Anaerobic Digestion of Corn Stover for Improved Biomethane Yield: Effect of Organic Nitrogen Sources (Soybean Curd Residue and Fish Waste)

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Authors’ contributions
This work was carried out in collaboration between both authors. Author CCO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FJCO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT
The present study investigated the effect of organic nitrogen sources, soybean curd residue (SCR), and fish meal (FW) on the anaerobic digestion of corn stover for biomethane production. The bioreactors were seeded with the corn stover (corn cob and corn sheath), soybean curd residue (SCR), and fish waste (FW) at different combinations: (CC/SCR), (CC/FW), (CS/SCR) and (CS/FW), including CC and CS alone. The fermentation was for 31 days under mesophilic conditions. Characteristics of the substrates indicate that CC and CS are good carbon and energy sources, but low in nitrogen content. Conversely, SCR and FW are rich nitrogen sources, with low organic carbon content. There was a remarkable increase in biogas production in all treatments, except CC/SCR 75:25 and CC/SCR 85:15 in which inhibitory effect was observed. The highest percentage increase (138%) in biogas was recorded in CS/SCR 85:15 (2.86 dm³), and the least was CC/FW 75:25 with 1.49 dm³ (24.18% increase). Significant difference (P ≤ 0.05) in biogas yield was in the following: CC/SCR 50:50, CC/FW 50:50, CS/SCR 85:15, CS/FW 50:50, and CS/FW

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1. INTRODUCTION

Plant biomass wastes composed of cellulose, hemicellulose, and lignin are primarily referred to as lignocellulosic wastes and may be classified into different groups: wood residues (sawdust and paper mill wastes), grasses, waste paper, agricultural residues (including straw, stover, peels, cobs, stalks, nutshells, non-food seeds, bagasse, domestic wastes (lignocellulose garbage), food industry residues, municipal solid wastes, etc. Lignocellulosic biomass represents the largest renewable reservoir of potentially fermentable carbohydrates on earth, but it is largely wasted in the form of pre-harvest and post-harvest agricultural losses and wastes from food processing industries [1].

Lignocellulosic biomass, example, corn stover, rice straw and other agro-wastes have in recent time been of interest in renewable energy production, both for economic and environmental reasons. Lignocelluloses have complex chemical composition, including carbohydrates (lignin, cellulose and hemicellulose, pectin, proteins, salt, and minerals) [2]. Corn stover is a promising lignocellulosic biomass for renewable energy production. They are wastes generated from processing of corn for consumption. About 0.15 kg of cobs, 0.22 kg of leaves, and 0.50 kg of stalks are generated from every 1 kg of dry corn grains produced [3].

The rate of biodegradation of lignocellulosic wastes is generally limited because of their chemical structure and composition. From available reports, the hydrolysis of complex organic wastes to soluble compounds is the rate-limiting step of anaerobic digestion processes for wastes high in solid content. Several physical, chemical, and biological pre-treatments are therefore needed to improve substrate solubility and accelerate the biodegradation rate of solid organic waste [4].

Soybean curd residue (SCR) (okara in Japanese) is the major waste product that emanate from soybean processing into different end-products. Poor management of SCR is a potential threat to the environment and humans because of its high susceptibility to putrefaction [5]. It is well documented that SCR is a rich source of protein, and contains high quality protein (27% protein by dry basis), especially essential amino acids. The protein content of SCR has been reported to be of better quality than that from other soy products; for example, the protein efficiency ratio of SCR is 2.71 compared with 2.11 for soymilk, but the ratio of essential amino acids to total amino acids is similar to tofu and soymilk [5].

In recent decades, studies have been intensified on biological processes that convert biomass to energy, and hence provide a source of fuel. One of the most important and prominent of these processes is the anaerobic digestion (AD) of organic waste to obtain biogas, a product of the metabolic action of methanogenic microbial consortia [6]. Anaerobic digestion (AD) is a biochemical process in which complex organic compounds are broken down in an oxygen-free environment by different types of anaerobic bacteria resulting in biogas production [7]. It involves a four-stage process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis that engage four different bacterial groups (hydrolytic, acidogenic, acetogenic, and methanogenic). Each of these bacteria has different physiology and nutritional requirements [8]. While methanogenesis has been reported to be the rate-limiting step for easily biodegradable organic substrates, hydrolysis is the rate-limiting step for complex organics. This is due to the formation of toxic by-products such as complex heterocyclic compounds and undesirable volatile fatty acids [9].

