



This work is licensed under  
Creative Commons Attribution  
4.0 International License.

DOI: 10.53704/fujnas.v1i1.31

A publication of College of Natural and Applied Sciences, Fountain University, Osogbo, Nigeria  
Journal homepage: [www.fountainuniversity.edu.ng/journal/fujnas](http://www.fountainuniversity.edu.ng/journal/fujnas)

## Effects of Organic Nitrogen and Carbon Supplementation on Biomethanation of Rice Bran

<sup>1\*</sup>Ganiyu, O. T. and <sup>2</sup>Oloke, J. K.

<sup>1</sup>Department of Biological Sciences, Fountain University, Osogbo, Osun state, Nigeria.

<sup>2</sup>Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

### Abstract

The effects of organic nitrogen, sucrose and starch on the bio-methanation of rice bran were investigated using digesters of 1-litre capacity operated at ambient temperature (30 - 32°C) and pH of 7 for 20 days. Rice bran and organic nitrogen supplement were varied at ratios 2:1, 3:1, 4:1 and 5:1, while 20g of sucrose and 20 g of starch were added to rice bran as supplements. Fresh strained cow rumen liquor was used as inoculum. There was no significant difference ( $P < 0.05$ ) between non-supplemented and sucrose supplemented feedstock. Starch supplemented feedstock produced more biogas than the sucrose supplemented and the non-supplemented feedstock ( $P > 0.05$ ). Varying the organic nitrogen had no significant effect on the biogas yield ( $P < 0.05$ ).

**Keywords:** Inoculum; feedstock; sucrose; starch; poultry droppings

### Introduction

Waste generation is an unavoidable component of our daily life. Almost all human activities generate waste. Wastes can be grouped based on the source into: i) domestic, ii) municipal, iii) agricultural and iv) industrial (Kalia, 2007). Agriculture and municipal sectors are the main contributors to the wastes produced. Anaerobic treatment is the use of biological processes, in the absence of oxygen, for the breakdown of organic matter and the stabilization of these materials, by conversion to methane and carbon dioxide gases and a nearly stable residue. In the year 2003, the economic and social council of the United Nations adopted a survey, presented in 1978 to the committee on Science and Technology for development, listing the ongoing research and development in unconventional sources of energy.

The use of farm wastes to produce methane has also been identified in the United Nations World plan of action for the application of Science and Technology to development (Yadvica *et al.*, 2004). Agriculture and food industries produce high quantities of organic residues which could be used as raw materials for biogas production. Salminen, Rintala, Harkonen, Hogmander and Oikari (2001), Tani, Sakamoto, Kishimoto and Umetsu (2006) and Wang *et al.*, 2010 found that anaerobic decomposition of organic material contributes to the reduction of residues and increases the energy value of farming by producing biogas and utilizing biodigester residues to fertilize the soil instead of

\*Corresponding author: Tel: +234-7034650862  
Email: address: [tajudunni@yahoo.com](mailto:tajudunni@yahoo.com)

using mineral fertilizers. Effective biodegradation of organic wastes into methane requires the coordinated metabolic activities of different microbial populations (FAO, 1992).

Today, anaerobic digestion is widely accepted as a sound technology for many waste treatment applications. Wastes from agriculture and agro-based industries have been a major source of biogas especially in areas where agriculture forms important part of the economy. These wastes if allowed to litter the environment could pose a lot of health problems as they are potential growth media for pathogenic microorganisms (Uzodinma *et al.*, 2007). The final product of anaerobic fermentation of biomass is a flammable gas (biogas) which is composed of 50 - 75% methane ( $\text{CH}_4$ ), 25 - 45% carbon dioxide ( $\text{CO}_2$ ), 2 - 7% water ( $\text{H}_2\text{O}$ ), trace amounts of oxygen ( $\text{O}_2$ ), nitrogen ( $\text{N}_2$ ), ammonia ( $\text{NH}_3$ ) and hydrogen sulphide ( $\text{H}_2\text{S}$ ).

