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Biomethanation Of Fungal Predigested Caster Seed Cake In Acclimatized Seed

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ABSTRACT

Mixed fungal pretreatment of caster seed cake was carried out and then introduced in acclimatized seed for biogas production. It exhibited a considerable biogas production yield on caster seed cake with 10% TS. Fungal pretreatment followed by autoclaving failed to boost biogas production. Acclimatized seed used in this study served as enriched microbial consortia and water-soluble nutritional factors for enhancing the biomethanation process. The maximum biogas production volume and production yield were 105 L and 0.190m^3/Kg TVS added with 53% methane content respectively. The highest percentage of TS(79%) reduced at 36d in digester with 15%. No critical fluctuations in pH, acidity and total volatile acids until the course of digestion reported. COD destruction in digested slurry reached maximum to 60%. Soluble sugars and soluble proteins almost disappeared in feedstock at the end of digestion. The degradation rate of hemicelluloses was greater than cellulose. In 10% caster seed cake, nitrogen content disappeared maximum in digester in acclimatized seed. Thus, in accordance to all the experimental data, suggested anaerobic digestion of caster seed cake in acclimatized seed could be suitable for large scale biomethanation process.

KEYWORDS

Anaerobic digestion;
Caster seed cake;
Acclimatized seed;
Fungal pretreatment;
Methane;
Biogas production.

INTRODUCTION

Anaerobic digestion is a biological process, in which bacteria decompose organic material to yield biogas in absence of oxygen. India is a pioneering country in biogas production. During the last fifty years, many domestic community and industrial digesters of Khadi and Village Industries Commission(KVIC) and Janata (Chinese type) have been implemented using cattle dung as a feedstock[1]. Due to shortage of cattle dung, more research activities on searching alternative feedstock have been engaged. More than 1000 million tones of agro-industrial, biomass, and food processing wastes are available in India, out of which lignocellulosic wastes derived from cereals, pulses, oil seeds and plantation crops account for 318 million tons[2]. Among agro-industrial residues, oil industrial by-products like groundnut shell and caster seed cake are occupied demand status to convert to bio-fuel as compensating electrical need in industrial sectors.

Anaerobic digestion has gained importance not only as a technology of waste treatment, but also for energy production in the form of methane[3]. However, the physical and chemical properties of feed materials affect their rate of bioconversion[4] during anaerobic digestion. Mushroom compost[5], fungal digested (*Aspergillus, Penicillium, Sporotricum*) agro-industry wastes[6-7], microbial pretreated wheat bran[8], chemical pretreated plant materials[9], and fungal pretreated oil-industry wastes[10] have already studied in the vision of enhanced biomethanation. Acclimatization is the process of organism being forced to adjust to change in their environment. Nevertheless, the combination of fungal pre-digestion and acclimatization strategies have not done yet. Since, the present paper demonstrated the feasibility of considering caster seed cake as feedstock in batch digester and the advantageous of acclimatized seed on biomethanation as well as anaerobic digestion of organic constituents.

MATERIAL AND METHODS

Feedstock preparation

Caster seed cake was collected locally and dried at room temperature. It was powdered and sieved by passing through a standard sieve to make as 0.5mm size. It was dried at 105^{0}C for 5-7 h in oven before biomethanation.

Fungal pretreatment

100g caster seed cake(0.5mm mesh) was placed in 2 L Erlenmeyer flask moisturized with distilled water and sterilized at 121^{0}C for 30min. The flask was inoculated with mixed fungal spore suspension (*Aspergillus* conidiospores) and then incubated at 30^{0}C. After 10 d incubation, fungal biomass was blended with caster seed cake and dried at 100^{0}C for 6h.

Acclimatization process

Buffalo dung was collected locally and mixed with tap water(1:2 ratio). After blending with tap water this mixture was introduced in 5L bottle digester and then incubated at 38^{0}C. The digested dung slurry was recovered sequentially by replacing with feed material (0.5mm size). It was acclimatized with caster seed cake for 3 months.

