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네이피어 풀줄기의 혐기성 분해: 외부에서 가해준 철과 니켈의 효과

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Anaerobic Digestion of Napiergrass Stem: the Effect of Exogenous Iron and Nickel

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요 약. 네이피어 풀줄기의 메탄 전환수율에 대한 니켈과 철의 효과(각기 독자적 사용 또는 혼합사용 경우)를 조사하기 위해 실험실 규모의 혐기성 분해 실험을 수행하였다. 철(iron (III) chloride, $FeCl_3$), 니켈(nickel (II) chloride, $NiCl_2$), 및 철-니켈 혼합물(a 1:1 $FeCl_3$ and $NiCl_2$ molar mixture)을 기질에 첨가하였다. 분해실험을 진행하기 위해 사용한 미생물은 새로 도축한 5년생 숫소의 첫째위장액에서 얻은 것을 사용하였다. 생분해로부터 얻어진 가스를2일 간격으로 30일간 채집하였고 메탄 수율을 기체크로마토그래피를 사용하여 측정하였다. 이 실험결과로부터 누적메탄수율, 메탄생성속도(메탄생성 이전) 시간 지연 등은 철, 니켈, 철-니켈 혼합물을 가함에 의해 촉진된다는 것을 알았다. 네이피어 풀줄기의 누적메탄수율은 이들 무기화합물들의 첨가양의 증가함에 따라 증가함을 또한 발견하였다. 게다가 니켈이온을 첨가하였을 때 최고의 메탄수율이 얻어졌다. 철과 니켈 이온들을 각기 기질에 가하였을 때 메탄생성 이전 시간 지연이 8일에서 6일로 감소하였고, 철-니켈 혼합물의 경우 4일로 더욱 감소하였다.

주제어: 네이피어풀, 메탄 수율, 철, 니켈, 혐기성 분해

ABSTRACT. Batch laboratory-scale anaerobic digestion experiments were carried out with the aim of investigating the effect of nickel and iron, singly and in combination, on the methane yield of napiergrass stem. Iron, nickel and an iron-nickel mixture were added to the substrate as iron (III) chloride ($FeCl_3$), nickel (II) chloride ($NiCl_2$), and a 1:1 $FeCl_3$ and $NiCl_2$ molar mixture, respectively. The inoculum used to inoculate the digestion experiments was obtained from the rumen fluid of a freshly slaughtered 5 year old male bovine. The biogas produced was collected for 30 days by 2 days intervals and the methane yield was measured using gas chromatography (GC). The results obtained show that the cumulative methane yield, rate of methane production, and time lag before methane production were enhanced with the addition of iron, nickel, and the iron-nickel mixture. The study also show that the cumulative methane yield of the napiergrass stem increased as the amount of added $FeCl_3$, $NiCl_2$, and $FeCl_3$-$NiCl_2$ mixture increased. Furthermore, the cumulative methane yield was highest when nickel ion was added. Addition of iron and nickel ions to substrate reduced the time lag before methane production from 8 days to 6 days and addition of the iron-nickel ions mixture to substrate reduced the time lag to 4 days.

Keywords: Napiergrass, Methane Yield, Iron, Nickel, Anaerobic Digestion

INTRODUCTION

It has long been known that energy can be derived from biorenewable resources. Towards the end of the last century, the production of energy from biorenewable resources has changed from a playground of few researchers to a proven technology. This change is due to record-high oil, gasoline, and natural gas prices, couples with a steady stream of blistering news accounts of real or possible global-warming emergencies.[1] One of the premier energy potentials of biorenewable resources is the production of methane from energy crops.[2,3] Literature search reveals that the production of methane via anaerobic digestion of energy crops is one of the most energy-efficient and environmentally benign ways of generating energy.[4]

