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Research Article

CO-UTILIZATION OF PRETREATED *PARTHENIUM HYSTEROPHORUS* L., WEEDS ALONG WITH BOVINE ANIMAL FAECES FOR BIOMETHANATION

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ABSTRACT

In India, *Parthenium hysterophorus* L. is a common weed occupying more than 35 million hectares of agricultural land. To address its impact on agricultural output, biodiversity implications and allelopathic effect, a systematic study was undertaken to examine the biomethanogenic property. As biogas is a sustainable alternative energy fuel, in this study the weed biomass was subjected for biomethanation process in various forms and at various compositional parameters. Biochemical methane potential (BMP) vials were inoculated with slurry from on-going plant-litter based biogas plant, at inoculum substrate ratio of 0.5%, 1% and 2%. The admixtures of weed biomass and co-additives ratios were optimized at 1:0.25, 1:0.5 and 1:1. The physical parameters maintained were 35 days hydraulic retention time, pH 7.2 and 38 – 40°C ambient temperature. There was a significant difference with respect to physico-chemical properties of the untreated (UT) and pretreated (PT) *Parthenium*. A substantial increase in biogas production after pretreatment was observed. The co-additives mixed in different ratios enhanced the capacity of the weed to produce biogas. The study reveals *P.hysterophorus* showed best results in the pretreated samples and as well as in combination with co-additives highlighting the provision of improving the BMP for *P.hysterophorus* with additives for sustainable resource utilization.

INTRODUCTION

Biogas technology has established itself as a technology with great potential to serve as an alternate energy source as there is an ever increasing demand for energy due to the depletion of the existing fossil fuel. Initially this technology was limited to only cow dung as potential feedstock, very famously called as gobar gas. However, the availability of sufficient quantity of this feedstock was one of its major limitations. To overcome this problem, utilization of organic wastes other than cattle dung was considered. Energy crops are increasingly being used as feedstock for biogas production[1,2]. Since energy production from crop biomass is in high demand, crops digestion processes with high efficiency and performance are a must. Anaerobic digestion (AD), process of organic matter decomposition by a microbial consortium, in an Oxygen free environment results in biogas production. On the other-hand, the weed management has taken a toll on the country's economy. Failing the weed prevention programmes and weed eradication programmes, researchers are now looking at the various possibilities of weed management programmes[3]. *P. hysterophorus*, one such weed which occupies the majority of the agricultural land and poses a serious threat to the agricultural yield other than its allelopathic effect and ecological impact was studied for its biomethanogenic potential[4]. This study aims at improvising the biomethanogenic potential of *P.hysterophorus* by various pretreatment methods, which otherwise showed a poor BMP in its naïve form (in this study) and by optimizing the co-additive admixtures. Weed, as substrate for AD is inefficient because the nutrients and minerals required for bacterial growth are not present at sufficient levels[5]. In addition to this, the physicochemical, structural and compositional factors, hinders the hydrolysis of cellulose. Thus, this study explores the various possibilities for improved BMP of *P. hysterophorus*.

MATERIALS AND METHODS

Substrate collection and processing

The weed material *P.hysterophorus* chosen for this study as substrate was collected in the month of March 2013 from Hesaraghatta, Bangalore (Latitude: 13.110312N, Longitude: 77.485971E). The substrate when collected was in its flowering stage. As a part of processing, the entire plant was uprooted, thoroughly washed under running tap water, cut into small pieces of 4-5 inches in length and sundried for 12 consecutive

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days[6]. It was later subjected to various pretreatments before biomethanation.

Pretreatments of substrate

The processed substrate was subjected to first level of pretreatment where-in the plant biomass was milled to attain a particle size of 3-5mm and stored at room temperature until use. In the second level of pretreatment the powdered biomass was treated with alkali (PT1), acid (PT2), water (PT3), biological (PT4) and whey water (PT5) at the rate of $4mlg^{-1}$ total solids (TS) at RT (28±2^{o}C) for 144hrs (6days) with periodic mixing[7].

Inoculum collection

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 located at Indian Institute of Science, Center for Sustainable Technologies, Bangalore, India (Latitude: 13.016944N, Longitude: 77.567118E). 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 4^{o}C until use.

Biomethanation of substrate

The batch fermenters used in this study were 100ml BMP vials (Fig: 1). During the loading operation, the bottles were gassed out with O_2 free N_2, corked with rubber cork and crimped with the aluminium crimps. The biomethanation was spread over a period of 35 days.

