Experimental studies on co-digestion of KOH pretreated wheat straw and dairy industry wastewater for enhanced biogas production

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Research Article

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Abstract

The dairy industry is one of the biggest industries in the world due to tremendous demand for milk and milk products, thereby produces huge amount of waste water as well. This wastewater contains various nutrients, high chemical oxygen demand (COD) and biological oxygen demand (BOD), in addition to organic and inorganic substances. Among the various methods of treatment of wastewater, anaerobic digestion is considered to be one of the best methods, as it contributes to energy production to resolve the energy crisis. To attain maximum production of biomethane co-digestion of substrates containing rich amount of carbohydrates and essential nutrients is beneficial. Thus, in the present experiment untreated and alkaline pretreated samples of wheat straw were co-digested with dairy wastewater. The results showed that biomethane production from slurry containing dairy wastewater co-digested with KOH pretreated wheat straw inoculated with cow dung has maximum yield (624 ml/g VS). To study the experimental data of biogas production, Gompertz model was applied and resulted that the rate of biogas production ($\mu_m$mld^{-1}) was found highest with dairy wastewater co-digested with 2% KOH pretreated wheat straw, while rate of biogas production was found lowest with untreated wheat straw.

1. Introduction

In an agriculture country like India, less profit in the farming sector, depletion of natural resources and energy crisis are becoming the major challenges for sustainable agriculture. Approximately 500 million tons/year of agriculture waste is produced in India out of which 91 Mt is burnt every year(Hiloidhari et al., 2014). Among the various crop grown, crop residues released from rice, wheat and sugarcane is burnt in larger extent. The crop residues burning instantly release air pollutants such as particulate matters, oxides of carbon, oxides of sulphur, oxides of nitrogen, volatile organic compounds, non- methane hydrocarbons into the ambient environment. It also reduces the fertility of soil by lowering the soil organic carbon, nitrogen and other essential nutrients. In view of the harmful impacts burning of crop residues, stakeholders have adopted alternative ways for its sustainable management such as nutrient recovery, animal fodder, bioenergy generation etc.

Dairy industry is another major agricultural operation with a value of Rs 7, 01,530 Crore (in 2017-2018), which is higher than the value of output of rice and wheat. With the largest bovine population, India ranks first at global level in the milk production since 1998. Despite of the significant contribution in the Nation’s economy, dairy industry is regarded as one of the most polluting food industries(Hung et al., 2005). Dairy wastewater comprises large extent of lactose, other organic compounds, inorganic nutrients which results in high biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Various treatment methods (physical, chemical and biological) have been suggested to remove higher concentrated material like oil, greases, and lactose from wastewater before its final discharge. Biological treatment methods have been observed as most economical and efficient way for dairy industry waste water (DIWW) treatment. Biological processes such as activated sludge process, rotating biological disc and anaerobic digestion are the most common methods for DIWW treatment. Among these processes, anaerobic digestion has been observed as a more efficient way of treatment than the others due to its higher treatment efficiency and energy recovery.

Also, to achieve the sustainability in agriculture sector, an integrated approach is required for the management of different agriculture waste materials. Researcher have evaluated that the overall bioenergy potential of India is about 25,700 MW that involves municipal solid waste, crop residues, agricultural waste, wastewater etc. [4]. In the process of production of biogas from agricultural waste, researchers have analysed various pretreatment methods such as physical by changing size and other conditions, chemical using acidic and alkaline medium and biological using enzymes, fungi or bacteria. Jaffar et al. [5] studied the efficiency of KOH pretreated wheat straw for biogas production and achieved 45% improvement in the cumulative biogas production than the raw wheat straw. Liu et al. [6] reported that 20% KOH (for 1 hour) pretreated wheat straw loading at ambient temperature resulted in the maximum methane yield and therefore recommended for the wheat straw pre-treatment loading. On the other hand significant methane yield has been also achieved from anaerobic digestion of DIWW by various researchers [6-7].Co-digestion of DIWW with various organic substrates (slaughter house sludge, food leftover waste, municipal sludge, and industrial waste sludge) has been found more effective than the anaerobic digestion of DIWW as a single substrate [8-9]. The efficiency of co-digestion is directly related to nutrient content, pH buffering capacity, C/N ratio, composition of seed inoculums, substrate ratio and reactor conditions [10].