Napiergrass, *Pennisetum purpureum*, is one of the most important energy crops producing high herbage yield per unit area of land.[5] Napiergrass is a tall, predominantly vegetative-propagated, perennial indigenous to sub-Saharan Africa, which can produce biomass of 20~30 tons per hectare per year.[6] Methane production potential of napiergrass via anaerobic digestion is documented.[7] The production of methane via anaerobic digestion of napiergrass, like other biomethanation processes, is a complex group of biochemical reactions, which rely on the interaction of different groups of microorganisms and enzymes. Various factors affect these biochemical reactions, microbial interactions and enzymic activities. These factors, which have profound impact on methane yield, include type of inoculum, pH and temperature of substrate, carbon – nitrogen ratio of substrate, volatile fatty acid content of substrate, pretreatment of substrate, presence of salts, and trace metals ions, etc..[7-14]

The importance of trace metals ions, singly or in combination, in anaerobic digestion of energy crops has been reported.[13,14] The results of these previous studies suggest that trace metals ions substantially increase the methane yield of biomass by activating the metalo-enzymes presence during the digestion process.[15,16] Whilst the importance of trace metals ions in anaerobic digestion is documented in literature, the body of literature reporting the effect of trace metals ions on the methane yield of energy crops during anaerobic digestion is scanty. In fact, research on the significance of these ions on methane yields of energy crops during anaerobic digestion has been neglected. This neglect is due to the presence of seemingly adequate trace metals in energy crops and/or the practice of blending poor-quality energy crops with trace metals-rich animal manure or sewage. The main purpose of this work was to evaluate the effect of iron and nickel ions, singly and in combination, on the methane yield of napiergrass stem. Sub-objectives include (i) compare the effect of iron and nickel ions, singly and in combination, on the methane yield of napiergrass stem; (ii) investigate the effect of varying amounts of iron and nickel ions on methane yield of napiergrass; and (iii) determine the chemical characteristics of napiergrass.

MATERIALS AND METHODS

Chemicals

All the chemicals used in the study were analytical grade, purchased from Merck (Darmstadt, Germany), and used without further purification.

Source of substrate

The stems of the 6 month old napiergrass used in this study were obtained in October 2007 from a private farm in Benin City, Nigeria. The napiergrass stems were taken to the laboratory and chopped to approximately 3 cm particle size. The chopped samples were dried at room temperature to a constant weight and ground in a Wiley mill to pass through a 1 mm screen. The ground sample was frozen and stored at -20 °C. Before analyses and anaerobic digestion experiments, the samples were allowed to thaw overnight at 4 °C.

Pre-treatment of substrate

A 20 g of the ground sample and 4 g of sodium hydroxide pellet were put into a 250 mL conical flask. Deionised water was then introduced into the flask until the sample was submerged. The slurry was thoroughly stirred with a magnetic stirrer at 250 rpm to ensure complete dissolution of sodium hydroxide pellet. The conical flask containing the slurry was corked and left to stand for 24 h in a water bath maintained at 35 °C. At the end of the pre-treatment process, 40 mL of 2.5 M hydrogen chloride acid was added to bring the pH of the slurry to 7.0. The neutralized slurry was further stirred and left to stand for another 24 h at room temperature before the anaerobic digestion experiment; after which the slurry was filtered and then air-dried to obtain the substrate.

Source of inoculum

The inoculum used for the anaerobic digestion of substrate was obtained from rumen compartment of a freshly slaughtered 5 year old male bovine that weighed 178 kg. The bovine, which was maintained on a steady meal of napiergrass and groundnut residue for 3 months, was slaughtered at the Edo State Abattoir, Ikpoba, Benin City, Nigeria. A 40 mL of the rumen fluid was filtered through a glass wool into conical flask and 30 mL of 1:4 potassium dihydrogen phosphate/potassium bicarbonate buffered solution of pH 7.2 was added. The buffered inoculum was stirred and incubated at 37±1 °C until digestion experiment.

