

Effect of the solid content on biogas production from *Jatropha curcas* seed cake

Nusara Sinbuathong¹, Junko Munakata-Marr², Boonsong Sillapacharoenkul³,
Suphang Chulalaksananukul⁴

Abstract—This paper describes anaerobic conversion of *Jatropha curcas* seed cake at 30°C in a five - liter working volume reactor. The aim of this work was to investigate the effectiveness of employing a batch process to produce biogas and evaluate the performance of the process in terms of methane yield and extent of degradation. Initially fresh cow dung slurry culture was inoculated in five reactors for culture development. The seed cake was prepared as a slurry at ratios of 1:20, 1:10, 1:6.67, 1:5 and 1:4 by weight with tap water. Over a 60-day operating period, a slurry of seed cake in water at a ratio of 1:20 showed the highest methane yield of 296 liter methane at STP/kg COD degraded (or 156 liter at STP/kg of *Jatropha curcas* seed cake added) and was achieved with 52% COD removal efficiency. The results revealed that *Jatropha curcas* seed cake treated anaerobically can be a good source of methane production. At the tested concentration, the appropriate seed cake-to-water ratio for methane production should be in the range of 1:20 to 1:10.

Keywords—Agricultural waste, biogas, *Jatropha curcas*, methane yield, solid content

1 INTRODUCTION

Several biological processes to convert biomass to energy, and thus provide a source of fuel, have been studied in recent decades [1,2]. One of the most important processes for this purpose is the anaerobic digestion of organic matter to obtain biogas (consisting mainly of CH₄ and CO₂) as a product of the metabolic action of methanogenic microbial consortia. Another benefit of utilizing biomass to generate energy is that the solid residual product from anaerobic degradation can be used as organic fertilizer [3]. Every year around the world several million tons of agricultural wastes are disposed [4] through land application and land filling, despite the high potential of this global waste as a biorenewable energy resource that can be transformed into high-value by-products. Disposing of this waste creates increasing demand for land in most urban regions. Land is an important limited and scarce resource, and shortage of land is a potential problem [5].

Jatropha curcas de-oiled cake is one of the agricultural wastes considered as an energy source. *Jatropha curcas* is a drought-resistant shrub belonging to the family *Euphorbiaceae*, which is cultivated on a large scale

in Central and South America, Southeast Asia, India and Africa [6]. From the Caribbean, where this species was used by the Mayans [6], *Jatropha curcas* was probably distributed by Portuguese seafarers via the Cape Verde Islands and Guinea Bissau to other countries in Africa and Asia [7]. *Jatropha curcas* can easily be propagated by cuttings [8]. *Jatropha curcas* is a succulent that sheds its leaves during the dry season. The *Jatropha curcas* plant has high agro-industrial potential because of its various potentially beneficial products. For example, the oil extracted from the seeds can be used as a substitute for diesel after transesterification [9,10]. Such biodiesel has currently high demand in the United States and Europe and is being heavily promoted in countries such as India [11]. *Jatropha curcas* is well adapted to arid and semi-arid conditions and often used for erosion control [7]. All parts of *Jatropha curcas* have been used in traditional medicine and for veterinary purposes for a long time [10]. The first commercial applications of *Jatropha curcas* were reported from Lisbon, where the oil imported from Cape Verde was used for soap production and for lamps. The seed cake was used as a fertilizer for potatoes. Even today, *Jatropha curcas* is mainly cultivated for the production of oil as a fuel substitute. Uses of various *Jatropha curcas* components are shown in Fig. 1 [12].

Researchers have generally focused on the production of biodiesel from *Jatropha curcas*, while few studies have investigated biogas production from *Jatropha curcas* seed cake. Singh, et al. [13] and Sinbuathong, et al. [14] studied biogas production from *Jatropha curcas* seed cake enriched with cattle dung and operated by semi-continuous single stage operation. Authors of both studies conclude that *Jatropha curcas* seed cake is a good source, due to the high conversion rates and efficiencies obtained.