Microbial growth in anaerobic digester and biogas yield is largely a function of the biodegradability and nutrient composition of the organic matter in the feedstock. The biogas yield of any organic substrate is also dependent on its carbon to nitrogen (C/N) ratio. The presence of

75:25. The composition of the biogas revealed that the treatment improved biogas production as well as biomethane content, the highest being 69.44% in CS/SCR 85:15. Regression analysis of cumulative biogas yield as a function of time (t) in the different treatments that had a significant difference in biogas yield showed a good correlation between biogas yield (GY) and time (t). Improving the biodegradability of lignocellulosic wastes could lead to a boost in the development of anaerobic digestion and biogas production technology. To improve their biodegradability during anaerobic digestion, both pre-treatments and supplementation have vital roles to play.
nitrogen source(s) in the feedstock is necessary for the synthesis of amino acids, proteins, and nucleic acid. It is also needed for the formation of ammonia to neutralize volatile fatty acid produced during the fermentation process and to maintain neutral pH conditions of the digesting slurry [10]. However, an excess of nitrogen in the feedstock can exert a toxic effect on bacteria by the formation of a very high level of ammonia. A suitable amount of nitrogen therefore is required to provide sufficient nutrients while avoiding ammonia toxicity [11].

Process instability, poor digester performance, and low biomethane production have been associated with single-substrate digestion. This could probably be because of imbalance in nutrient composition and insufficient trace elements that regulate enzyme activities in the methanogens, the key players in biogas production. Research studies have shown that the best practice is to co-digest food wastes with sewage sludge or animal waste. Co-substrate digestion has largely been beneficial in maintaining process stability, either by balancing the nutrient composition, providing the required C/N ratio, and buffering action [12]. Studies have also shown that improvement in the biodegradability of lignocellulosic wastes results in an efficient bioconversion of cellulose and hemicellulose to biofuels such as ethanol, methane, and hydrogen. However, even with successful pretreatments, the physicochemical characteristics of lignocellulosic biomass and inhibitory products are still not completely known. Different pretreatment measures are known to improve the bio-digestibility of lignocellulosic materials [13].

Co-fermentation, commonly known as co-digestion has been posited to increase the load of mixed nutrients and accelerates the breakdown of macromolecules in substrates by bio-stimulation studied during the last 15 - 20 years [9]. It is a well-investigated approach to anaerobic digestion, by blending organic wastes in different ratios, thereby increasing the load of degradable organic matter and keeping the C/N ratio within the desired range of 25-30 [14,15,16]. Nitrogen sources have been poorly investigated in anaerobic digestion processes even though they are known as one of the possible process limiting factors (in the hydrolysis phase), but also as nutrient sources to the microbial consortium in the anaerobic digestion of organic wastes with subsequent methane production. In this study, the carbohydrate-rich corn stover (corn cob and corn sheath) was supplemented with organic nitrogen sources, Soybean curd residue (SCR), and Fish waste at different ratios with the aim of improving biodegradability and ultimately enhancing methane yield.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Bioreactor feeds used in this study were corn stover (CC and SC), soybean curd residue (SCR), and fish waste (FW). The corn stover was collected from different sources at Obinze and other host communities of Federal University of Technology, Owerri, Imo State, Nigeria. The samples were sun-dried separately for 10 days to a moisture content of 9.63% and 9.58% for corn sheath and corn cob, respectively. The samples were milled to finely reduced particle size in an animal feed-processing company in Owerri, Imo State, and subsequently stored in air-tight polyethylene bags to preserve the substrates.

The soybean Curd residue (SCR) was sourced locally from producers of soybean milk. The sample, after the collection was sun-dried to a moisture content of 11.40%. The fish wastes (from smoked fish) were collected from a local market, after the day's business. It was further dried, ground, and then stored in an air-tight container.

2.2 Proximate Composition of the Substrates

The proximate composition of the substrates was determined by adopting the methods described by AOAC [17]. The parameters include the moisture content (MC), ash, total solid (TS), volatile solids (VS), crude fat, crude fiber, crude protein, carbon/ nitrogen ratio, etc.  