Co-digestion of DIWW with wheat straw for biogas production is a novel approach for integrated agricultural waste management. Thus, the present work is an attempt to evaluate the potential of DIWW and wheat straw co-digestion for biogas production. The study also involves investigation on the KOH based pre-treatment of wheat straw and its influence over the yield of biogas production.

2. Materials And Methods

2.1. Substrates collection and characterization

The Dairy industry wastewater used in present study was collected from Haryana Dairy Development Cooperative Federation Ltd, Ballabhgarh (Faridabad) India. The wastewater was collected from the outlet point of the dairy wastewater treatment plant in a representative manner. Collected sample of dairy wastewater was stored in refrigerator at 4°Ctemperature. DIWW was analysed for pH, Total solids, suspended solids, volatile solids, nitrate, phosphate, chloride, hardness, BOD and COD by following the methods prescribed by APHA [11].

For co-digestion with dairy wastewater, substrate (lignocellulosic biomass) selected is wheat (*Triticum aestivum*) straw, which was collected from a crop field located in Faridabad, India. Collected wheat straw was further processed for drying under the solar radiation. Dried wheat straw was grounded to obtain uniform size by a Muller with 100 cross-section strainer to keep away from the impedance of substrate size on the yields, and afterward dried at 65^0C at the steady mass before test use and pre-treatment. The wheat straw sample was stored in impenetrable bags for further characterisation and examination. Analysis of cellulose, hemicelluloses, and Klason lignin was carried out using quantitative acid hydrolysis under standard conditions(Guerra-Rodríguez et al., 2012). All chemicals used in the chemical analysis were of analytical reagent grade and purchased from Merck (Germany), Sigma (USA) or BDH chemicals (Mumbai, India). Deionized water was used throughout the experiments. Standardized reagents for titration purpose were prepared by using primary analytical reagent grade. The fermentative inoculum was obtained from operating agriculture biogas plant located in Faridabad. The inoculum was stored under anaerobic condition at room temperature. Wheat straw, inoculum and slurry were characterized for pH, Total solids (TS), Total Kjeldhal nitrogen (TKN), Volatile solids and Chemical oxygen demand (COD) according to the methods adopted by Sharma et al.(A. Sharma et al., 2020). Various celluloses e.g. Glucan, Xylan, Galactan and Arabinose, and other constituents like hemicelluloses and lignin were found out by the extraction method for raw fiber content determination as per the procedures proposed by Van Soest et al.(Van Soest et al., 1991).

2.2 Pretreatment of Wheat Straw

Alkaline pretreatment was conducted with concentration of 2% KOH (w/v). The pretreatment method has been selected on the basis of previous works reported by many researchers(Cheah et al., 2020; Y. Chen et al., 2020; Rani et al., 2022; Thomas et al., 2018) The alkaline pretreatment was carried out in a 500 ml flask with 30 g wheat straw soaked in the selected concentration of KOH for 5 days and incubated at 30^0C. After completion of the pretreatment process, wheat straw was filtered, air-dried and stored in air tight containers till its application for anaerobic digestion.

2.3 Anaerobic digestion experiments

Anaerobic digestion experiments were performed in triplicates at room temperature (25-30^0C). Aspirator glass bottle (1 liter) with working volume of 0.7 liter was used as bio-reactor. The aspirator bottle was initially purged with nitrogen gas to achieve anaerobic condition inside the reactor. Biogas production was observed in five set of experiments. In the first set (A1) DIWW and cow dung digested anaerobically to determine the potential of DIWW for biogas production. In the second set (A2), potential of untreated WS for biogas production was evaluated by anaerobic digestion of untreated WS and cow dung. In third set (A3), potential of pretreated wheat straw for biogas production was analyzed by the anaerobic digestion of pretreated wheat straw and cow dung. In fourth (A4) and fifth (A5) set of experiment DIWW and cow dung were digested anaerobically with pretreated wheat straw and untreated wheat straw respectively. The Wheat straw (pretreated or untreated) and cow dung ratio was maintained 1:1, while substrate to distilled water/DIWW ratio was maintained to 1:10 in respective set of experiments. All digester were tightly sealed with rubber septa and screw caps and were gently shake for one minute every day prior to the estimation of biogas volume. The volume of biogas produced from each digester was determined by water displacement method.