Reactor setup 1: Inoculum substrate (I/S) ratio on methane production

In this experiment the effect of inoculum substrate ratio on methanogenic potential was studied with respect to that of untreated dried *Parthenium* (UT) and the pretreated biomass (PT1, PT2, PT3, PT4 and PT5). The inoculum substrate ratios were maintained at the concentration of 0.5% (a), 1.0% (b) and 2.0% (c) of TS. The fermenters were incubated at optimum temperature of 37^{o}C to 40^{o}C over a period of 35 days.

Reactor set-up 2: Cow dung (CD) as co-additive

This describes the role of cow dung as co-additive along with *P. hysterophorus* in the biomethanation process. The ratios of the co-additive added were 1:0.25 (I), 1:0.5 (II), and 1:1 (III) for all three I/S ratios maintained in reactor set-up 1. Three operational conditions of the anaerobic digestion process were uniformly maintained through-out the experimental set-up.

Reactor set-up 3: Goat dropping (GD) as co-additive

In this reactor set-up, goat dropping was used as co-additive. For each I/S ratio followed in reactor set-up 1, the co-additive added was at I, II and III ratio of total solids. The operational conditions were maintained same as that of reactor set up 1.

Biogas analysis

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 at 60^{o}C. Methanator temperature was set at 360^{o}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

Gas samples were taken at 5-day interval for composition analysis by gas chromatography. To qualitatively analyze the methane content in the volume of gas produced for various fermentation protocols used in this study, a HS-GC method of compositional analysis was used and the results are given below.

The results obtained for compositional analysis of biogas using HS-GC were evaluated based on 1) the effect of I/S ratios (a, b and c) of various pretreated *Parthenium* biomass on methane yield 2) the effect of pretreatments on methane yield at I/S ratios of a, b and c 3) the effects of the co-additives on the production of methane in the biomethanation.

In addition, the results were evaluated to identify the co-additive and its co-digestion ratio that results in the most efficient digestion and yield maximum methane yield. Preliminary studies from the available literature were conducted in order to select the two different naturally available co-additives (CD and GD). The results were evaluated in terms of percentage of methane produced by compositional analysis of biogas over a HRT of 30 days at 5 day interval.

Effect of I/S ratio on methane yield

Fig: 2, shows the effect of I/S ratio on methane yield. From the data obtained, except for the substrate sample UT, all the other substrate samples PT1, PT2, PT3, PT4 and PT5 revealed that I/S ratio of c resulted in higher methane yield in comparison to that of I/S ratio of a and b. Hence proving that the biogas produced at I/S ratio c has the maximum methane content in percentage.

Effect of pretreatments on methane yield

Fig: 3(i)-(iii), shows the effect of pretreatments on methane yield. From the data obtained it is established that, PT4 has resulted in maximum methane yield at all the three I/S ratios a, b and c. There was consistently high percentage of methane yield observed for the PT4 from day 5 to day 25, proving the fact that the pretreatment PT4 can be used in converting the *Parthenium* biomass to potential feedstock.

Effect of co-additives on methane yield

To get an overall analysis on the effect of co-additives CD and GD on methane yield a graph was plotted with the data obtained. The graph Fig: (4(i)-(vi)) is used to interpret the data for each pretreated substrate sample where-in both the co-additives were compared with that of control. The graph also gives the interpretation of the co-digestion ratio at which the efficiency of the biomethanation process resulted in high methane yield. It is proved that the percentage of methane content in the biogas produced using the PT4 substrate was high in comparison to that of other substrate samples irrespective of the I/S ratios, co-additives and co-digestion ratio used. Fig: 4(v) shows the methane yield in percentage for the substrate sample PT4, where-in compared to that of the control both the co-additives have proved to have yielded high methane yield. Among the two co-additives used for the sample PT4, the GD has shown an insignificantly high methane yield when compared to CD. Among the three co-digestion ratios used (I, II and III), the co-digestion ratio of I for CD co-additive and the co-digestion ratio of III for GD have resulted in maximum yield. Thus the two samples, PT4cCDIII and PT4cGDI proved to have a microbiome that has efficiently involved biomethanation of the *Parthenium* biomass.

DISCUSSION

The composition of a substrate is very important for the microorganisms in the AD process for process stability and biogas production rates. The substrates should provide all the nutritional requirements of the microbial consortium present inside the digester.