2.3 Bioreactor Design and Operation

The experimental design and operation were adopted from that described by Opurum et al. [18]. 2015. Batch mode bioreactors of ten liters (10 L) capacity (8 L effective volume) were used in conducting the experiments. The bioreactors were self-designed and fabricated with polyvinyl chloride material. A thermometer was fitted on the lid of each reactor to monitor temperature changes in the digesting slurry, and an outlet with a regulator at the base for sample collection to monitor the pattern of pH changes during anaerobic digestion. The biogas harvesting system has an inverted bucket with a regulator at the outlet, which enables the trapping of biogas (Fig. 1).
2.4 Preparation of Slurry and Charging of the Bioreactors

The bioreactors were loaded with different percentages (50:50; 75:25 and 85:15) of corn stover (CS and CC) and the organic nitrogen sources (SCR and FW). The different substrate combinations include: (CC/SCR), (CC/FW), (CS/SCR) and (CS/FW).

Into a clean 12 L capacity bucket, 260:260 g (520 g final weight) of CC/SCR was weighed and 6.4 L of water was used to prepare the slurry which was fed into labeled bioreactors after blending mechanically. In the bioreactor for 75:25, 346.67:173.33 g of CC/SCR was weighed and the slurry was prepared as described above. In 85:15, 390:130 g of CC/SCR was used in the preparation of the slurry. The same method was adopted for CC/FW, CS/SCR, and CS/FW. The control bioreactors each contained only corn cob (CC) and corn sheath (CS). The different slurry preparations were fed into their respective bioreactors and labeled accordingly. The bioreactors were pitched with the inoculum, the methanogen source, and the reactors were corked airtight. The water displacement system used to harvest the biogas was connected to the bioreactor via a one-quarter inch gas outlet hose from the bioreactor [19].

Anaerobic digestion was at ambient temperature that ranged between 25 - 35°C. In the course of the digestion, the bioreactor contents were manually agitated to forestall stratification and more importantly enhance the rate of contact between the microorganisms and the substrates. The volume of biogas produced daily was determined by measuring the displaced water every 24h. The anaerobic digestion lasted for thirty-one (31) days hydraulic retention time (HRT), during which changes in pH of the digesting slurry were monitored using a digital pH-meter.

2.5 Determination of Biogas Composition

Compositional analysis of the produced biogas was carried out at the National Centre for Energy Research and Development, University of Nigeria, Nsukka, Enugu State, Nigeria. A biogas analyzer was used to determine the methane (CH₄), carbon dioxide (CO₂), and carbon monoxide (CO) content.

2.6 Analysis of Data

The biogas yield in the test parameters and the control were analyzed using the students’ T-test implemented with Microsoft Excel 2003. Regression analysis with SPSS software was used to model the cumulative biogas yield as a function of hydraulic retention time (HRT), with the equation:

\[ C_Y = a + bt + \varepsilon \]  (1)

Where: \( C_Y \) = gas yield.
3. RESULTS

The determined proximate composition of the different feedstocks used in this study is presented in Table 1. The result indicates that corn cob (CC) and corn sheath (CS) are good carbon and energy sources with 57.81 and 48.32% organic carbon content, respectively, but low in nitrogen. Conversely, fish waste (FW) and soybean curd residue (SCR) are rich nitrogen sources (FW, 6.06% and SCR, 1.96%) but low in organic carbon content. The C/N ratio of CC and CS is very high (CC, 47:1 and CS, 54:1) while SCR and FW are very low (SCR, 9:1, and FW, 4:1). Similarly, the substrates exhibited a remarkably high level of volatile solid (VS) and organic carbon contents.

The anaerobic digestion pattern of corn cob supplemented with different ratios of soybean curd residue against the hydraulic retention time (HRT) and the pH changes are shown in Fig. 2. In CC/SCR 50:50 and 75:25 the pH fluctuation was in the range of 6.38 - 4.20 and 4.74 - 4.08, respectively. The peak of biogas production was recorded on days 1 and 2 followed by a sharp drop in daily biogas production throughout the study period. The lag phase lasted for four days in CC/SCR 85:15, a peak of biogas production was observed on the 7th day with a subsequent decrease in gas production, and the pH was observed to be remarkably lower than the optimum.

