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Research Paper

Impact of C/N Ratio Of Pretreated *Parthenium Hysterophorus l.* on Biomethanation

Life Science

KEYWORDS : Biomethanation, Carbon-Nitrogen ratio, Parthenium hysterophorus, Pretreatment.

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ABSTRACT

The objective of this study was to know the effect of Carbon-Nitrogen ratio (C/N ratio) in pretreated Parthenium hysterophorus L., on the microbes involved in the biomethanation. Experiments were performed based on completely randomized design with five pretreatments of P. hysterophorus i.e. alkali, acid, water, biological and whey water which is replicated thrice to obtain the C/N ratio. The results showed that the C/N ratio obtained were statistically significant with respect to that of the pretreatments which was further subjected for anaerobic digestion. The results also indicated that the C/N ratio is independent to that of the pretreatment with respect to that of the CH_4 yield.

I. Introduction

In India, *Parthenium hysterophorus* L., is the most widespread weed which is very difficult to eradicate due to its fast growth. This has called for a suitable management method of the weed, with due respect to that of large scale utilization. Biomethanation being the most sought after method for weed management was under study for many decades. There are various parameters that governs the efficient way of utilizing *Parthenium* weed as potential feedstock for biomethanation like, Inoculum Substrate (I/S) ratio, C/N ratio, lignocellulosic content, pH, temperature etc (Dioha.I.J. et al., 2013). Among the various parameters, the Carbon and Nitrogen content present in the *Parthenium* plays an important role by influencing the methane production. Carbon and Nitrogen provide essential elements for synthesis of amino acids, proteins, nucleic acid and also maintains neutral pH conditions essential for fermentative process by microorganisms. The optimum C/N ratio being 20-30:1 (Wang. X et. al., 2012) was achieved in this study by using various pretreatment methods. The aim of this study is to analyze the effect of C/N ratio of pretreated *P. hysterophorus* and its efficiency in producing methane gas.

II. Materials and methods

Processing the Substrate:

The substrate *P. hysterophorus*, was collected during its flowering stage from Hesaraghatta, Bangalore. The entire plant was uprooted, thoroughly washed, cut into small pieces of 4-5 inches in length, Sun dried for 12 consecutive days, milled to attain a particle size of 5mm and stored in closed container at room temperature until use.

Pretreatment protocol:

The pretreatment protocols, Alkali (PT1), Acid (PT2), Water (PT3) and Biological (PT4) were followed as described by Ramya. R et al., (2014). In addition to the above said pretreatments, another pretreatment was performed using whey water and labeled as PT5. The Dried *Parthenium* (DP) was pretreated with whey water at the rate of 4mlg-1 Total Solids (TS) at Room Temperature (RT) (28±2ºC) for 144hrs (6 days) with periodic mixing. The whey water was collected from K.C.Das Pvt Ltd., Bangalore. The initial pH at the beginning time of PT5 was found to be 4.0 which was neutralized to pH 7.0 at the end of the treatment period. These pretreated samples were refrigerated for further analysis.

Inoculum:

Completely digested slurry was obtained from a $10m^3$ biogas plant containing the leaf litter and municipal waste, operating at an ambient temperature (mesophilic) with 30 days retention time. The biogas plant is a floating dome-Khadi and village industries commission model (KVIC) with no provision for mixing. The collected slurry was filtered through a muslin cloth and stored at 4ºC until use. (Courtesy: Indian Institute of Science, Center for Sustainable Technologies, Bangalore, India)

Biogas digester:

Simple fermenters were designed for the laboratory scale set up. Vials of 100ml capacity were chosen, which were corked and crimped with the aluminum crimp to maintain the internal anaerobic condition. The fermenters were operated as batch fermenters. After the vials were crimped, O_2 free N_2 gas was flushed to maintain an oxygen free internal environment. (Courtesy: Institute of Wood Science and Technology, Bangalore, India).

Biomethanation study:

The laboratory scale mesophilic digester was operated at 37ºC

UT (Untreated *Parthenium*): Using *Parthenium* biomass as substrate.

Different concentrations of UT i.e 0.5%, 1%, 2% was added to a 100ml capacity vial along with 50ml of initial inoculum. The vial was flushed with O_2 free N_2 gas at the pressure of 1 Kg/cm^2 for one minute after crimping, to provide an internal anaerobic condition. The methane content was quantified using HS-GC (Head Space-Gas Chromatograph) at a time interval of 5 days with 30 days of Hydraulic Retention Time (HRT).

PT (Pretreated *Parthenium*): Using pretreated *Parthenium* biomass as substrate.