1 Scientific Equipment Center, Kasetsart University Research and Development Institute, Kasetsart University, Bangkok 10900, Thailand. E-mail: rdinrs@ku.ac.th

2 Environmental Science and Engineering Division, Colorado School of Mines, Golden, Colorado, 80401, US. E-mail: junko@mines.edu

3 Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand. E-mail: bsk@kmutnb.ac.th

4 Department of Chemical Engineering, Faculty of Engineering, Mahidol University, Salaya campus, Nakornpathom, 73170, Thailand. E-mail: egscl@mahidol.ac.th

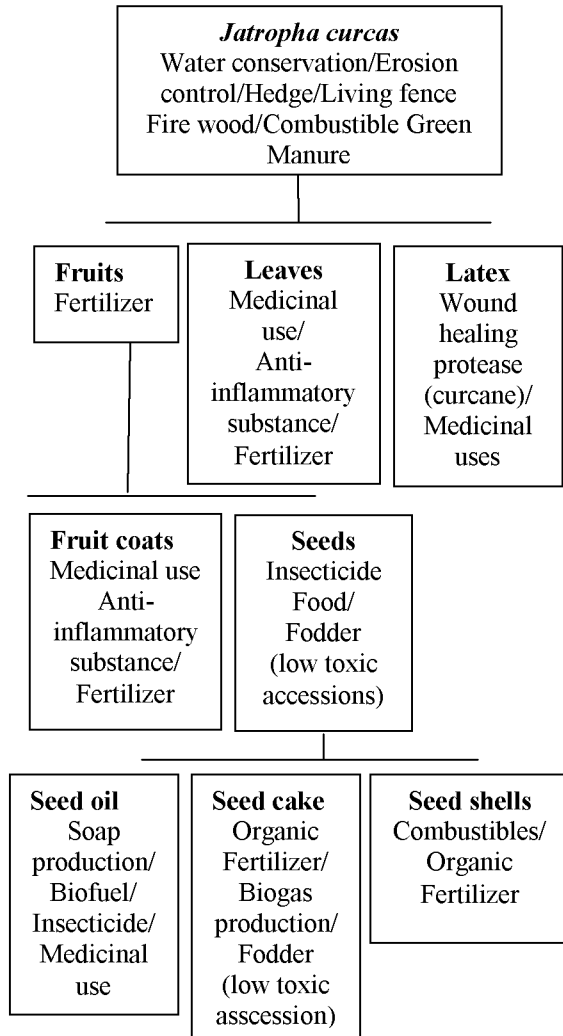


Fig.1. Exploitation of *Jatropha curcas* components (adapted from [12])

Anaerobic digestion processes can be classified into three main categories: batch, single stage and multi-stage. In batch fermentation, the reactor is loaded once and discharged until the end of the anaerobic process takes place. Because of its simplicity and portability, batch reactors are a good option for treating biowaste. Because plant solids will not flow through pipes, this type of solid waste substrate is best treated in a single batch digester. The tank is opened, old slurry is removed and the new charge is added. The tank is then resealed and ready for operation.

A slurry of *Jatropha curcas* seed cake is acidic [15]. Knowledge of the optimum solid content for batch digestion is necessary to prevent system failure due to acidic pH. The objective of the current study was to evaluate the biogas production, as well as to investigate, in general, the performance of digestion in terms of CH₄ yield and organic waste reduction utilizing *Jatropha curcas* seed cake in a batch anaerobic digesting system when varying the solid content under mesophilic conditions.

2 MATERIALS AND METHODS

2.1 Seed Cake Characterization

Samples were chemically characterized because the most important parameters of biogas production are the composition and quantity of feedstock. Both parameters were influenced by the feedstock used and the solid content applied. The analyses of the seed cake include moisture content, total solid (TS), total volatile solid (TVS), organic carbon, organic matter, nitrogen and phosphorous. The moisture content of the samples was determined by oven-drying to a constant weight at 105°C. Total solid equaled from 100% - %moisture content. Total volatile solid was obtained by igniting the total solids in a muffle furnace at 550°C. Organic carbon in the sample was measured using the Walkley-Black method [16,17]. Organic carbon was oxidized with a mixture of potassium dichromate and sulfuric acid; the excess potassium dichromate was titrated with ferrous sulfate. The organic matter content of soil was indirectly estimated through multiplication of the organic carbon content by 1.72 [18]. Nitrogen was determined by the Kjeldahl methods by digesting samples to convert organic N to NH₄⁺-N and determining NH₄⁺-N in the digest [18]. Total phosphorous was determined by digesting samples with sulfuric acid and analyzed by ascorbic acid method [19].