2.4 Kinetic study of biogas production

To understand the kinetics of biogas production from each set of experiment, modified Gompertz model was employed on experimental data to evaluate the cumulative biogas production (Altas, 2009; Brown et al., 2012) and other kinetic variables.

$$\mathrm{Y(t)} = \mathrm{Pexp}\{-\exp\left[\frac{\mu_{\mathrm{m}}}{\mathrm{P}}(\lambda - 1) + 1\right]\}$$

Where, Y (t) is the cumulative biogas yield (ml) with respect to time t (days), P is the maximum biogas production potential (ml), μ_m is maximum biogas production rate (ml/day), λ is lag phase (days). Evaluation of kinetic parameter is essential for optimal design of digester and for efficient operation of large scale biogas plant. In present study, the equation was estimated by using nonlinear curve fitting by using PAST 4.03 statistical software.

2.5 Statistical analysis

Experimental data shown in this study is the mean of three measurements of analysis of parameters. Standard deviation and Pearson correlation of operating parameters were analyzed by using Microsoft Excel.

3. Result And Discussions

3.1 Physicochemical properties of DIWW

The physicochemical characteristics of collected DIWW (Table 1) revealed that the wastewater is composed of readily and slowly biodegradable organic compounds. The wastewater was slightly alkaline in nature and higher value of BOD indicates that the wastewater is rich in degradable organic substances, which has proven its utility as a feedstock for biogas production. High load of inorganic nutrient in wastewater is due to the presence of cleaning agent (soap/detergent) in wastewater, which may cause inhibition to the growth of methanogens (Boggula et al., 2020).

Table 1	Characteristics of dairy industry wastewaters (composition in mg/l, except pH)		
S. No	Constituents	Dairy Wastewater	Standards for effluent discharge
1.	pH	7.2	6.5-8.5
2.	Total solids (mg/l)	1840	—
3.	Suspended solids (mg/l)	550	150
4	Volatile solids (mg/l)	112	—
5	Nitrate N (mg/l)	80.5	—
6	Phosphates (mg/l)	8.5	—
7	Chlorides (mg/l)	96	—
8	Hardness (mg/l)	530	—
9	BOD (mg/l)	1380	100
10	COD (mg/l)	2850	—

3.2 Effect of alkaline pretreatment on characteristics of lignocellulosic biomass

The constitution of wheat straw show lignin in maximum amount and its complexity result into low yield of anaerobic digestion process. So, to reduce the amount of lignin present and complicated structure of celluloses and hemicelluloses of biomass alkaline pretreatment with 2% KOH was performed keeping the same conditions of experiment as per suggested by previous experimental study(Rani et al., 2022)

To determine the effect of the applied pretreatment method, analysis of the chemical characteristics of untreated wheat straw and pretreated wheat straw was performed and listed in table 1 along with characteristics of cow dung. Compare with the untreated wheat straw, total organic carbon, cellulose, hemicelluloses and lignin contents were significantly decreased in the pretreated wheat straw. Remarkable reduction in chemical oxygen demand was found in prreated wheat straw, which may be attributed to the precipitation of complex compounds due to alkaline pretreatment (T. Liu et al., 2019). Similar trend of reduction in parameters in KOH pretreated wheat straw has been observed by Memon and Memon (Loow et al., 2016)

Table 2
Characteristics of substrates and inoculums before anaerobic digestion

Parameters	Untreated WS	Cow Dung	Pretreated WS
pH	7.1±0.14	6.2±0.12	7.6±0.12
TS %	76.4±1.22	32.4±0.11	63.1±1.1
VS%	86.23±1.35	21.2±0.01	70.65±1.6
TKN %	0.4±0.01	0.53±0.01	0.38±0.03
TOC %	18.82±0.22	9.75±0.21	16.78±0.18
COD(mgL-1)	200.0±4.3	80±1.2	110±4.8
C/N ratio	47.05±0.78	18.40±0.57	44.16±0.62
Celluloses%	39.5±0.56	19.6±0.16	24.6±0.14
Hemicelluloses%	26.8±0.34	21.8±0.24	19.4±0.22
Lignin%	6.6±0.12	1.9±0.14	1.2±0.16