Biomethanation process

Batch digestion was conducted in laboratory scale glass reactor with 15% of fungal predigested feed stock with 10% of the predigested slurry. In the mean while, caster seed cake(with 10 and 15 %) was loaded in 5L digestion bottle. 4L of above acclimatized seed with less than 0.2% TS was added into each digester instead of tap water. The predigested buffalo dung was collected from our biogas plant(25m^3) and centrifuged, and then filtered through a glass wool column. 1%(with 1% TS) of this seed culture was used to start-up the anaerobic digestion process. All digesters were incubated at 38^{0}C for 40d.

Gas measurement

The gas production was measured daily by raising volume of acid saline(pH 4, 20% NaCl) in a gradually marked aspirator bottle[11] and weekly average was calculated for volume of biogas production expressed as L/ Kg TS added.

Constituent analysis

Total solids in slurry and total acidity and inorganic phosphorus in filtrates were analyzed by standard methods[12]. Organic carbon and total nitrogen in biomass were determined by Walkely and Black method and kjeldhal method, respectively[13]. Cellulose was measured by Updegraff method(1969)[14]. Hemicellulose in digested(4g) dried solid was stream distilled with 0.01 N HCl. The pooled distillate was precipitated with 0.7% phloroglucinol reagent for 12h and precipitate dried at 105^{0}C, 12h. The percentage of hemicellulose was calculated as dry weight basis according to Krober's formula[15]. The soluble sugars in digested slurry(free from solids) was inverted with concentrated HCl overnight and neutralized by 2.5 M Na_2CO_3 solution, and then total reducing sugars were determined with dinitrosalicyclic acid reagent using glucose as a standard[16]. Total soluble sugars were determined by addition of non-reducing sugars and reducing sugars. 3M NaOH digested slurry(5min) was filtered and then soluble proteins were estimated by Lowry method (1956)[17] using BSA as a standard.

GC analysis

Methane content in the biogas produced was analyzed by using a gas chromatograph (Sigma 2000, Perkin Elmer), equipped with thermal conductivity detector and a 2m porapak (80-100 mesh) column. Nitrogen was used as carrier gas at a flow rate of 30mL/ min^{-1}. The oven, injector and detector temperatures were 70, 100 and 170^0C respectively.

RESULTS AND DISCUSSION

As a matter of practice, it is important to maintain by weight a C/N ratio close to 20 for an optimum rate of digestion. Thus, the chemical compositions and complexity of feed materials are determining the rate of anaerobic digestion. As listed in TABLE 1, the experimental feedstock caster seed cake has 98% TS, 91%TVS and 19:1 C:N ratio. It was composed of 21% cellulose, 12% hemicellulose, 18% starch, 4% tannin, 2.3 % organic nitrogen and 0.38% organic phosphorus in its cellular constituents. It invoked to extend our study for utilizing caster seed cake as a substrate in methane enhancement.

As shown in TABLE 2, a maximum cumulative biogas volume (42 L) recorded with 45% methane, when digester loaded with fungal pretreated caster seed cake (unautoclaved). In contrast, autoclaving did not show its effectiveness on caster seed cake for biogas production. It reveals that autoclaving itself serving as a mode of pretreatment may to some degree convert the polymeric macromolecules to smaller subunits, which in turn are utilized by fungi during pretreatment. Since, the rest of nutrients in caster seed cake may not satisfactory for boosting anaerobic bacterial growth.

The optimum biogas production was achieved with 8% TS of cattle dung[18] and with 12% TS of cow dung[9], and of market wastes(pretreated with chemicals)[13]. In this study, the optimum biogas production on caster seed cake (with 11-16 % TS) was observed in acclimatized seed and the resulted biogas yield was 0.171-0.190m^3/KgTVS. The maximum biogas production volume was recorded to be 105 L on caster seed cake with 15%(TABLE 3).