Nigeria is one of the major producers of food in the world and yet the value in food products is very low (FAO, 1992). This results in tremendous monetary loss to the marginal farmer and the nation. Mostly, it is the lack of energy to power appropriate food processing industries in rural areas that causes this loss. Cost benefit analysis of methane generation vary widely, depending upon the uses and actual benefits of biogas production, public and private costs associated with the development and utilization of methane, and on the technology used to generate methane. Several factors affect the economics of biogas generation. These factors include pH, temperature, and carbon/nitrogen ratio of input material, types of feedstock and retention time (National Academy of Sciences, 1997). The relationship between the amount of carbon and nitrogen present in organic materials is often expressed in terms of the Carbon/Nitrogen (C/N) ratio. A C/N ratio ranging from 20 to 30 is considered optimum for anaerobic digestion (FAO, 1996). If the C/N ratio is very high, nitrogen will be used up rapidly by methanogens for meeting their protein requirements and will no longer react on the left over carbon content of the material. As a result, gas production will be low. On the other hand, if the C/N ratio is very low,

nitrogen would be liberated and accumulated in form of ammonia. Ammonia will increase the pH value of the content in the digester and pH value higher than 8.5 will start showing toxic effect on methanogen population (FAO, 1996). Animal waste, particularly cattle dung, has an average C/N ratio of about 24. The plant materials such as straw and sawdust contain a higher percentage of carbon. The human excreta have a C/N ratio as low as 8 (Karki and Dixit, 1984). Materials with high C/N ratio could be mixed with those of low C/N ratio to bring the average ratio of the composite input to a desirable level. In China, as a means to balance C/N ratio, it is customary to load rice straw at the bottom of the digester upon which latrine waste is discharged. Similarly, at Machan Wildlife Resort located in Chitawan district of Nepal, feeding the digester with elephant dung in conjunction with human waste enabled to balance C/N ratio for smooth production of biogas (Karki *et al.*, 1994).

Over the last few years, during which methane fermentation have been used for waste treatment and fuel production, several processes have been developed, each having its own potential for treatment of industrial, agricultural or municipal wastes, with a variety of organic concentrations and characteristics. The principal focus of this study is to optimize conditions for anaerobic degradation of rice bran and evaluate the process and operational parameters for the bi-methanation of wastes to biogas. The overall objectives are to evaluate and monitor process and operational parameters which influence the depolymerization of complex polymers such as rice bran, evaluate fermentability of some organic wastes in mixtures in order to elucidate optimal biomethanation and good biogas yield and investigate the effects of disaccharide (sucrose) and polysaccharide (starch) on biogas yield from rice bran.

## Materials and Methods

### Digester

The digesters consist of seven 1-litre transparent glass bottles with plastic cap. The bottles were locally sourced. The digesters were connected by means of rubber tubings to inverted

burettes inside a plastic bucket filled with water. The burettes were held in place by means of clamps and stands. The burettes were used for measuring biogas production (Plate 1 and 2). Other materials used are digital pH meter (Jenway 3505), hose pipes, digital weighing balance and blender (National).

#### *Feedstock Materials*

The feedstock materials used were rice bran collected from a rice mill in Ogbomoso and then milled to 60-mesh particle size and stored in plastic containers. Poultry droppings were obtained from Best Food Farm at Ikose near Ogbomoso. Sucrose and starch were of analytical grade.

#### *Organic Nitrogen Supplement*

Poultry droppings were oven dried at 87°C for 16 hours. The dried droppings were then blended using National blender and stored at room temperature (26°-30° C) in plastic containers. Dried poultry droppings serve as the organic nitrogen supplement.

#### *Inoculum*

The microbial inoculum was obtained from the rumen of a freshly slaughtered cow. It was collected from the slaughter house at Oja tuntun in Ogbomoso and brought to the laboratory in sealed air-tight containers. This was strained in cheese cloth and the liquor used as digester inoculum.

#### *Digester Operation*

Normal digester operation was carried out with 100g of rice bran, 33.33g of poultry droppings, 500cm<sup>3</sup> of sterile pre-warmed water, all thoroughly mixed before 50cm<sup>3</sup> of inoculums was added according to the method of Okaka, Ekpunobi, Ezeonu and Ofih (1997) and the digester tightly covered. The pH of the mixture was measured using 'Jenway 3505' pH meter and adjusted to 7. Temperature of the environment was monitored twice daily using a 100 °C Mercury in glass thermometer. Rubber tubing connected the digester to an inverted burette filled with water inside plastic buckets. The inverted burette

was used to measure the amount of gas produced daily using downward displacement of water. The amount of biogas produced is equivalent to the volume of water displaced in the burettes. The burettes were refilled daily after taking the readings. The digesters were stirred once daily by shaking and swirling (Okaka *et al.*, 1997).