2.2 Sample Preparation and Feed Solution

Jatropha curcas seed cake was collected from Prathumthani province, Thailand. The seed cake was stored in a plastic bag at room temperature and was blended prior to experiment. After blending, the seed cake was mixed with tap water at ratios of 1:20, 1:10, 1:6.67, 1:5 and 1:4 by weight to create a slurry. The slurry was stored overnight before adding to each reactor. The initial pH of the slurry was 5.5 and then was adjusted to 7 with sodium bicarbonate.

2.3 Inoculum, Test Bioreactors and Operation

Fresh cow dung was collected and brought back to the laboratory in bags. For experiments, sufficient water was added to cow dung at a ratio of 1:1 by weight to produce a slurry. This slurry was used to inoculate test bioreactors without acclimation. Biomass was measured as mixed liquor volatile suspended solids (MLVSS) by standard methods [20] before inoculating to each reactor in order to start each test bioreactor with the same cell mass.

To compare the effect of the solid content on biogas production, five reactors were constructed from six-liter capacity polyethylene terephthalate (PET) plastic bottles with five-liter working volume. Each was equipped with two outlet ports,

one for liquid sample withdrawal and the other for gas venting. The reactor was connected to a gas collection system, which was based on water displacement by the exiting gases (Fig. 2). Sulfuric acid of 0.05 molar was used for the displacement by gas in the gas collection system. The experiments were conducted in batch mode after inoculation as described above at an initial concentration of 13.8 g MLVSS /l. The *Jatropha curcas* slurry at ratios of 1:20, 1:10, 1:6.67, 1:5 or 1:4 was added to different test reactors. Because digester start-up is an especially critical step, the initial pH of the slurry was controlled with sodium bicarbonate during the start-up period (the first 48 hours) in order to maintain neutral conditions. Alkalinity, as added via bicarbonate, is important to neutralize the acidic slurries (initial slurry pH was 5.5) added to the reactor. pH was measured every day in the start-up period and then at least every other day. pH was not adjusted after the startup period. The ambient temperature of all reactors was 30°C.

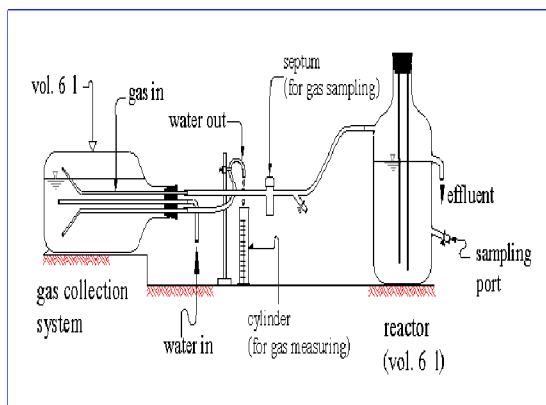


Fig. 2. Bioreactor for the digestion of *Jatropha curcas* seed cake.

The total gas production at ambient temperature was recorded daily based on water displacement by the collected gases and the CH₄ content was then determined by a Shimadzu GC-14B gas chromatograph equipped with a thermal conductivity detector (TCD). The gas volumes were calculated to the standard temperature (0°C) and pressure (760 mm Hg) (STP) and recorded at this condition. The initial COD of each reactor was analyzed, and during the bioreaction time, digested slurry samples (5 ml) were withdrawn periodically and analyzed for COD, TVS and pH. Samples for COD analysis were acidified, shaken for 2 hours to remove sulfide and analyzed according to the APHA Standard Method procedure [20]. Experiments were conducted in duplicate; results are reported using the mean of the experimental values. The batch reactors were operated for 60 days when the biogas production had leveled off in all reactors.

