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Bio-Methane Production Potential Assays of Organic Waste by Anaerobic Digestion and Co-Digestion

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Authors' Contribution
NB conducted the research and wrote the manuscript. NA and SZHS took part in the execution of the study. JIQ supervised the study. AH critically reviewed the article.

Key words
Anaerobic digestion, Anaerobic co-digestion, Biogas production, Food waste, Dairy manure

ABSTRACT

The present study was conducted to investigate biochemical methane production potential (BMP) of the most abundant feed-stocks i.e., dairy manure (DM) and food wastes (FW) in agrarian countries by anaerobic digestion (AD) and co-digestion (Co-AD) under mesophilic (35 ± 2 ℃) settings at substrate to microorganism (S / M) ratio of 1.0 based on volatile solids (VS) for 30 days. Solids [Total solids (TS), volatile solids (VS), fixed solids (FS) and their ratios as (VS / TS)] determinations were also carried out for process monitoring. The highest cumulative biogas yield of 459.4 mL /g VS was observed in the co-digestion of DM + FW with 58.63 and 41.36 % CH_4 and CO_2 contents. BMP of the microcrystalline cellulose (MCC), FW and DM were observed as 401.2, 389.61 and 358.7 mL /g VS with CH_4 and CO_2 contents of 54.1 and 35.9, 51.13 and 42.2, 56.66 and 40 %, respectively. The volatile solids reductions were 82, 79, 59 and 71 % for MCC, FW, DM and FW + DM, respectively. These results indicate that FW and DM are very desirable substrates for AD and their Co-AD upturns the biogas generation efficiency. Thus, we can conclude that BMP assays are extremely useful to determine the amount of bio-methane and bio-degradability of the organic substrates and would facilitate in the preliminary selection systems for the field scale applications especially in framework of developing countries.

INTRODUCTION

Developing countries are facing acute energy crisis and Pakistan is not an exception. However, country has reserves of oil [0.31 billion barrels (Bb)], gas [30 trillion cubic feet (TCF)], coal [185 billion tons (Bt)] and shale gas [51 trillion cubic feet (TCF)]. Being an indigenous resource natural gas has substituted oil. Large scale switching from oil to gas took place by domestic, power, industrial and transport sector due to its cost control and environmental protection factor. Consequently, massive load on limited reserves of natural gas triggered its fast diminution (Haq and Hussain, 2008; Farooqui, 2014).

Expanding prerequisites of natural gas increased the demand/supply gap which resulted in curtailing the major users mainly transport sector (CNG stations), power plants and industries, throughout the winter season to ensure domestic supplies. At present, situation is even worse with the power supply shortage of 5-7 GW bringing the severe blackouts. Currently around one fourth population of Pakistan is deprived of electricity and natural gas. The most important cause for such a massive energy shortage is its substantial dependency on imported oil for power production (Haq and Hussain, 2008; Farooqui, 2014). Pakistan heavily depends on the import of fossil fuels to meet its 80 % energy requirements by spending approximately 7 billion US$ per annum. The substitution of conventional fuels to the alternate renewable and sustainable energy resources like biogas provides best option in such scenario. Biogas is sustainable energy solution for developing countries, especially Pakistan, which has natural gas based economy (Amjid *et al*., 2011).

Biogas is derived from biomass (almost all kinds of organic wastes, agricultural residues or energy crops, woody biomass like forestry residues, sewage sludge and manure) by anaerobic digestion. Its major constituents are CH_4 and CO_2. Trace components that encompass biogas are, hydrogen sulfide, siloxanes, water vapor, oxygen, carbon monoxide, hydrocarbons, ammonia and nitrogen. Renewable natural gas offers an environmental friendly approach of switching from fossil derived natural gas. It is a flexible energy vector for heat, electricity and fuel applications with associated benefits of wastes consumption (Ryckebosch *et al*., 2011; Strauch, 2012).