We have studied the effect of acclimatized seed alone with 1% biogas plant slurry(control). Though control produced total biogas volume and methane content only 280mL and 52% respectively, acclimatized seed alone did not serve supplementary as carbon source to biogas production. Moreover, nutrients in caster seed cake are thrived by fungi used during pretreatment with great extent. Therefore, in anaerobic digestion process nutrients are unavailable for the growth of anaerobic bacteria. Thus, acclimation is the period of time that

TABLE 1 : Physiochemical analysis of caster seed cake

Constituent	%
TS	98
TVS	91
Total ash	09
Water insoluble matter	80
Cellulose	21
Hemicellulose	12
Starch	18
Pectin	1.2
Total soluble sugars	2.6
Total nitrogen	2.3
Crude protein	14
Oil and fats	10
Coloring matters	1.5
Organic phosphorus	0.3
Tannin	4.0
Organic carbon	40
C: N ratio	19

TABLE 2 : Effect of fungal pretreatment on biogas production and anaerobic digestion of organic constituents in caster seed cake(15%) at 36 days in batch digester

	Digester I	Digester II
From digested slurry		
pH	8.0	7.6
Total alkalinity (g L^{-1})	9.0	11.9
Total acidity (g L^{-1})	4.5	4.3
TFA (g L^{-1})	3.4	4.1
Reduction (%) of		
TS	39	52
Cellulose	45	41
Hemi cellulose	82	86
Total nitrogen	29	25
Final soluble protein	0.10	0.20
COD (g O_2/Kg)	149	150
Total gas production (L)	28	42
Methane content (%)	33	45

Digester I: Autoclaved and fungal predigested caster seed cake
Digester II: Unautoclaved and fungal predigested caster seed cake

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TABLE 3: Effect of acclimatized seed on biogas production and anaerobic digestion of organic constituents in caster seed cake at 36 days in batch digester

	Digester I	Digester II
From digested slurry		
pH	7.5	7.5
Total alkalinity (g L^{-1})	10.6	12.6
Total acidity (g L^{-1})	3.8	4.0
Reduction (%) of		
Ts	70	79
Cellulose	34	46
Hemi cellulose	96	98
Total nitrogen	31.8	8.6
Final soluble protein(g L^{-1})	1.0	3.3
Final reducing sugars(g L^{-1})	0.05	0.12
Total gas volume(L)	63	105
Production yield (m^3/KgTVS)	0.171	0.190
Production yield (m^3/KgTS)	0.157	0.175
Methane content(%)	44	53

Digester I: 10% Fungal predigested caster seed cake in acclimatized seed, Digester II: 15% Fungal predigested caster seed cake in acclima tized seed

also not shifted to either above or below the optimum range (pH 7.0-8.0).

As it has high protein content, digesters can be maintained even for long period. This attributes buffering capacity to digesters, and to establish the microbial populations. Volatile fatty acids relationships are important in anaerobic digestion of biological wastes, as they are direct precursors of methane[10,22,23]. TVA as acetic acid in digested slurry recorded as 3.0-4.0g/L so that such concentration not showed harmful effect on anaerobic growth (TABLE 4), showing digesters are in stable mode.

The reduction of cellulose and hemicellulose in pretreated digester was 33-46% and above 90% respectively. The degradation rate of hemicellulose present in caster seed cake was greater than cellulose content, which was comparatively better than the results attrib-

TABLE 4 : Effect of acclimatized seed on pH, TS, TFV, COD, and methane content during anaerobic digestion caster seed cake in batch digester

Digestion time	pH		Final TS(%)		TVF(gL^{-1})		%COD destruction		% CH_4	
(Days)	10%	15%	10%	15%	10%	15%	10%	15%	10%	15%
	0.0		7.35	7.20	11	16	4.3	4.7	-	-
5.0	7.48	7.60	5.7	7.5	8.7	9.1	18	28	27	24
10.0	7.68	7.42	4.2	4.3	6.4	5.9	30	35	40	38
15.0	7.50	7.70	4.2	3.5	4.1	4.0	45	46	44	53
20.0	7.44	7.60	3.9	3.4	3.2	2.8	51	56	36	42
35.0	7.52	7.68	3.0	3.35	7.2	7.68	58	60	32	36

needed for existing consortia to metamorphoses into consortia structure can more efficiently utilize the given substrate. During this time the organisms increase in mass and number to dominate the environment accordance to the chemical composition of the substrate[19-20].