3 RESULTS AND DISCUSSION

In this study, analysis of the seed cake showed that it had 8.3% moisture and 91.7% total solid (TS), of which 95.0% was volatile solid (VS). The cake was fairly high in organic matter (68.7%) and contained nitrogen and phosphorous of 3.3 and 0.88%, respectively. Nitrogen and phosphorous are the major biological nutrients in the seed cake. Thus the *Jatropha curcas* seed cake has good potential for biogas generation. The results from the laboratory analysis are summarized in the table below.

Table 1. Chemical properties of *Jatropha curcas* seed cake

Parameters	Content (% by weight)
Moisture	8.3
TS	91.7
Organic carbon	39.9
Organic matter	68.7
Nitrogen	3.32
Phosphorous	0.88

3.1 Biogas production

The cumulative total biogas, measured in liters, is shown in Fig 3. The cumulative total biogas from reactors that contained seed cake to water at ratios of 1:10 was higher than that of 1:6.67 and 1:20, and was approximately 2 times higher than that of 1:4 and 1:5.

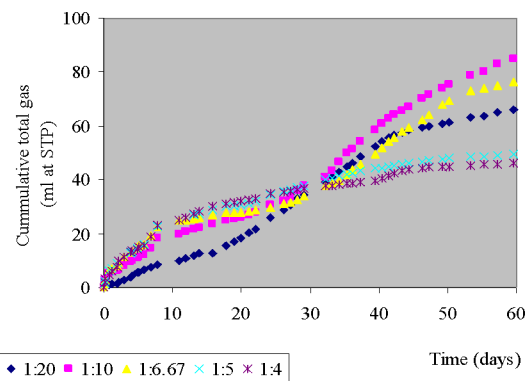


Fig.3. The time course of the cumulative total gas production for different bioreactors that contained specified dilutions of *Jatropha curcas* seed cake slurry.

Fig. 4 depicts the percentage of CH₄ in the gas that was produced in reactors containing a slurry of *Jatropha curcas* seed cake at dilutions of 1:20, 1:10, 1:6.67, 1:5 and 1:4. In the first 20 days of operation, the content of CH₄ gas in the reactor that contained the slurry ratio

1:20 was 60%, highest of all reactors. After 30 days of operation, the CH₄ gas content in the three reactors with lowest solids content (seed-cake-to-water ratios 1:6.67, 1:10 and 1:20) was indistinguishable (Fig 4). However, the CH₄ gas content in these three reactors was much higher than the other two reactors that contained seed-cake-to-water ratios 1:4 and 1:5. The figure reveals that increasing solid concentration (from 1:20, 1:10, 1:6.67 to 1:5 and 1:4) resulted in a decrease in the percentage of CH₄ production and/or a delay in production. *Jatropha curcas* seed cake that was more highly concentrated than 1:6.67 significantly caused both a delay and a diminished production of CH₄ (Fig. 4). The lowest CH₄ content was only 20%, which was obtained from the reactor that contained the slurry at the ratio of 1:4 (Fig. 4).

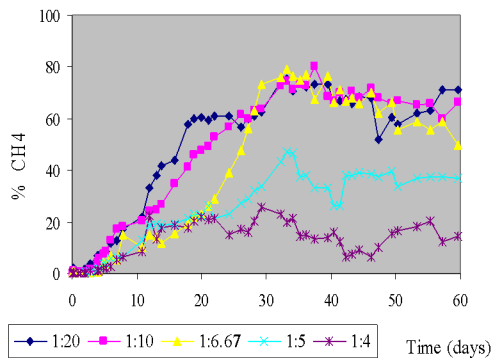


Fig. 4. The time course of the percentage of CH₄ for different bioreactors that contained specified dilutions of *Jatropha curcas* seed cake slurry.

Cumulative CH₄ production in the bioreactors is displayed in Fig. 5. The volume of CH₄ produced in the bioreactors that contained the slurry seed cake at 1:20 and 1:10 dilutions was comparable and was higher than that of 1:6.67. *Jatropha curcas* seed cake at the ratio of 1:10 appeared to cause a slight delay in CH₄ production compared with the slurry seed cake of 1:20, while CH₄ production from the reactor that contained the slurry seed cake of 1:6.67 was more significantly delayed but rapidly increased to levels nearly comparable to 1:20 ratios. *Jatropha curcas* seed cake slurry at the dilutions of 1:5 and 1:4 caused both a delay and a decrease in CH₄ production. The decrease in CH₄ content with less effect on the total biogas production with increasing solid content was probably due to production of other gases for bioreactors with dilutions of 1:5 and 1:4 (Fig. 3 compared with Fig. 5). This study shows that digestion using the higher solid content than 1:6.67 resulted in loss in biomethanation process efficiency.