The following parameters were varied and gas production was monitored for 20 days

- Organic nitrogen component were varied at ratios 2:1, 3:1, 4:1 and 5:1 using five digesters, with all other parameters being constant.
- Digesters were supplemented with 20g of starch and sucrose to determine their effects on biomethanation of rice bran.

#### *Statistical Analysis*

The student t-test was used to ascertain if there were significant differences between the means of gas evolved at ( $P < 0.05$ ).

#### **Results**

Figure 1 shows the effect of organic nitrogen on biodegradation of rice bran. At rice bran to organic nitrogen ratio 2:1, there was an efficient gas production from day 1 to day 11 but from day 12 production of gas gradually declined. For the rice bran to organic nitrogen ratio 3:1, the volume of gas produced from day 1 to day 8 was steady but decreased steadily after day 8. About the same results were obtained for rice bran to organic nitrogen ratios 4:1 and 5:1. The total volume of gas produced were 879.00, 800.70, 757.90 and 712.80 cm<sup>3</sup> for rice bran to organic nitrogen ratios 2:1, 3:1, 4:1 and 5:1 respectively (Table 1). Statistical analysis of the means showed no significant differences in their means ( $P < 0.05$ ).

The effect of the addition of sucrose and starch to the feedstock on the production of gas is shown in Figure 2. Starch supplemented feedstock started producing gas from day 1 (54.50cm<sup>3</sup>), while sucrose supplemented feedstock and unsupplemented feedstock started gas production from day 4 and day 3 respectively. The total volume of gas produced were 461.70,

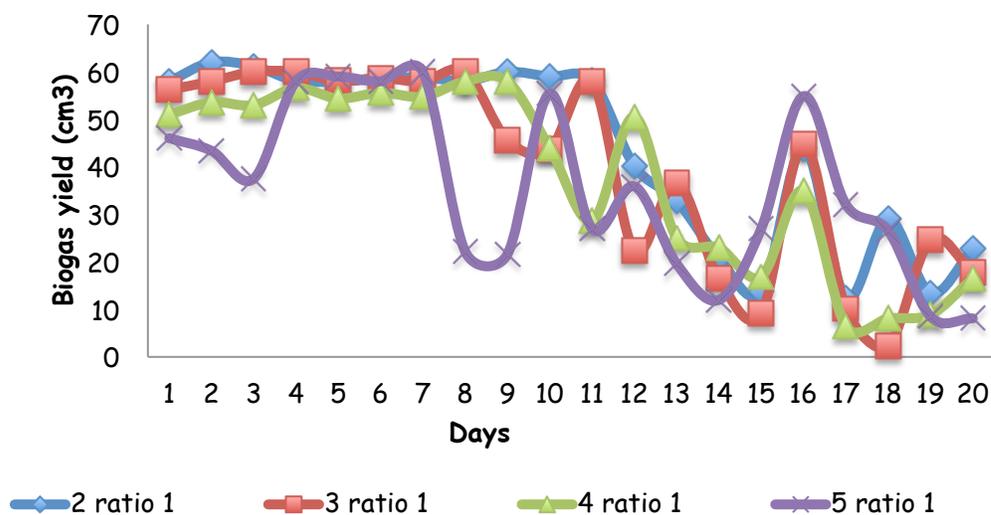


Figure 1: Effect of organic nitrogen on biodigestion of rice bran

Table 1: Cummulative biogas yield at different ratios of rice bran to organic nitrogen for twenty days.

| Ratio of rice bran to organic nitrogen         | 2:1    | 3:1    | 4:1    | 5:1    |
|--|--------|--------|--------|--------|
| Total Vol of gas produced (cm <sup>3</sup> )   | 879.00 | 800.70 | 757.90 | 712.80 |
| Mean volume of gas produced (cm <sup>3</sup> ) | 43.95  | 40.03  | 37.89  | 35.64  |