The reduction in total solids is higher and faster in thermophilic digesters than mesophilic digesters, but maximum gas production is achieved only in mesophilic condition[3,21]. Like that, a maximum percentage of reduction in total solids was found in digester having 15% at 36 d under mesophilic condition(TABLE 3). Fungal treatment followed by acclimatization strategy showed greater reduction in total solid than fungal treatment alone, suggesting the possibility of adopted anaerobic microbial sources in acclimatized seed would promote substantial methane yield.

The methane production is totally inhibited if the pH is maintained below the value of 4.0[22]. As shown in TABLE 4, alkalinity exhibited in digested slurry was favorable for anaerobiosis and pH of the slurry was uted to wheat bran residue by Nirmala Bardiya and Gaur[8]. COD destruction in digested slurry was observed in each digester. It reported that COD destruction reached maximum to 58 and 60% in digester having 10 and 15% caster seed cake in acclimatized seed, respectively (TABLE 4). Soluble sugars and soluble proteins in digested slurry almost disappeared from caster seed cake with a great level. Soluble protein and nitrogen losses ranged from 19-26% and 25-30% respectively. Similar results have been observed in crop residues with poultry wastes as reported by El.Shinnawi (1989)[4].

CONCLUSION

Seeding of the digester commonly involves the use of an adequate population of both the acid forming and methanogenic bacteria. As a general guideline, the seed material should be twice the volume of the fresh manure slurry during the start-up phase with a gradual decrease in addition over a 3 week periods[19-20]. The acclimatized seed supposed to contain more amounts of water-soluble matters include sugars, amino acids and free fatty acids. Since acclimatization was carried out with same feed material, it may highly possible to have anaerobic microorganisms that are being adopted in the new environment. Acclimatized seed with caster seed cake showed a significant biogas volume(105 L) with 53% methane, but seed alone produced only 280 ml of cumulative gas, it suggesting acclimatized seed was served as a major microbial source. Accumulation of volatile fatty acids in digesters with attempt of fungal pretreatment followed by acclimatized seed is milder than fungal treatment alone. These results pillar the concept that acclimatized seed will foster a stable digester processing regime. Fungal pretreatment is somewhat less important than acclimatized seed treated caster seed cake in reference to biogas yield. The reduced retention time, theoretically calculated detention time and high methane content are achieved due to water soluble matters digested initially causing a sharp decline in TS upholds the view.

Microbial pretreatment could not improve the biogas yield. The nutrients are utilized for biomass or dissipated as carbon dioxide as an end product. This may be attributed by decreasing the retention time just sufficient for induction and release of hydrolytic enzymes and baring the feed stock utilization during pretreatment. Thus, microorganisms may monitor the level of products in the environment and are able to regulate synthesis of the enzyme accordingly the external macromolecule is hydrolyzed and catabolized in the most efficient manner. Enzyme will probably play a key role in the introduction of environmental friendly processes that form cleaner product in much milder, efficient and economical way traditional chemical pretreatment process based on large amounts of toxious solvents, acids, alkali or other corrosive agents. Overall, we conclude that acclimatization followed by microbial pretreatment is the one of the important strategies by which the efficiency of methane generation as well as bioconversion of organic constituents present in caster seed cake can be improved. Thus, it could also be reduced the environmental crises caused by using chemical pretreatment during solid wastes recycling processes. Apart from this, it is easily available and quite cheap, thus it can be considered as an alternative feed stock for biogas production.

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