Anaerobic digestion of substrate

The digestion of the substrate was carried out in a laboratory-scale batch experiments. The digestion experiments were conducted in duplicate in a 500 mL glass bottles on a shaking water bath (70 rpm) at 37±1 °C. A 50 mL of the inoculum and 0.5 g of the substrate were added to the bottles. 0.1 g $FeCl_3$ was added to the content in the bottle. The experiment was also carried out with different masses (0.2 g, 0.3 g, and 0.4 g, respectively) of $FeCl_3$. The contents of the bottles were flushed with N_2/CO_2 gas mixture for 10 minutes and the bottles sealed with rubber stoppers. The bottles were connected to a calibrated wet gas meter to collect biogas produced. The methane content of the biogas was measured for 30 days by 2 days intervals. The digestion was also carried out with different masses (0.1, 0.2, 0.3, and 0.4 g) of $NiCl_2$, and 1:1 molar mixture of $FeCl_3$ and $NiCl_2$. The digestion experiment was also carried on a substrate without added trace metal salts, which served as control. Assay with inoculum only was carried to minus the methane yield of inoculum from that of the substrate.

Methane analyses and calculations

Biogas samples were taken with a pressure lock syringe and their methane content was measured with gas chromatography (Perkin Elmer Model Clarus 500). The GC was equipped with a flame-ionisation detector and 30 m × 0.53 mm Perkin Elmer alumina column. The carrier gas was argon and its flow rate was 0.8 mL min^{-1}, oven temperature was 100 °C, injection port temperature was 250 °C and detector temperature was 225 °C. The methane yield of substrate was calculated as m^3 CH_4 kg^{-1} VS_{added} minus the methane yield of the inoculum.

Determination of chemical characteristics of napiergrass stem

Volatile fatty acids (VFAs) were measured with a GC (Perkin Elmer Autosystem XL) equipped with flame-ionisation detector. The GC's column was 30 m × 0.32 mm × 25 μm PE FEAP column, carrier gas was helium and its flow rate was 0.8 mL min^{-1}, injection port and detector temperature were 225 °C, while the oven temperature was 100~160 °C (20 °C/min). Total solids and volatile solids were determined according to the Standard Methods[17] and Metrohm 774 pH-meter was used in pH measurement. pH of sample was measured from slurry of substrate and deionised water. Carbon and nitrogen were analyzed with elemental analyzer

Table 1. Chemical characteristic data of napiergrass

Chemical characteristics	Amounts
TS (% FM)	18.62
VS (% TS)	92.81
VFA (g/kg FM)	0.42
pH	6.60
C:N (Carbon : Nitrogen ratio)	18.82
Total carbohydrate (% TS)	9.63
Crude fat (% TS)	3.20
Crude fiber (% TS)	24.69

(CHN2400, Perkin-Elmer, Wellesley, MA, USA). Crude fats, crude fiber and carbohydrates were determined according to the Standard Methods.[18] The results of the chemical characteristics of napiergrass stem are given in *Table* 1.

RESULTS AND DISCUSSION

The results of the study concord with results of previous studies that metal ions enhance methane yield of biomasses undergoing anaerobic digestion.[13,15,16,19] The results of the study, which are presented in *Figs.* 1-4, show that iron and nickel, which were added as $FeCl_3$ and $NiCl_2$, respectively, can have effect on the methane yield and rate of methane production during anaerobic digestion of napiergrass stem.

Generally, the study shows that iron and nickel, either singly or in combination, improved the methane yield of napiergrass stem. The rate of methane production was also enhanced during the digestion. The study also shows that the metal ions reduced the time lag before methane production. Overall, it was observed that addition of nickel to the substrate during anaerobic digestion was more beneficial to methane production than addition of iron or the iron-nickel mixture. The role of these metals in improving the methane yield of napiergrass was not

