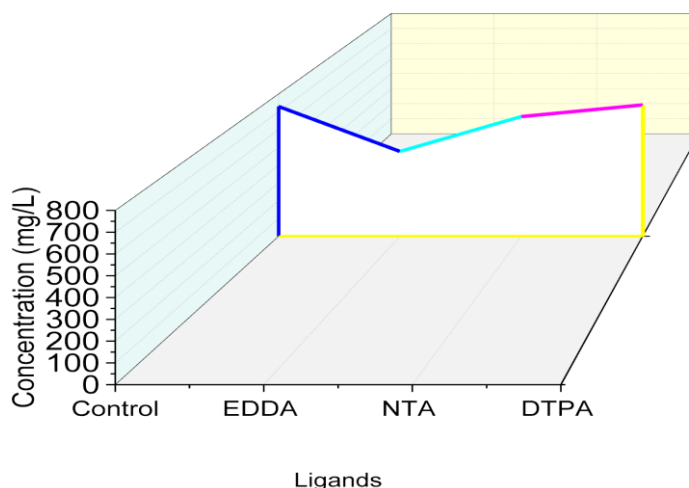


Figure 4: Result Showing the Effect of Addition of Chelating Ligand on VFA Concentration

The final VFA concentration for EDDA containing digester systems was recorded as 476.05 mg/L; NTA had a final VFA concentration value of 671.96 mg/L, while DTPA recorded a final VFA value of 737.63 mg/L against the control which had a concentration of 644.48 mg/L. This low VFA concentration levels in EDDA, NTA and DTPA is implicated in the high CH₄ gas yield observed with these digester systems. In general, the VFA concentrations were significantly lower than 2000 mg/L with pH range between 6-7.5 (see Table 2); indicating that all the anaerobic digester systems performed appreciably above average.

Table 2: Result Showing the Effect of Addition of Chelating Ligand on pH CM Substrate

WEEK	EDDA	NTA	DTPA
1	6.04	6.00	6.05
2	7.37	7.38	7.54
3	7.50	7.37	7.49

4.4 Conclusion

In conclusion, the results show that the addition of EDDA, NTA, and DTPA to digester systems helped improve the CH₄ gas produced significantly by 2-15%. Thus, the use of chelating ligands has been shown to be a promising and sure way of improving the percentage of CH₄ gas produced from solid waste digester systems.

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