A lot of methods of chemical pretreatment have been reported by many researchers and out of which alkaline pretreatment is significantly good (Barakat et al., 2014; Peng et al., 2020). Rani et al.(Rani et al., 2021) showed alkaline pretreatment by using calcium hydroxide solution loosen the complex lignin structure of lignocellulosic biomass. Silverstein et al.(Silverstein et al., 2007) investigated that out of 4 chemical pretreatments (NaOH, H_2SO_4,H_2O_2, and O_3) of cotton stalks, NaOH pretreatment delignified the biomass at maximum extent (65.63% at 2% NaOH, 90 min, 121°C). Sharma et al.(R. Sharma et al., 2013) showed that KOH is better than NaOH in generating high sugars during hydrolysis. Alkaline pretreatment of wheat straw with KOH resulted in significant changes in physical and chemical properties of the substrates and increase their biodegradability. As the structure of lignocellulosic biomass is hard enough to be fully degraded by microbes, so, pretreatment is required to overcome this hurdle. Pretreated substrates can be easily decomposed by microorganism due to loosening of structure therefore reduction in COD values in study may be due to the depolymerization of complex organic compound. Table 2 shows the change in characteristics of Wheat straw due to alkaline pretreatment. Here, it has been noted that alkaline pretreated wheat straw has higher biodegradability to yield higher biogas production in compare to untreated wheat straw.

Pretreatment with KOH breaks the complex lignocellulosic component into three polymeric constituents i.e. cellulose, hemicelluloses and lignin(Ward & Kumar, 2010). In the process of disruption, crystalline structure of compound is reduced. After KOH pretreatment, lignin content was reduced by 36.36% and celluloses and hemicelluloses were enhanced by 15.44%and 28.35% respectively with 2% KOH pretreated wheat straw. These results indicate that KOH pretreatment induces better degradation of carbohydrate than that of untreated wheat straw. Jaffar et al., 2016 also reported maximum reduction in TS and VS by 86% and 89% respectively with wheat straw pretreated with 6% KOH with pretreatment time of 3 days. Value of COD present indicates the presence of complex organic compound that cannot be easily degraded. For this proper pretreatment time plays a very important role in degradation of complex carbohydrates in lignocellulosic biomass. Recent studies have suggested that lower concentration of chemicals with proper time of interaction of attacking molecules of chemical compounds used for pretreatment with glycosidic bonding of polysaccharides enhance the chances of biomethane production.COD reduction in 2% KOH pretreated wheat straw was found by 45% while with 4% KOH it was found by 20%. It has been found that value of COD indicates the biogas production potential of the selected substrate; however it is found that that higher COD value indicates the presence of complex organic compound that cannot be easily decomposed by microorganism, therefore reduction in COD values in present study may be due to the depolymerization of complex organic compound. This can also be proved by Table 3 which shows the amount of celluloses undergo depolymerisation due to alkaline treatment. The value of glucan, xylan, galacton, and arabinose is maximum but lignin is minimum in 2% KOH pretreated WS,

Table 3
Carbohydrates depolymerisation and lignin in untreated and alkaline pretreated samples

	Untreated WS	**2% KOH pretreated WS**	**4% KOH pretreated WS**
Glucan	30.6±0.2	36.3±23	34.2±14
Xylan	14.6±0.12	10.2±0.16	11.7±0.14
Galactan	0.27±0.02	0.18±0.03	0.22±0.04
Arabinose	2.3±0.2	2±0.1	2.2±0.2
Lignin	18.1±1.2	12.8±1.1	13.6±1.2

3.3 Biogas production from different set of experiments

The daily and cumulative biogas production from different set of experiment is shown in Fig. 1 and Fig. 2. Similar trend in terms of increase and decrease of biogas production was observed with all set of experiments. The lowest volume of biogas production (418 ml) was obtained with A2 experimental set up i.e. anaerobic digestion of untreated wheat straw and cow dung. While a slight increase in the biogas production (427 ml) was observed from A1 experimental set up i.e. anaerobic digestion of DIWW and cow dung. Compare to the A1 and A2, higher volume of biogas production (480 ml) was observed with A3 i.e. anaerobic digestion of pretreated wheat straw with cow dung. Remarkable increment in biogas production was observed with co-digestion experimental set up i.e. A4 and A5. The maximum biogas production (624 ml) was obtained with co-digestion of DIWW, pretreated wheat straw and cow dung.

The biogas production has been increased from slurry containing untreated wheat straw and reached to maximum value from slurry containing alkaline pretreated wheat straw by anaerobic process. This can be explained as co-digestion enhance the biodegradable content of slurry and hence the yield of anaerobic digestion and pretreatment helps to expose the complex material to methanogens by enhancing depolymerization of celluloses, hemicelluloses and lignin.