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Pakistan holds a strong potential for biogas production. It is the 36th largest country by an area cover of 796,095 km^2 and the 6th most populous country in the world. Consumption of either biomass i.e., animal dung (1480 kg) or firewood (2325 kg) or crop residues (1160 kg) for country's averaged sized household is reported on annual basis to congregate the energy needs. These efforts and expenditures can be effectively replaced with efficient source of renewable biogas. The population growth rate and solid waste generation are directly linked. Being a populous and agro-industrial-livestock based economy, huge amount of municipal solid waste, food waste, crop residues and animal manure categories are available to handle in an environmentally compatible way. Solid waste generation of 67,500 tons per day is reported (Zuberi and Ali, 2015; Yasar *et al*., 2017). According to Dawn News (2014), the country's agricultural biomass resources are reported to be around 80 million tons which hold the potential to substitute 25 million barrels of oil equivalent energy. Food waste is estimated to be 36 million tons on annual basis (Dawn News, 2018). There are around 159 million cattle generating daily manure of 652 million kg from buffalos and cows only which in turn could produce16.3 m^3 day^{-1} biogas and 21 million tons organic fertilizer annually, compensating 20 and 66 % nitrogen and phosphorous, respectively (Yasar *et al*., 2017).

In the past some projects of installing biogas digesters at rural household level were launched which gone off due to removal of external subsidies (Asif, 2009). Thus present study was aimed to develop the simplest and cost effective schemes for mesophilic digesters that could be scaled up based on results of present investigation. Biochemical methane potential assays for dominant waste categories of agro-industrial countries i.e., food waste and dairy manure by anaerobic digestion and co-digestion were conducted in batch experiments. Substrates and effluents were also characterized by analytical measurements for pH and solids as monitoring parameters and to evaluate the bio-degradabilities. Experimental layout was configured to mimic the conventional mesophilic AD.

MATERIALS AND METHODS

Substrate and seeding culture collection /pretreatment

The food waste was collected from university's cafeteria and stored at 4 ºC. Collected food waste sample contained mixed proportions of different fruit, vegetable and kitchen wastes (leftovers of orange, banana, apple, peas, spinach, coriander, carrot, potato, chickpeas, cucumber, rice, tomato, cabbage, wheat flour, egg shells, tea, coffee, salads, burgers and sandwiches). A screening operation was performed within 24h to confiscate the coarse waste products such as bone pieces, wood, metal and plastics for reducing sampling errors and impairment of homogenization equipment. The feed-stocks were then ground and homogenized by mincer for size reduction to 1mm. Dairy manure samples were obtained from a local dairy farm and scraped off from the food trails followed by their immediate storage at 4 ºC. The seeding culture was collected from primary sludge treating mesophilic digester of wastewater plant. Its pre-incubation step includes 7 days to ensure the degradation of residual matter after screening from 5 mm sieve. Before feeding the digesters, substrates and seeding culture were sampled for analytical measurements.

Biochemical methane potential assays

Batch digestion tests were carried out in triplicates using 1000mL laboratory digesters. The digesters were maintained at mesophilic temperature range (35 ± 2 ºC) and pH 7 with the effective volume of 0.5 L and retention time of 30 days. Each substrate and their mixture were fed in the digester in consort with sustaining substrate to seeding culture ratio (S / M) at 1.0 on VS g L^{-1} basis. Analyses included five experimental lines consisting of i blank (inoculum only) to correct the gas volume ii positive control using microcrystalline cellulose (Sigma MCC, aldrich) as a reference substrate (Angelidaki *et al*., 2009) iii Food waste (FW) iv Dairy manure (DM) v Food waste and dairy manure (FW + DM). The working volume of up to 500 mL was maintained by using autoclaved water. All the digesters were vacuum-packed with rubber bungs and screw controls. Headspace of the batch digesters was purged with nitrogen. Manual mixing of the reactors were performed on daily basis for 30s followed by biogas measurement. Biogas potential and biochemical methane potential (BMP) analyses were performed by water displacement set up with 1000 mL graduated cylinder. CO_2 and H_2S scrubbing was performed with 3 M NaOH to estimate the CH_4 according to Anaerobic Lab Work (1992).