The pretreated *Parthenium* biomass was loaded with 50ml of inoculum to attain an inoculum sustrate ratio (I/S ratio) of 0.5%, 1% and 2%. The substrate was neutralized prior to the biomethanation study. Dewatering was done for the pretreated plant biomass in hot air oven at 60ºC.

Analytical methods:

All compositional analysis was done in quadruplicate and data were corrected to a 100% dry matter (DM) basis. Since there was only a single sample of plant biomass, statistical analysis of the compositional data was not possible. The untreated *Parthenium* (UT) and pretreated samples (PT1, PT2, PT3, PT4 and PT5) were tested for Total Organic Carbon (TOC) by (USP – Pg 257), N_2 by APHA Standard methods, 1995.

Monitoring parameters:

The composition of the biogas collected in the head space of the digester was analyzed using a Nano HP-1 Model BG 1000 Gas

Chromatograph equipped with a Flame ionization detector (FID) and Thermal conductivity detector (TCD). Argon (Ar) was used as carrier gas with flow rate of 8ml/min. Oven temperature was set up to 60°C. Methanator temperature was set at 360°C. All the digesters were operated in triplicate. Biogas was sampled by directly inserting the intra venous syringe into the Anaerobic Digestion (AD) vial and volumetric composition of biogas was analyzed by using gas chromatography. Gas chromatograph (Nano HP-1) equipped with FID was used to analyze gas composition.

RESULTS AND DISCUSSION:

Table 1 reveals the methane yield of PT samples at different I/S (0.5%, 1% and 2%) and C/N ratios. C, H, N, O and S are the main elements to be present in a substrate for an effective microbial growth. In anaerobic digestion, the chief elements involved are C, H, and O for the production of CH_4 and CO_2. On the other hand, excess amount of N and S incorporation would rapidly reduce into ammonia and sulphides which are toxic for methanogens, acting as inhibitors arresting the digester. The optimal C/N ratio for biomethanation is in between the range of 20-30:1 (Marchaim U, et al., 1993 and Dioha. I.J. et al., 2013).The correlation between the C/N ratio and the methane yield at different substrate concentrations in the present study were analyzed separately using IBM SPSS Statistics 22.0 software.

Fig 1, 2 and 3 shows the effect of C/N ratio and methane yield obtained for UT and all five PT samples having 0.5%, 1.0% and 2.0% of I/S ratio. There was better methane production in all the PT samples when compared to the UT (< 3.5%) in all the three concentrations.

From Fig.1, it was observed that PT1, PT2 and PT5 C/N ratio fell in the optimal range and the corresponding methane yield was 8.48%, 13% and 9.86% respectively. However, the PT3 having a high 92.67:1 C/N ratio far beyond the optimal range 20-30:1, produced the highest methane yield of 15.48% at 0.5% I/S ratio. The literature review has showed that the plant waste and by-products of straw having a higher C/N ratio beyond the optimal range had a better methane yield (Wellinger, 2013). However, the bivariate Pearson correlation analyzed between the C/N ratio and the methane yield of 0.5% substrate for all treatments showed a relatively independent value. $r = 0.533$, $p>0.05$, proving that the C/N ratio is independent to that of the pretreatment with respect to CH_4 yield.

From Fig 2, it was noticed that UT showed the least methane production, while PT1, PT2 and PT5 whose C/N ratio fell between the optimal range produced 17.37%, 9.6% and 6% of methane respectively for 1.0% pretreated *Parthenium* substrate concentration. Interestingly the high C/N ratio obtained for PT3 did not curb the production of methane and PT4 showed the highest methane yield (21.11%) inspite of C/N ratio being slightly less than the optimal range. The Pearson correlation (r) was found to be 0.374 and $p>0.05$ proving that even here at 1.0% of I/S ratio, the C/N ratio is independent to that of the pretreatment with respect to CH_4 yield.

Fig. 3 shows the association between the C/N ratio of the pretreated samples and their relative methane yield in individual bars. As expected, UT substrate showed very less production of methane, while PT4 showed the maximum methane yield around 24%. Likewise in other treatments PT1, PT2 and PT5 possessing C/N ratio within the beneficial range produced 21.7%, 18% and 12.87% of methane. PT3 having the C/N ratio of 92.67 also gave on par methane yield, proving that even in the range of 10-90:1 of C/N ratio, methane is still produce-able in anaerobic digester (Yen, H.W. et. al., 2007). Statistical bivariate correlation showed that Pearson correlation, $r = 0.352$, where $p > 0.05$ indicating that there is no significant correlation between the C/N ratio and methane yield produced in all the treatments having 2.0% of I/S.