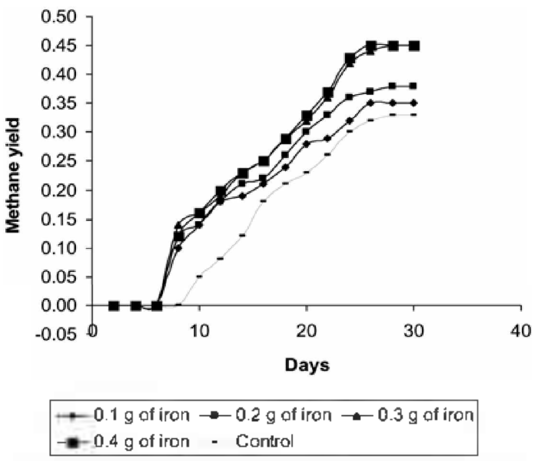

Fig. 1. Effect of various amounts of $FeCl_3$ on the mean cumulative methane yield (m^3 CH_4 kg^{-1} VS_{added}) of napiergrass stem collected for 30 days by 2 days intervals.

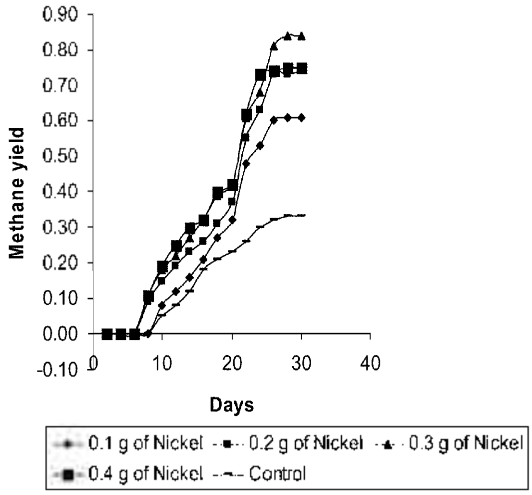

Fig. 2. Effect of various amounts of $NiCl_2$ on the mean cumulative methane yield (m^3 CH_4 kg^{-1} VS_{added}) of napiergrass stem collected for 30 days by 2 days intervals.

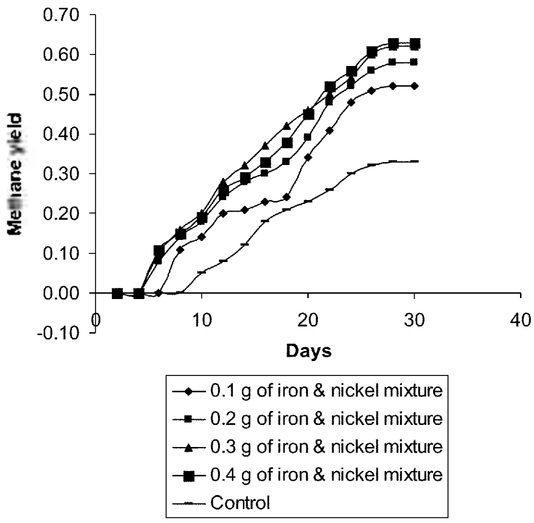

Fig. 3. Effect of various amounts of $FeCl_3$ and $NiCl_2$ mixture on the mean cumulative methane yield (m^3 CH_4 kg^{-1} VS_{added}) of napiergrass stem collected for 30 days by 2 days intervals.

surprising as metals have been reported to increase methane yield during anaerobic digestion of biomass.[13,15,16,19] Geetha et al., attributed enhanced methane yield during anaerobic digestion in the presence of metal ions to activation of metalo-enzymes by the metal ions.[16] These metalo-enzymes catalyze biomethanation of biomasses.

The results as presented in *Figs.* 1-3 indicate that the methane yield increased as the amount of added metal ions increased. Compared with the control, addition of 0.1 g of $FeCl_3$ to the substrate resulted in an increase of 6.06% in methane yield. This relative increase was further increased to 15.15 and 36.00% when the amount of added $FeCl_3$ was increased to 0.2 and 0.3 g, respectively. Further increase did not increase the methane yield beyond the relative percent increase of 36.00%. The effect of the amount of metal ions was more remarkable with nickel than with iron and iron-nickel salts mixture. The relative percent increase was 84.85% when 0.1 g of $NiCl_2$ was added to the substrate; this relative percent increase rose to 121.21% and 154.55% when the amount of added $NiCl_2$ was increased to 0.2 and 0.3 g, respectively. A trend similar to that obtained with $FeCl_3$ was observed with the $FeCl_3$-$NiCl_2$ mixture except that the relative percent increase in methane yield was higher with the mixture. The effect of amount of trace metals added on methane yield suggested that an optimum quantity of trace metal is needed to obtain the best yield of methane.