Analytical methods

Feedstock, seeding culture and biogas effluents were analytically characterized. Solid analysis (TS, VS, FS, VS / TS) and pH measurements were carried out following the standard procedures described in American Public Health Association (1998). All the constituents of batch reactors were also measured for solids at the end of digestion assays to estimate the percent reduction.

Statistical analysis

Single factor ANOVA was applied followed by Tukey Pairwise comparisons on average yields of biogas

Table I. Average solids [Total solids (TS), volatile solids (VS), fixed solids (FS) and their ratios as (VS / TS)] and moisture contents (MC) of organic substrates [food waste (FW), dairy manure (DM)] and anaerobic seeding culture. Values represent average of three measurements ± standard deviation.

Waste streams	TS%	VS%	FS%	MC%	VS/TS%
FW	28.3±4.51	23.9±0.89	4.4±0.05	91.56±1.24	84.45±0.21
DM	13.2±1.90	10.5±1.03	2.7±1.11	87.34±0.25	79.54±0.31
Seeding culture	TS/g.L	VS/g.L	FS/g.L	MC%	VS/TS%
Mesophilic inoculum	11.25±0.08	7.55±0.09	3.7±0.11	93.14±1.58	67.11±0.21

and methane using Minitab 18 for the determination of significant differences among various substrates. Statistical significance was considered for the variance analysis at p <0.05.

RESULTS

Feedstock / seeding culture characteristics

The solid analysis of the feed-stocks and anaerobic seeding culture was performed (Table I). All the calculations were carried out on the basis of wet weight. The percentages of TS, VS and FS were 28.3, 23.9 and 4.4 for food wastes samples. The dairy manure samples exhibited lower TS, VS and FS values as compare to food waste. Solid concentrations of dairy manure samples comprised 13.2 % TS, 10.5 % VS and 2.7 % FS. The VS / TS ratios and MC were 84.45, 79.54, 91.56 and 87.34 % for food waste and dairy manure, respectively.

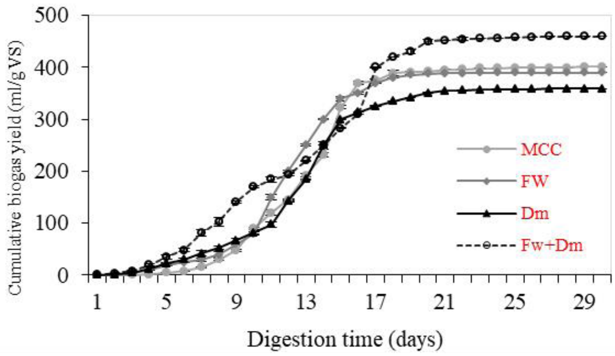

Fig. 1. Cumulative biogas yields from microcrystalline cellulose (MCC), food waste (FW), dairy manure (DM) and co-digestion of food waste and dairy manure (FW+DM). Each data point represents average measurement of three digesters with standard error of mean.

Biochemical methane potential (BMP) assays

Cumulative biogas yields for the anaerobic digestion and co-digestion of FW and DM under mesophilic conditions at S / M of 1.0 were determined. Biogas generation rates remained at low level during the last ten days of digestion for all the experimentation lines (Fig. 1). During the experimental period on the whole 401.20, 389.61, 358.70 and 459.40 mL g^{-1} VS of biogas produced from MCC, FW, DM and FW + DM, respectively. Average daily biogas production rates (mL / L. d) were also assessed (Fig. 2). The MCC results represented the confirmation for the microbial activity of the inoculum. After a small lag phase of 2-4 days most of the biogas was produced during 5-20 days for the anaerobic digestion process of individual lines i.e., food waste and dairy manure. In anaerobic co-digestion reactor biogas production process initiated without lag phase and projected upward until the 20^{th} day.