Likewise, composition of the microbiome was also found to be an important factor in the biomethanation. The source of inoculums, the adaptability of the inoculums and I/S ratios are few parameters that governs the process stability of the biomethanation process. With low I/S ratios, the methanogenic population is insufficient in preventing the VFA accumulation causing the imbalance in the biomethanation resulting in low methane yield8. Establishment of a proper microbiome within the bioreactor is an important factor in the biomethanation process[9]. The bioreactors predominantly being fed with substrate, with very low start-up culture have failed with respect to that of methane yield[10]. It was found that optimum I/S ratio is necessary as it impact the rate of reduction of volatile solids and there-by increasing the rate of methanogenesis during AD[11].

Recommended I/S ratio for liquid AD that have been reported in literature ranges from 0.1 to 4 [12,13,14,15,16], however, the ideal range being between 0.5 to 2.3[17,18,19]. In this study, anaerobic degradability of pretreated *Parthenium* biomass in different I/S ratios (with-in the ideal range of I/S ratio) shown as average mean of CMY (cumulative methane yield) in Vg^{-1} of TS was depicted in fig: 2. Based on this study, recommended I/S ratio for liquid state anaerobic digestion of Parthenium biomass (pretreated feedstock) is 2%, which is in agreement with that of various studies conducted in the past[20,21,22]. The data obtained affirm that the inoculums used substantially improved the performance of the process.

Here systematic comparison on the performance of these pretreatment methods for application of *Parthenium* lignocellulosic biomass for biogas production was experimented based on the HS-GC method of enumerating the methane produced. Thus, this analysis on pretreatment describes the AD process, structural and compositional properties of lignocellulosic biomass and various pretreatment techniques in the pretreatment process, parameters, performance and advantages versus drawbacks.

From the data obtained (Fig: 3(i – iii)), PT4 has resulted in significantly higher CMY in comparison to the control (UT) and other samples (PT1, PT2, PT3 and PT5) at all I/S ratios (maximum being at I/S ratio of c). This indicates that the biological treatment (aerobic microbial treatment) has proved to be an efficient method of pretreatment for the *P. hysterophorus*, subjected for biomethanation process. This pretreatment can be preferred over mechanical, thermal and chemical pretreatment techniques as it cuts down the requirement of high energy input, expensive instruments, chemicals and cost for an improved biomass conversion. There are intensive researches carried out in the past in identifying the microbes that can be efficiently used for the biological treatment process[23,24,25,26]. However in this study the plant's (*P. hysterophorus*) native flora has been considered as a mode of biological treatment and found to be efficient among the other pretreatments experimented. In general, although there are reports regarding the requirement of long incubation time for the biological pretreatment[26], in this study the biological treatment for performed on par with the other pretreatment processes, yet showed a better performance in terms of CMY. In-spite of being an environmental friendly approach, biological pretreatment breaks down chemicals that might otherwise inhibit methanogenesis in the anaerobic digester.

Earlier, AD of cattle manure has been conducted as a first laboratory experiment by Humphrey Davy in 1808. Since then, AD has been mainly used for municipal waste treatment using the cattle manure as a start-up culture, as the availability of the feedstock (cattle manure) was scare in many parts of the world. Later, with the progression made in this field of research, scientists started looking into alternative substrate source to be used as a feedstock for the AD process to over-come the shortage of cattle manure being used as feedstock in the production of biogas. As the lignocellulosic plant materials were available in plenty and various researches have reported the efficiency of this to be used as a potential feedstock, the application of AD process was viewed in different perspective. This perspective called for various optimizations of process-parameters. Thus in this study though *P. hysterophorus* was reported earlier as a promising substrate[21], to enhance the CMY various process parameters like I/S ratio and pretreatment protocols were analyzed.

Another method to improve methane production of lignocellulosic material on a dry weight substrate basis is by optimizing the VS concentration of it by co-digesting the substrate with co-additive materials that have higher methane potential. This concept of co-digestion can increase methane production by balancing the nutrient content of the substrate and reducing the negative effects of inhibitor compounds of substrate in the AD process[27]. In co-digestion process, different organic biomass are combined to generate a homogeneous mixture as an input to the anaerobic reactor in order to increase process performance[28,29] and avoid nutrient addition when a co-digested waste contains nutrients in excess[30,31].