No significant ( $P > 0.05$ ) between the means

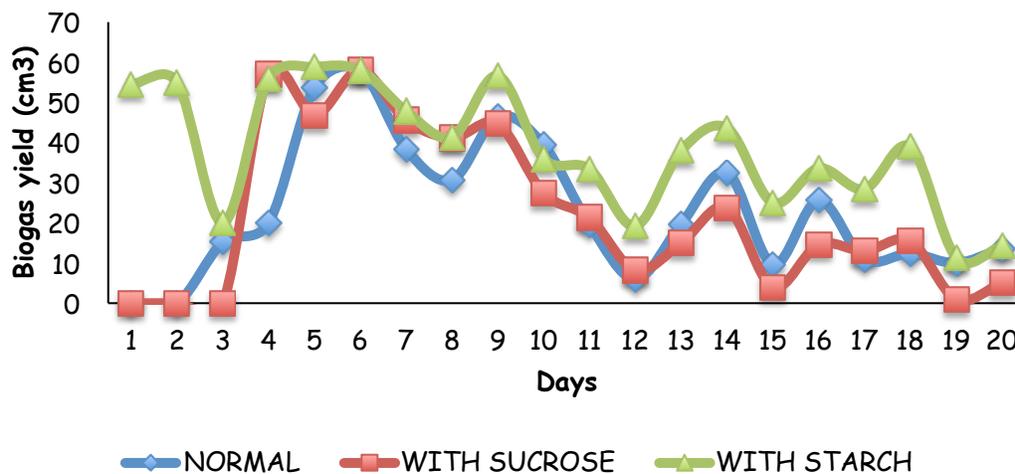


Figure 2: Effect of adding sucrose and starch on the yield of biogas.

442.00 and 769.80cm<sup>3</sup> from non-supplemented, sucrose supplemented and starch supplemented feedstocks respectively (Table 2). Statistical analysis of the means of gas produced showed significant differences ( $P > 0.05$ ) between the means of the gas produced by non-supplemented

feedstock and starch supplemented feedstocks. There was no significant difference ( $P < 0.05$ ) between the means of the gas produced by the non-supplemented feedstock and the sucrose supplemented feedstock.

Table 2: Cumulative biogas yield from non-supplemented, sucrose supplemented and starch supplemented feedstock for twenty days.

| Nature of feedstock material                  | Non-supplemented   | Sucrose-supplemented | Starch-supplemented |
|---|--------------------|----------------------|---------------------|
| Total vol. of gas produced (cm <sup>3</sup> ) | 461.70             | 442.00               | 769.80              |
| Mean vol. of gas produced (cm <sup>3</sup> )  | 23.08 <sup>a</sup> | 22.10 <sup>a</sup>   | 38.49 <sup>b</sup>  |

Means in rows with the same alphabet (a) are not significantly different ( $P>0.05$ ), means in rows with different alphabets (a,b) are significantly different ( $P<0.05$ )

## Discussion

Nitrogen is important in biodigestion of wastes to produce biogas anaerobically because nitrogen is necessary for the growth and multiplication of anaerobic microorganisms. Nitrogen present in the feedstock has two benefits: (a) it provides an essential element for synthesis of amino acids, proteins and nucleic acids; and (b) it is converted to ammonia, which as a strong base, neutralizes the volatile acids produced by fermentative bacteria, and thus helps maintain neutral pH conditions essential for cell growth. Varying the amount of nitrogen supplement added to the rice bran did not significantly affect the amount of gas produced. The total volume of gas produced are 879.0, 800.70, 757.90 and 712.80cm<sup>3</sup> from the feedstock to organic nitrogen ratios 2:1, 3:1, 4:1 and 5:1 respectively, showing reduction in the biogas yield as nitrogen level increased. At rice bran to organic nitrogen ratio 2:1, there was an efficient gas production from day 1 to day 11 but from day 12, production of gas gradually declined, this may be as a result of decreasing concentration of organic nitrogen since nitrogen is needed by methanogens for protoplasm development (FAO, 1992). FAO in 1992 also reported that too much of nitrogen can lead to accumulation of ammonia and ammonium which are toxic to methanogens and may lead to stoppage of methanogenesis. Although nitrogenous supplements enhanced biogas production, the quantity should not be in excess because of toxicity of the medium, which could stop the multiplication of some methanogenic bacteria. An over abundance of nitrogen in the substrate can lead to excessive ammonia formation, resulting in toxic effects as observed by Fry in 1974. Thus, it is important that the proper amount of nitrogen be in the feedstock, to avoid either nutrient limitation (too little nitrogen) or ammonia toxicity

(too much nitrogen).