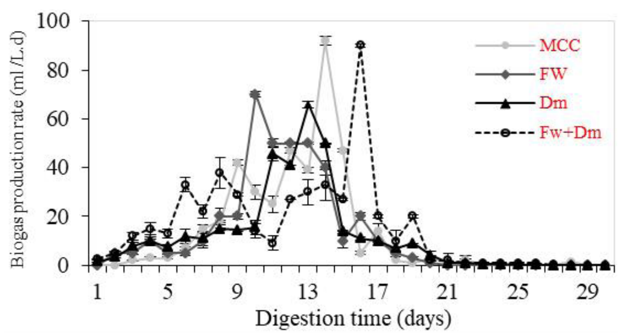

Fig. 2. Biogas daily production rates (mL / L.d) during anaerobic digestion of microcrystalline cellulose (MCC), food waste (FW), dairy manure (DM) and co-digestion of food waste and dairy manure (FW+DM). Each data point represents average measurement of three digesters with standard error of mean.

Estimating CH_4 contents and VS % reduction

The CH_4 and CO_2 contents of the biogas produced from MCC, FW, DW and their mixture were determined (Fig. 3). Methane contents of all the experimental lines were initially lower for about five days and gradually increased with time. The highest CH_4 contents of 58.63% on average were found for FW and DM co-digestion after 30 days with the 41.36 % CO_2 contents. Anaerobic digestion of single substrates revealed 56.66, 51.13 and 54.10% CH_4 and 40, 42.2 and 35.90 % CO_2 contents for

DM, FW and MCC, respectively.

The average pH values showed no significant difference before and after the digestion process (Fig. 4). The volatile solids reductions were 82, 79, 59 and 71% for MCC, FW, DM and FW + DM, respectively.

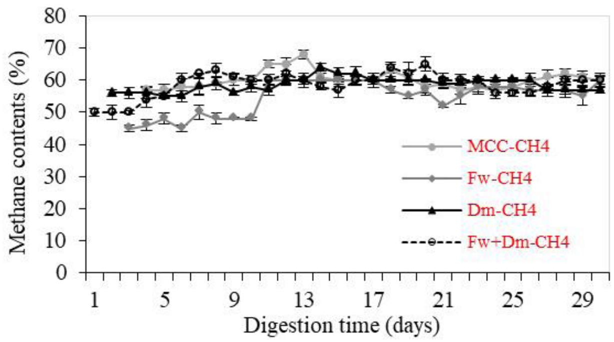

Fig. 3. Percent (%) methane (CH_4) contents of biogas produced from anaerobic digestion of microcrystalline cellulose (MCC), food waste (FW), dairy manure (DM) and their mixture (FW+DM). Each data point represents average measurement of three digesters with standard error of mean.

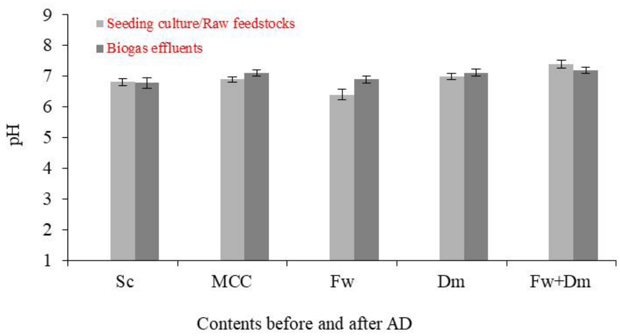

Fig. 4. Average pH measurements of seeding culture, raw feed-stocks and biogas effluents. Each data point represents average measurement of three digesters with standard error of mean.

Variance analysis

The results showed that variability of biogas yield for MCC, FW, DM and FW+DM was statistically significant (F-Value, 304205.43 and P-Value, 0.000). The methane yields were significantly different only for the FW and FW+DM (F-Value, 4.91 and P-Value, 0.032).