Besides increasing methane production of the substrate, the addition of co-additives can also stabilize the AD process if added in a controlled way[32]. Therefore, co-digestion of plant biomass with cattle manure seems an attractive option of making biogas plants economically viable. Since, the animal manure has a high methane potential per unit of fresh weight[33], it can alternatively be used as co-substrate with weed biomass in the AD process. Co-digestion studies of grass silage, sugar beet tops and oat straw with cow manure by Lehtomaki *et al.*, 2007[34], corn stover along with cattle manure by Li *et al.*, 2009[35], agro-waste and energy crops with cattle manure by Cavinato *et al.*, 2010[36], have reported the positive impact of the co-digestion on the AD.

In this study co-digestion has resulted in a significant increase of the biomethane potential of the *P.hysterophorus*, when the substrates (with different pretreatments at different I/S ratios) were prepared in mixtures with CD and GD (co-additives) at proper co-digestion ratios in the biomethanation process. The beneficial effects of the co-digestion could be probably due to the optimization of the nutritive balance in the substrate's mixture (when co-digestion nitrogen rich substrates with carbon rich substrates) or could probably due to the synergistic effects of microflora present in the co-additive adding up to the inoculation load or due to the dilution of potential toxic compounds, thus resulting in better biogas yield. The higher specific methane yield of 54.6% (Day 20) and 50.07% (Day 20) were achieved by co-digesting CD (at co-digestion ratio of III and I/S ratio of c) and GD (at co-digestion ratio of I and I/S ratio c) respectively (Fig: 4(v)). These co-additives also act as substrate-specific biocatalyst which helps in reducing the lag period of biomethanation during the start up. The results obtained using CD as co-additive is in agreement with various studies, which have proven that co-digestion of CD along with plant lignocellulosic mass has resulted in improving the biodigestability and increased methane yield of the lignocellulosic mass[37,38,39,40,41]. Likewise, the results obtained using GD as co-additive has also proven to increase the biomethanation potential of the lignocellulosic biomass when co-digested than alone which is in agreement with earlier studies carried out by Zhang *et al.*, 2013[42], on three crop residues namely wheat straw, corn stalks and rice straw.

From the HS-GC data obtained for compositional analysis of biogas produced for pretreated samples at different I/S ratios along with co-additives, indicated that the pretreated sample (PT4), at an I/S ratio of c resulted in higher methane yield when compared to that of all other treatments and I/S ratio (Fig: 4 (i-vi)). A detailed probe in the HS-GC data obtained for PT4 (Fig: 4 (v)), shows that the methane content of *P.hysterophorus* biomass can be improved to 60% which is otherwise 35% (UT sample – Fig: 4(i)). The methane content in the raw biogas varies greatly from depending upon the substrates used. Cow-dung, being the commonly used feed stock in AD process reported to produce biogas with methane content of 50 – 60%. Hence, optimizing any organic fraction to produce biogas with average methane content of 60% would remain the need of the hour. Thus in this study using various pretreatment methods, I/S ratios, co-additives and co-digestion ratios, the requirement is met. As the calorific value plays a key role in making the biogas an apt alternative fuel, with better combustion property, improving the methane content in the biogas becomes the prime importance.

Fig: 4 (v), reveals the experimental set-up, where highest methane content is obtained by using *P. hysterophorus* biomass as the potential substrate. Thus proving the fact that *P. hysterophorus* can be used as potential feedstock in obtaining a biogas and be used promisingly as an alternative energy fuel. The samples PT4cCDIII and PT4cGDI has resulted in highest CMY with maximum methane percentage (Fig: 4 (v)) and thus kindling the inquisitiveness of understanding the microbiome involved in this biomethanation process.

CONCLUSION

This study demonstrated the usefulness of biogas technology for the safe disposal and efficient management of the weed *P. hysterophorus*. It was concluded from the study that among the pretreatment protocols carried out PT4 at the I/S ratio of 2%, the co-additive ratio of 1:1 for cow dung and 1:0.25 for goat dropping showed the best methane yield. On the basis of observations, the performance of the digesters with all the pretreated samples showed increased methane yield when compared to untreated sample. Moreover, the co-additives cow dung and goat dropping play an important role in the AD by contributing the microbiome and thus increasing the methane yield[43,44]. In India, the weed management is a big challenge with respect to agriculture. Biomethanation with optimum pretreatment and a suitable microbiome proves to be the best option for the *P. hysterophorus* management.

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