The composition of the organic matter added to a digestion system has an important role on the growth rate of the anaerobic bacteria and the production of biogas. The use of kitchen wastes and wastes from food processing industries have been suggested as good feedstock for biogas production (Kalia, 2003). The large volume of gas (769.80cm<sup>3</sup>) produced by starch supplemented feedstock compared to 442.00cm<sup>3</sup> produced by sucrose supplemented and 461.70cm<sup>3</sup> by the non-supplemented feedstock attest to the fact that starchy feedstock are better for biogas production and it may be advisable to first convert cellulosic materials to starchy derivatives before being used to obtain better yields because starch is an intermediate product in the biomethanation of lignin and cellulose to produce biogas (Chongrak, 1988; Best, 1988). Optimum period for starch-supplemented feedstock was between day 1 and 9 and for sucrose-supplemented feedstock between day 4 and 9 while for the unsupplemented feedstock it was between day 5 and 10.

## Conclusion and recommendations

The results show that though nitrogen is needed by Methanogens, its ratio should be minimal and monitored in order to avoid toxicity of the biodigester which may lead to stoppage of biodigestion and reduction in biogas yield. Starchy feedstocks are better used for biogas generation than cellulose, lignocellulose and sucrose feedstock because they will enhance the generation of gas almost immediately and production of more biogas over a long period of time. It is recommended that rice bran to organic nitrogen ratio 2:1 be used for charging biogas plant when rice bran is to be used as feedstock material. Biogas is produced as a result of the

metabolic activities of Microorganisms, regular biogas production could be mastered when the living conditions, operational parameters and mechanisms of metabolism of the fermenting Microorganisms are recognized.

## References

- Best, D. J. (1988). The Environment and Biotechnology. In I.J., Higgins *et al* (eds.), *Biotechnology: Principles and application* (pp. 222-231). Blackwell Scientific Publication.
- Chongrak, P. (1988). *Organic waste recycling*. Published by John Wiley and sons.
- FAO, (1992). *Biogas processes for sustainable development*. FAO Agricultural services Bulletin No. 95, Rome
- FAO, (1996). *Report on the Meeting for the Development of a National Biogas Policy Framework and Celebration of the 10,000th Biogas Plant Construction with BSP Support*. FAO, Kathmandu, 7 February 1996.
- Fry, L. G. (1974). *Practical Building of Methane Power Plants for Rural Energy Independence*, Santa Barbara, California.
- Kalia, V. C. (2003). *Future Fuels via Microbial Digestion of Wastes*. United Nations Development Programme.
- Kalia, V. C. (2007). *Microbial Treatment of Domestic and Industrial Wastes for Bioenergy Production: In Applied Microbiology, Microbial Biotechnology and Genomics*, Institute of Genomics and Integrative Biology, CSIR, Delhi University Campus, Mall Road, Delhi - 110 007
- Karki, A. B. and Dixit, K. (1984). *Biogas Fieldbook*. Sahayogi Press, Kathmandu, Nepal.
- Karki, A. B., Gautam, K. M. and Karki, A. (1994). *Biogas Installation from Elephant Dung at Machan Wildlife Resort, Chitwan, Nepal*. Biogas Newsletter, Issue No. 45.
- National Academy of Sciences (1997). *Methane Generation from Human, Animal and Agricultural Wastes*, NTIS Accession No PB-276-469, NAS, Washington, DC.
- Okaka, A. N. C., Ekpunobi, M. I., Ezeonu, F. C. and Ofih, J. O. (1997). Optimization of biogas production from corn-cob. *Proceedings of an international conference on biotechnology for development in Africa; priorities for the early twenty-first century*, Enugu, Nigeria.
- Salminen, E., Rintala, J., Harkonen, M., Hogmander, H. and Oikari, A. (2001). Anaerobically digested poultry slaughterhouse wastes as fertilizer in agriculture. *Bioresource Technology* 78, 81-88.
- Tani, M., Sakamoto, N., Kishimoto, T. and Umetsu, K. (2006). Utilization of Anaerobically digested dairy slurry combined with other wastes following application to agricultural land. *International Congress Series 1293*, 331-334.
- Uzodinma, E. O. U., Ofoefule, A. U. Eze, J. I. and Onwuka N. D, (2007). Optimum mesophilic temperature of biogas production from blends of agro-based wastes. *Trends in Applied Science Research* 2, 39-44.
- Wang, L., Li, Y., Chen, P., Min, M., Chen Y., Zhu, J. and Ruan, R. R. (2010). Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. *Bioresource Technology* 101, 2623-2628
- Yadvika, S. T. R., Kohli, S. and Rana, V. (2004). Enhancement of biogas production from solid substrates using different techniques: a review. *Bioresource Technology* 95(1), 1-10.