DISCUSSION

Anaerobic digestion (AD) technology is extensively used to treat organic waste streams generating biogas for fuel, electricity and heating applications. Anaerobic digesters using animal manure as substrate have widely been explored but are not favorable from standpoint of investment return, lower biodegradability and biogas yield as compare to food waste. In the present investigation, TS, VS and FS values were comparatively higher than dairy manure. From an economic perspective the higher organic fraction of the food waste represents the higher energy content expedient for biogas generation. The highest cumulative biogas yield of 459.4 mL / g VS for FW + DM co-digestion over 30 days was recorded in the present study. These results indicated that economics of the dairy digesters can be improved to obtain enhanced biogas generation rates by co-digestion of dairy manure with highly degradable substrate such as food waste. Anaerobic co-digestion may increase process efficiency owing to healthier balance of nutrients and carbon as reported by Mshandete *et al*. (2004); Parawira *et al*. (2004) and El-Mashad and Zhang (2010). Angelidaki and Ellegaard (2003) reported that feed-stocks which are easily biodegradable e.g., food waste can boost the microbial tolerance towards inhibitory compounds by nurturing the active biomass volumes in the digesters. The results of the present study are almost similar with the trends described by El-Mashad and Zhang (2010), performing mesophilic co-digestion of dairy manure and food waste which gave higher biogas yields for mixtures than the individual waste streams. Anaerobic co-digestion studies of Bouallagui *et al*. (2003) also reported the similar substrates exploitations where the mesophilic AD processing cow dung, fruit and vegetable wastes (FVW) in a tubular digester revealed that 4% to 6% increase of TS also increases the biogas production. While 6% to 8% increase in feed concentration decreased the conversion efficiency and at 10 % inhibition of the process occurred. Alike study of Islam *et al*. (2012) also reported the methane production from food waste and its co-digestion with swine manure at 35 °C analyzing various feed to inoculum (F / I) ratios and food waste to swine manure mixtures ratios with the loading quantities of 0.01 ml / g VS and 0.03ml / g VS. The highest biogas yield of 1148 mL / g VS for the co-digesting ratio 40:60 at 0.01ml / g VS was obtained. Methane contents of all the experimental lines in the present study were initially lower for about five days and gradually increased with time. This trend pointed to the possible buildup of intermediates at the initial phase which caused upset to the methanogenic bacterial activity. Similar patterns of corresponding phenomenon were observed by Wang *et al*. (1997) and Zhang *et al*. (2007). The pH values for all the experimental lines were not significantly different before and after the digestion process in the current study. This could be due to relatively higher salts, nutrient contents and alkalinity of the animal manure which provided essential buffer capacity for pH control during AD. This trend of nutrients compatibility was also reported by Mata-Alvarez *et al*. (2000) by nutrients addition via co-digestion.

CONCLUSION

Anaerobic digestion of organic wastes for biogas generation nexus energy-environment and economics by delivering solitary line answer to combat multiple complications predominantly fossil fuels extinction, growth in energy demands, inadequate waste management and greenhouse gases (GHGs) emissions. Implementation of this green technology with its associated benefits is foremost requirement in special context of developing countries like Pakistan where the aforementioned challenges are more intensified. The current study represents successful batch anaerobic digestion of five experimental lines at mesophilic temperature (35 + 2°C) along with the analytical determinations. Biogas and methane potential of DM and FW were determined alone and in the mixture at S/M ratio of 1.0 g VS L^{-1}. Results indicate that co-digestion of the FW and DM produced higher biogas yields as compare to individual digestion of FW and DM. Thus, co-digestion of highly bio-degradable substrates like food waste with a substrate providing right nutrient balance like dairy manure improved the biogas yield. Since food waste and dairy manure are two predominant biomass resources of populous and agrarian countries like Pakistan, thus this investigation provides the simplest schematic ground for scaling up the production of biogas for household or commercial applications.

Statement of conflict of interest

The authors declare that there is no conflict of interests.

REFERENCES

Amjid, S.S., Bilal, M.Q., Nazir, M.S. and Hussain, A., 2011. Biogas, renewable energy resource for Pakistan. *Renew. Sust. Energ. Rev.*, **15**: 2833-2837. https://doi.org/10.1016/j.rser.2011.02.041

Anaerobic Lab Work, 1992. *International course on anaerobic waste water treatment*. Wageningen University and IHE Delft, The Netherlands.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P. and Van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid wastes and energy crops: a proposed protocol for batch assays. *Wat. Sci. Tech.,* **59**: 927-934. https://doi.org/10.2166/wst.2009.040

Angelidaki, I. and Ellegaard, L., 2003. Codigestion of manure and organic wastes in centralized biogas plants. *Appl. Biochem. Biotech.,* **109**: 95-105. https://doi.org/10.1385/ABAB:109:1-3:95

APHA, 1998. *Standard methods for the examination of water and wastewater*: 18th ed. American Public Health Association, Washington, DC, USA.