Fig. 4 shows the comparision of the rate of methane production and methane yield of the substrate when 0.3 g of $FeCl_3$, $NiCl_2$ and $FeCl_3$-$NiCl_2$ mixture were respectively added to the substrate. The results indicate that the addition of 0.3 g of $NiCl_2$ to the substrate gave higher methane yield than the addition of either 0.3 g of $FeCl_3$ or 0.3 g of $FeCl_3$-$NiCl_2$ mixture. The order of increasing methane yield due to presence of 0.3 g of these metal ions was Fe < Fe-Ni mixture < Ni. Geetha and co-workers in their study of anaerobic digestion of water hyacinth and cattle waste blend observed that addition of nickel to biomass enhanced biogas yield better than addition of iron.[16] Jain et al. reported similar findings in their study of anaerobic digestion of *Azolla pinnata R. Br.* and *Lemna minor L.*; in fact, Jain and his co-workers showed that iron did not influence the yield of biogas.[19] The effect of nickel on the yield increase of methane better than iron suggests that the metalo-enzymes may have been more activated in the presence of nickel than in the presence of iron. The results in *Fig*. 4 also show

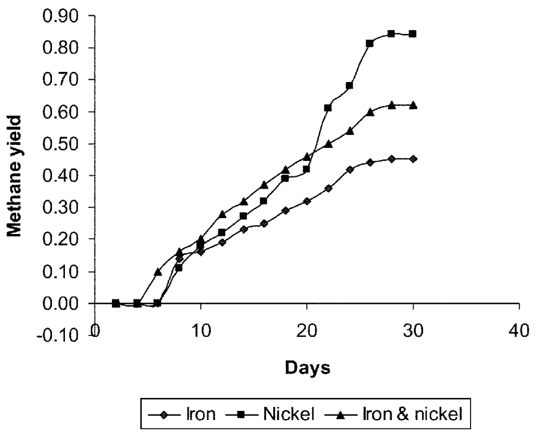

Fig. 4. Effect of 0.3 g of different metals salts on the mean cumulative methane yield (m^3 CH_4 kg^{-1} VS_{added}) of napiergrass stem collected for 30 days by 2 days intervals.

that the addition of iron to substrate increased the rate of methane production faster than the addition of the iron-nickel mixture to the substrate. The addition of iron to substrate also gave higher rate of methane production than the addition of nickel to substrate until the day 20 of digestion after which a steep rise in rate of methane production and methane yield of the substrate was observed with nickel. The observations concord with the report of Preeti Rao and Seenayya that addition of iron to biomass during anaerobic digestion gave faster biogassification than the addition of nickel.[20] In addition, the time lag before methane production was shorter in the presence of iron-nickel mixture than in the presence of nickel and iron. This shorter time suggests that iron and nickel may have had a synergistic effect on the metalo-enzymes activation.

CONCLUSION

This study describes the influence of iron and nickel on methane production potentials of napiergrass stem. Largely, the addition of iron and nickel, either singly or in combination, to napiergrass during anaerobic digestion enhanced the methane yield. While the addition of nickel gave higher methane yield than the addition of iron or iron-nickel mixture, the addition of iron-nickel mixture reduced the time lag before methane production better than the addition of nickel and iron. The findings are important since $FeCl_3$ and $NiCl_2$ would be simple, low-cost, nutritional additives to biomass anaerobic digestion. This is particularly important for biomasses that are deficient in micronutrients.

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