The mean cumulative biogas yield from each of the tested parameters within the study period (31 days HRT) was 2.29 dm³, 0.92 dm³, and 0.17 dm³ for the ratios: CC/SCR 50:50, CC/SCR 75:25, and CC/SCR 85:15, respectively. It could be observed that while the treatment in CC/SCR improved biogas yield, whereas there was an antagonistic effect in CC/SCR 75:25 and CC/SCR 85:15 (Table 2).

Plots of anaerobic digestion of corn sheath (CS) with different ratios of soybean curd residue (SCR) and daily biogas production pattern, with corresponding changes in pH, are shown in Fig. 3. Biogas production started on day 1 in the treatments CS/SCR 50:50 and 75:25, reached its peak on day 2 (0.495 and 0.435 dm³, respectively). With the decrease in pH below 5.5, the daily biogas production decreased below 0.1 dm³ throughout the hydraulic retention time. The biogas yield (cumulative) in CS/SCR 50:50, CS/SCR 75:25 and CS/SCR 85:15 are 1.68, 1.64 and 2.86 dm³, respectively.

Presented in Fig. 4 is the effect of supplementation of corn stover (CC and CS) with fish waste (FW). In all the tests, gas production started on day 1, the peak of production was recorded on day 3 and 4 in CC/FW 50:50 and 75:25, respectively. It could also be observed that a steady decrease in biogas production followed a decrease in the pH of the digesting slurry. A similar observation was made in the corn sheath (CS) treated supplemented with fish waste (FW). As indicated by the result of the standard deviation, there was a very low variation in the different bioreactors, and the data was used to introduce error bars in the graph.

The mean cumulative and percentage increase in biogas yield are summarized in Table 2. The treatment conditions reasonably increased biogas production in all the test parameters except CC/SCR 75:25 and CC/SCR 85:15 in which biogas yield was lower than that of control (1.02 dm³), indicating an inhibitory or antagonistic effect. The highest percentage increase in biogas was recorded in CS/SCR 85:15 (2.86 dm³ (138.51%)) and the least was observed in CC/FW 75:25 with 1.49 dm³ of gas yield (24.18% increase).

Comparative analysis (T-test $P \leq 0.05$) of the test results with the control indicated a significant difference in biogas yield in the following: CC/SCR 50:50, CC/FW 50:50, CS/SCR 85:15, CS/FW 50:50, and CS/FW 75:25.
The composition of the generated biogas (Table 3) showed that supplementation of corn stover (corn cob and corn sheath) with organic nitrogen sources (soy milk residue and fish waste) did not only improve biogas production but enhanced the methane content.

The results (Table 4) of the computer-aided regression analysis (SPSS software implemented) of cumulative biogas yield as a function of time (t) in the different treatments that had a significant difference in biogas yield showed a suitable correlation between biogas yield (CY) and time (t).

4. DISCUSSION

It is noteworthy that the efficiency of biogas production from organic wastes could be significantly improved by careful selection of appropriate substrates as base materials in bioreactor feeds, and selection of suitable co-substrates to blend at specific ratios. The result of the proximate composition of corn cob (CC) and corn sheath (CS) revealed that they good carbon and energy sources with considerably high organic carbon content (57.81 and 48.32% CC and CS, respectively) and volatile solids (VS), and hold prospect in bioenergy production. However, the low nitrogen content and high C/N ratio (CC 47:1 and CS 54:1) indicate the need for supplementation of corn stover with a feedstock that has substantial nitrogen content. It is evident from the findings of this study that in most cases, single-substrate digestion does not represent the most efficient approach to improved biogas production. This underscores the need to co-digest with other substrates to achieve a synergistic effect, balanced C/N ratio, macro and micronutrients, a suitable pH, improved buffering capacity, and dilute toxic compounds/inhibitors [20,21]. Carbon/Nitrogen (C/N) ratio is an expression of the relationship between the amount of carbon and nitrogen present in organic materials. A carbon to nitrogen (C/N) ratio in the range of 20 - 30 is considered optimum for anaerobic digestion and biogas production [19]. Therefore, organic materials with a high C/N ratio could be co-digested with those of low C/N ratio to bring to balance, the C/N ratio of the input feedstock to a desirable level [22].