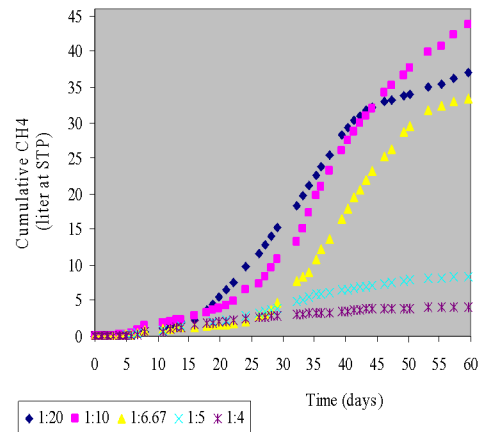


Fig. 5. Graph comparison of cumulative CH₄ generated in the systems at specified dilutions of *Jatropha curcas* seed cake slurry.

Methane production in bioreactors started after 10 days of operation. The rate of CH₄ production gradually increased during 10-25 days of digestion then sharply increased, and reached highest at 40-50 days (Fig. 6). For the slurry at the ratios of 1:20 and 1:10, the highest rate of CH₄ production was 7.5 liters (STP) /liter-day while at the ratio of 1:6.67 the rate was 6 liters (STP) /liter-day; the rate obtained at higher solids concentrations was much lower. So, the dilution of *Jatropha curcas* seed cake should be optimized to ensure high process rates of CH₄ production. The reduction of the rate of CH₄ production has serious consequences for plant throughput and economic operation, so these considerations are very important.

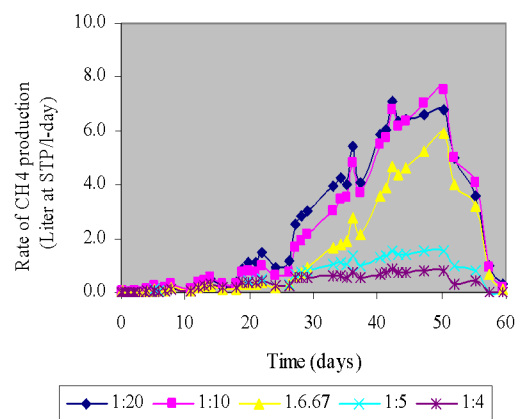


Fig. 6. Rate of CH₄ production over time in the systems at specified dilutions of *Jatropha curcas* seed cake slurry.

Kalia et al. [21] studied a batch culture anaerobic digestion of banana stem waste under mesophilic conditions at 37°C and compared the CH₄ production from different solid content. They found that the conditions of 2-4% total solids (TS) achieved higher CH₄ yield than the higher solid content of 16% TS.

This is comparable to the results observed in the current study, with 1:20 and 1:10 dilutions (equivalent to 4.8 and 9.0 % TS) generating higher CH₄ yield than at lower dilutions (TS > 13%).

3.2 Reactor pH

The initial pH of the *Jatropha curcas* seed cake slurry was 5.5 and then was adjusted to 7 with sodium bicarbonate at the start-up period. The reactor pH was measured for a total of 60 days (Fig. 7). The system pH gradually increased to a final pH of approximately 8.3 in the reactor systems at dilutions of 1:20, 1:10, and 1:6.67, while the reactor systems at the ratios of 1:5 and 1:4 generally remained at a pH of approximately 7.4.

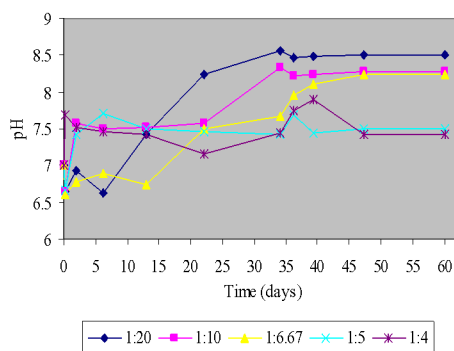


Fig. 7. pH of the reactors containing the slurry of *Jatropha curcas* seed cake at specified dilutions.