3.4. Kinetic study

The Modified Gompertz model has been applied to daily and cumulative biogas production data of each test group. The kinetic parameters of biogas production are shown in Table 4.

Table 4
Kinetic parameters calculated using modified Gompertz model of various samples

Parameters	A1	A2	A3	A4	A5
P (ml)	462.02	529.7	440.6	662.75	649.16
μ(ml/day)	4.037	4.926	4.88	4.00	4.71
λ	0.209	0.21	0.254	0.23	0.17

This shows that correlation coefficient for Gompertz model was found 0.99 for all test groups, which shows good fit of the Gompertz model with experimental data. Highest biogas production potential, maximum rate of biogas production and shortest lag phase were found with slurry of dairy wastewater and pretreated wheat straw inoculated with cow dung. Lowest value of biogas production potential and rate of biogas production was found with untreated wheat straw. Slurry of dairy wastewater inoculated with cow dung was also found with longer lag phase than the other combinations. Co-digestion of dairy wastewater with wheat straw was found to have positive influence over the yield of biogas production and kinetic parameters. However using pretreated wheat straw in place of untreated has enhanced the biogas production to maximum and showed shortest lag phase. Khalid et al., 2019 studied the anaerobic digestion of Switch grass and found that 3% KOH pretreatment enhances the biodegradability of biomass. Figure 3(a-e) represents comparison of actual and predicted biomethane production in different samples and it is evident that both the results are in accordance with each other.

3.5. Conclusion

Biogas production can be increased by co-digestion, pretreatment and optimization of conditions of anaerobic digestion. Co-digestion helps in balancing the nutrients, hence optimizing the conditions for methanogenesis by microbes. The results suggest that dairy wastewater can be effectively used in anaerobic digestion process if co-digested with chemically pretreated wheat straw using animal manure (cow dung) at a temperature 25-30^{0}C. This study further suggests that pretreatment breakdowns the complicated structure of wheat straw and enhances loosening of complicated structure. This increases depolymerization of celluloses and hemicelluloses into smaller units, hence increases their conversion into bio-methane.

Declarations

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Disclosure of potential conflicts of interest:

Authors and co-authors do not have any conflicts of interest.

Informed consent:

All authors and co-authors have given their consent for publication of manuscript in this journal.

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Figures

Figure 1

Daily biogas production via anaerobic digestion of samples

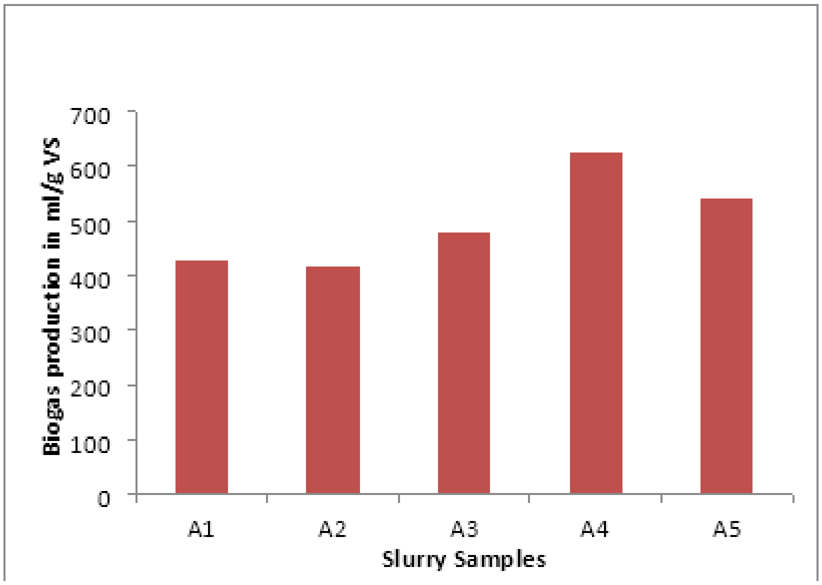

Figure 2

Cumulative biogas production via anaerobic digestion of samples

Figure 3

Cumulative Biogas production from anaerobic digestion from (a) dairy wastewater and cow dung (b) untreated wheat straw and cow dung (c) untreated wheat straw, dairy wastewater and cow dung (d) pretreated wheat straw, cow dung and dairy wastewater (e) pretreated wheat straw and cow dung