As could be observed in the control, gas yield in the anaerobic digestion of corn stover alone (CS and CC) was low. This could be because of the high C/N ratio, imbalance in nutrients and other mineral elements required for microbial growth and productivity which are not present at optimal levels in the corn stover. It was found in this study that depending on the ratio, supplementation of corn stover with organic nitrogen sources such as soy milk residue and fish waste could significantly improve biogas yield and increase the methane content. However, it was also found that at certain ratios (CC/SCR 75:25 and CC/SCR 85:15), the observed effect could be inhibitory to biogas production. The C/N ratio of corn cob and corn sheath was 47 and 54, while SCR and FW were 10 and 4, respectively. The addition of these nitrogen-rich organic materials, therefore, improved the C/N ratio of the slurry for anaerobic digestion. The improvement in biogas yield and methane content could be attributed to the positive synergistic effect of supplementing CC and CS with organic nitrogen (SCR and FW) in the different treatment ratios which resulted in a balanced nutrient composition of the bioreactor feeds, improved C/N ratio, increased organic load of biodegradable organic matter, which ultimately enhanced the biodegradability of corn stover (CC and CS) and hence, a significant increase in biogas production [23,24,25].

These findings are in agreement with the reports of lortyer et al. [26] and Aragaw et al. [27]. Evaluation of the effect of supplementation of fish pond effluent with cow blood showed a significant difference in cumulative biogas yield in all the treatments Opurum et al. [28]. On a laboratory scale; the effects of different nitrogen sources on biogas production were investigated. The results obtained showed that among the complex nitrogen sources used, yeast extract and casamino acids had the highest methane production, whereas no methane production was observed from the use of skim milk. L-arginine showed the highest methane production from the defined nitrogen sources with 1400 ml of methane per mole of nitrogen [29]. In line with the report of Zhu et al. [30], co-digestion of diary manure and Soybean straw increased methane yield compared to soybean straw alone. The biogas generated in this experiment burnt with deep blue flame, confirming the observed percentage methane content in the result of compositional biogas analysis which ranged between 59.01- 69.44%. The percentage methane content of the biogas assented to the report of Adamu et al. [31], analysis of the biogas they produced from abattoir waste showed 67.76 and 31.13% for methane and CO2, respectively.
Table 1. Proximate analysis of the feedstocks (%)

| Substrate | Moisture content | Ash | Fat | Crude protein | fibre | Nitrogen | Organic Carbon | Total CHO | C/N Ratio | TS | VS |
|-----------|-----------------|-----|-----|---------------|------|----------|----------------|----------|-----------|----|----|
| CC        | 9.58            | 5.78| 4.42| 7.61          | 34.66| 1.22     | 57.81          | -        | 47        | 90.39| 84.64|
| CS        | 9.63            | 3.88| 5.31| 20.79         | 27.70| 0.90     | 48.32          | -        | 54        | 90.37| 86.49|
| SCR       | 11.40           | 21.92| 2.35| 12.22         | 26.70| 1.96     | 19.22          | 34.08    | 10        | 88.60| 66.68|
| FW        | 29.02           | 9.55| 20.81| 37.89        | 2.42 | 6.06     | 23.88          | -        | 4         | 70.98| 61.43|

Table 2. Cumulative biogas yield from supplementation of Corn Stover with SCR and FW, and percentage increase in gas production

| Substrate Ratios (%) | Cumulative Yield (dm$^3$) | % Increase in gas Production. |
|----------------------|---------------------------|-------------------------------|
| CC (Control)         | 1.02                      | -                             |
| CS (Control)         | 1.20                      | -                             |
| CC/SCR 50:50         | 2.29                      | 124.51                        |
| CC/SCR 75:25         | 0.92                      | -                             |
| CC/SCR 85:15         | 0.17                      | -                             |
| CS/SCR 50:50         | 1.68                      | 39.87                         |
| CS/SCR 75:25         | 1.64                      | 36.57                         |
| CS/SCR 85:15         | 2.86                      | 138.51                        |
| CC/FW 50:50          | 2.07                      | 72.44                         |
| CC/FW 75:25          | 1.49                      | 24.18                         |
| CS/FW 50:50          | 2.56                      | 113.19                        |
| CS/FM 75:25          | 2.21                      | 84.38                         |