Asif, M., 2009. Sustainable energy options for Pakistan. *Renew. Sust. Energ. Rev.,* **13**: 903-909. https://doi.org/10.1016/j.rser.2008.04.001

Bouallagui, H., Cheikh, R.B., Marouani, L. and Hamdi, M., 2003. Mesophilic biogas production from fruit and vegetable waste in a tubular digester. *Bioresour. Technol.,* **86**: 85-89. https://doi.org/10.1016/S0960-8524(02)00097-4

Dawn News, Pakistan., 2014. http://www.dawn.com/news/1130471/renewable-energy-solutions-to-power-crisis.

Dawn News, Pakistan., 2018. https://www.dawn.com/news/1394618.

El-Mashad, H.M. and Zhang, R., 2010. Biogas production from co-digestion of dairy manure and food waste. *Bioresour. Technol.*, **101**: 4021-4028. https://doi.org/10.1016/j.biortech.2010.01.027

Farooqui, S.Z., 2014. Prospects of renewables penetration in the energy mix of Pakistan. *Renew. Sust. Energ. Rev.*, **29**: 693-700. https://doi.org/10.1016/j.rser.2013.08.083

Haq, D. and Hussain, K., 2008. *Energy crisis in Pakistan*. IPRI Factfile.

Islam, M.N., Park, K.J. and Yoon, H.S., 2012. Methane Production Potential of Food Waste and Food Waste Mixture with Swine Manure in Anaerobic Digestion. *J. Biosys. Eng.,* **37**:100-105. https://doi.org/10.5307/JBE.2012.37.2.100

Mata-Alvarez, J., Mace, S. and Llabres, P., 2000. Anaerobic digestion of organic solid wastes: an overview of research achievements and perspectives. *Bioresour. Technol.,* **74**: 3-16. https://doi.org/10.1016/S0960-8524(00)00023-7

Mshandete, A., Kivaisi, A., Rubindamayugi, M. and Mattiasson, B., 2004. Anaerobic batch co-digestion of sisal pulp and fish wastes. *Bioresour. Technol.,* **95**: 19-24. https://doi.org/10.1016/j.biortech.2004.01.011

Parawira, W., Murto, M., Zvauya, R. and Mattiasson, B., 2004. Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet leaves. *Renew. Energ.,* **29**: 1811-1823. https://doi.org/10.1016/j.renene.2004.02.005

Ryckebosch, E., Drouillon, M. and Vervaeren, H., 2011. Techniques for transformation of biogas to biomethane. *Biomass Bioenerg*., **35**: 1633-1645. https://doi.org/10.1016/j.biombioe.2011.02.033

Strauch, S., 2012. *Biogas upgrading technologies technical and financial aspects*. Green Gas Grids study tour Asten (A).

Wang, Y.S., Odle, W.S., Eleazer, W.E. and Barlaz, M.A., 1997. Methane potential of food waste and anaerobic toxicity of leachate produced during food waste decomposition. *Waste Manage. Res*., **15**: 149-167. https://doi.org/10.1177/0734242X9701500204

Yasar, A., Nazir, S., Rasheed, R., Tabinda, A.B. and Nazar, M., 2017. Economic review of different designs of biogas plants at household level in Pakistan. *Renew. Sust. Energ. Rev*., **74**: 221-229. https://doi.org/10.1016/j.rser.2017.01.128

Zhang, R.H., El-Mashad, H.M., Hartman, K.F., Wang, G., Liu, C., Choate and Gamble, P., 2007. Characterization of food waste as feedstock for anaerobic digestion. *Bioresour. Technol*., **98**: 929-935. https://doi.org/10.1016/j.biortech.2006.02.039

Zuberi, S.J. and Ali, S.F., 2015. Green house effect reduction by recovering energy from waste landfills in Pakistan. *Renew. Sust. Energ. Rev*., **44**: 117-131. https://doi.org/10.1016/j.rser.2014.12.028