Conclusion

The C/N ratio plays an important role in the process of anaerobic digestion. The extensive literature survey states that, for an increased methane production, C/N ratio that has been achieved via a co-digestion process was found to be an effective approach in AD from low yielding substrates (Wang. X., et. al., 2014). Co-digestion improves the C/N ratio to the optimum, increases the buffering capacity and also the biodegradability of the substrate. But there are few drawbacks using co-digestion which includes, increased digester effluent, chemical oxygen demand (COD), additional pretreatment requirements, increased mixing requirements, waste water treatment requirements, high utilization degree required (Lehtom ̈aki, A., et. al., 2007). In this study, various pretreatment methods were followed which led to the changes of the physico-chemical parameters that altered the C/N ratio of the *Parthenium* substrate. Pretreated *Parthenium* at different I/S ratios, has proved to yield methane at the C/N ratio ranging from 18-90:1.

Even with a wide range of C/N ratios obtained from the pretreated samples (18-90:1), there was efficient methane yield obtained in all the pretreated *Parthenium* compared to the Untreated one. Statistical analysis proved that, for any substrate concentration the relationship between the C/N ratio of the pretreated samples and the methane yield is independent and does not provide any significance even though C/N ratio is independently significant of the pretreatment for biogas production. Thus biogas can be effectively produced from pretreated biomass substrates as long as the C/N ratio is dependent on the pretreatments, even when there is no significance between the C/N ratio of the treated samples and the methane yield.

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Table 1: Methane yield of pretreated samples at different I/S and C/N ratio

TREATMENTS		UT	PT1	PT2	PT3	PT4	PT5	
I/S ratio (%)	0.5%	2.32	8.48	13.00	15.48	12.46	9.86	Methane yield (%)**
	1.0%	3.51	17.37	9.60	16.85	21.11	6.00	
	2.0%	1.81	21.69	17.99	20.96	23.92	12.87	
C/N RATIO*		14.99	29.24	20.94	92.67	17.97	19.71	

***Represents the mean value of four replicates with standard deviation.**

****Represents the mean value of three replicates with standard deviation.**

Figure 1 Relationship between C/N Ratio and Methane yield in 0.5% Treated Substrate

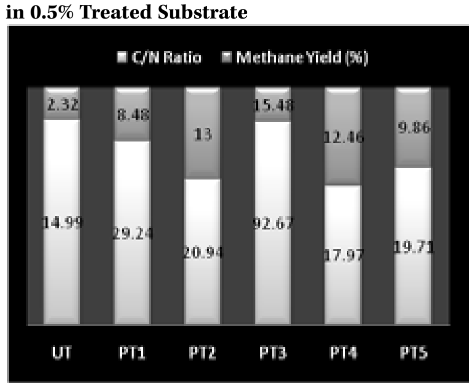


Figure 2 Relationship between C/N Ratio and Methane yield in 1.0% Treated Substrate

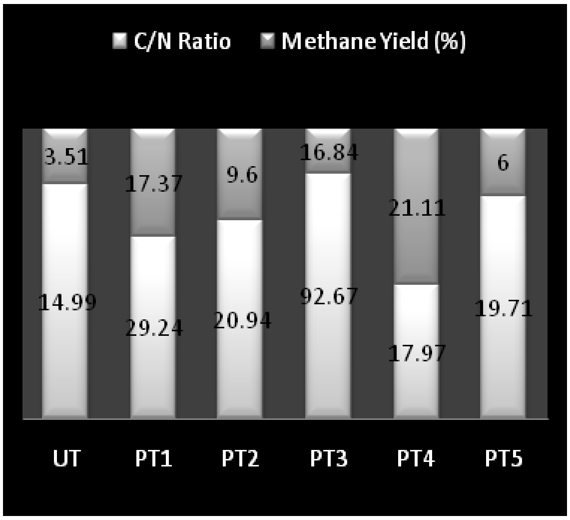
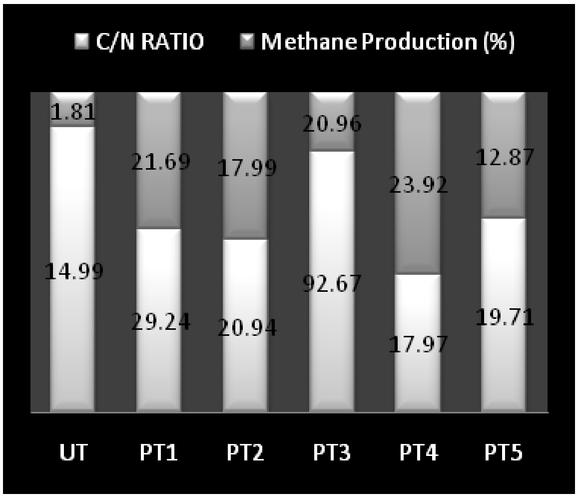


Figure 3 Relationship between C/N Ratio and Methane yield in 2.0% Treated Substrate



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