Table 3. Composition of biogas from the different parameters

| Parameters       | Methane (CH4) | Carbon (IV) Oxide | Carbon (II) Oxide |
|------------------|---------------|-------------------|-------------------|
| CC/SCR 50:50     | 62.77         | 34.09             | 2.01              |
| CS/ SCR 50:50    | 59.51         | 38.31             | 1.56              |
| CS/ SCR 75:25    | 62.68         | 33.04             | 2.87              |
| CS/ SCR 85:15    | 69.44         | 28.15             | 1.77              |
| CC/FW 50:50      | 69.00         | 28.22             | 1.32              |
| CC/FW 75:25      | 60.64         | 34.00             | 3.73              |
| CS/FW 50:50      | 67.08         | 30.22             | 1.57              |
| CS/FW 75:25      | 67.50         | 28.55             | 2.21              |
| CC (control)     | 59.01         | 37.00             | 2.05              |
| CS (control)     | 61.80         | 34.50             | 1.78              |
Table 4. Regression parameters

| Substrates            | a        | b        | Ɛ       | Ra^2 (%) | P-value |
|-----------------------|----------|----------|---------|----------|---------|
| Corn Cob (Control)    | 93.97    | -0.575   | 1.014   | 30.7     | 0.001   |
| Corn Sheath (Control) | 120.81   | -0.483   | 1.729   | 20.7     | 0.006   |
| CC/SCR 1:1            | 208.07   | -0.560   | 2.462   | 28.9     | 0.001   |
| CC/FW 1:1             | 141.94   | -0.770   | 0.725   | 57.8     | <0.001  |
| CS/SCR 3:1            | 170.82   | -0.493   | 2.081   | 21.7     | 0.005   |
| CS/FW 1:1             | 162.78   | -0.774   | 0.762   | 58.6     | <0.001  |

Non-linear regression equation could be reliably adopted in predicting biogas yield in relation to time (t). The results obtained indicated a strong relationship between the gas yield (CY) and time (t), which was similarly observed by Ofoefule et al. [32]. Lignocellulosic materials are present in many organic wastes and sometimes are the major fraction. Therefore, improving their anaerobic digestion could lead to a boost in the development of this technology. To improve the biodegradability of lignocellulosic wastes during anaerobic digestion, both pretreatments and co-digestion have vital roles to play [33].
Fig. 3. Pattern of anaerobic digestion of CS with different ratios of SCR, and pH changes of the digesting slurry. Some error bars are within data points.

Fig. 4. Anaerobic digestion pattern of CC and CS with different ratios of FW, and pH changes of the digesting slurry. Some error bars are within data points.
5. CONCLUSION

The findings of this work have shown that Corn stover is a carbohydrate-rich lignocellulosic waste, and therefore a good carbon source, but poor in nitrogen. Supplementation of this waste with organic nitrogen, soybean curd residue, and fish waste could stimulate and improve biodegradation, adjust the C/N ratio to the required range and ultimately lead to a significant increase in biogas production and improved biomethane yield. The highest cumulative yield in biogas was recorded in CS/SCR 85:15 (2.86 dm³), followed by CS/FW 50:50 (2.56 dm³). The biogas yield was statistically different in the treatment ratios CC/SCR 50:50, CC/FW 50:50, CS/SCR 85:15, CS/FW 50:50, and CS/FW 75:25 compared to the control. The regression analysis of biomethane yield as a function of time (t) in the different treatments revealed a significant difference in biomethane yield, with Ra² ranging between 21.7- 58.6%, an indication of a strong correlation between the biomethane yield (CY) and time (t).

Lignocellulosic organics abound in many organic wastes and in some cases the major fraction. Therefore, improving their biodegradability could lead to a boost in the development of anaerobic digestion and biogas production technology. To improve the biodegradability of lignocellulosic wastes during anaerobic digestion, both pretreatments and co-digestion/supplementation have vital roles to play. Further research studies on the supplementation of corn stover with different nitrogen-rich organic wastes are recommended as they are relatively inexpensive compared to steam explosion, chemical pretreatments etc. which may be capital intensive.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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