To relate pH (Fig. 7) to CH₄ production (Fig. 5), higher CH₄ production was observed in the reactors in which final pH exceeded 8 (the reactors that contained the slurry of seed cake at the ratios of 1:20, 1:10 and 1:6.67). During anaerobic digestion, organic substrate is first hydrolyzed to soluble organics, and then the short chain fragments are converted to simple organic compounds, predominantly volatile fatty acids (VFAs), which are ultimately converted to CH₄, CO₂ and other trace gases. In an efficiently operating anaerobic reactor, these three steps are in dynamic equilibrium; that is, the VFAs are converted to CH₄ at the same rate that they are formed. Relatively speaking, the reactors containing slurry ratios of 1:20, 1:10 and 1:6.67 appeared to operate more efficiently, at higher pH, than the reactors that contained the slurry of seed cake at the ratios of 1:5 and 1:4, in which the CH₄ production and pH were lower and the reactor performance in term of CH₄ production declined until CH₄ production ceased. In order to allow the CH₄-forming archaea to thrive, digesters should likely be fed with less amounts of substrate and maintained at an optimal pH, both of which may be specific to the organic waste and the microbial consortium.

3.3 Organic Substrate Reduction

COD (Fig. 8a) and TVS (Fig. 8b) decreased slowly but continually over a 60-day period. Bioreactor removal efficiencies (at day 60) for COD and TVS are summarized in Table 2.

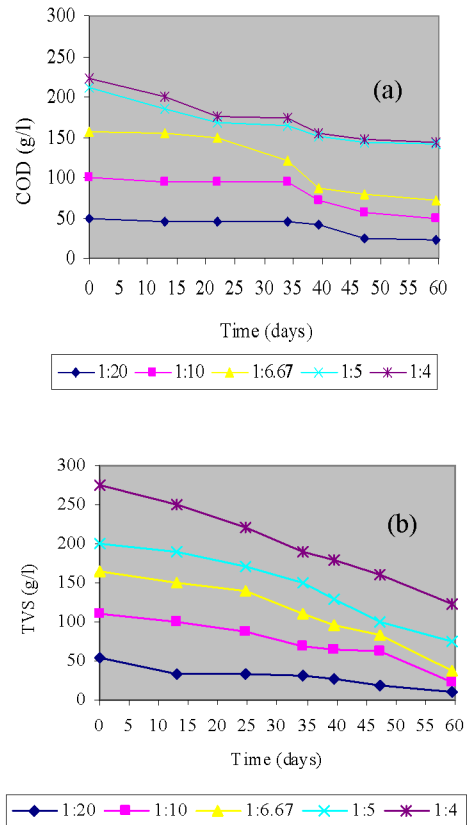


Fig. 8. The time course of (a) COD and (b) TVS of digested slurry in the reactors that contained *Jatropha curcas* seed cake at specified dilutions.

COD and TVS removal efficiencies were comparable for the reactors that contained the *Jatropha curcas* seed cake slurry at ratios of 1:20, 1:10 and 1:6.67. For the reactors that contained the slurry at 1:5 and 1:4 dilutions, the reactor performance was obviously lower than that of the other reactors. The consumed organic substrate (COD or TVS removal) was converted not only to CH₄ but to CO₂ and other gases. As discussed previously, at high solids content, the digester produces more CO₂ but less CH₄. Thus the system of high solid content consumed substrate but generated less CH₄.

3.4 Methane Yield

CH₄ yields, defined as the amount of CH₄ produced per kilogram of organic substrate degraded, calculated based on COD and TVS, are depicted in Figs. 9 and 10, respectively. The maximum CH₄ yield of 296 liter at STP/kg of COD degraded (164

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liter at STP/kg of TVS degraded or 156 liter at STP/kg of *Jatropha curcas* seed cake added to the reactor) was observed in the reactor that contained the *Jatropha curcas* seed cake at the dilution ratio of 1:20. This yield is lower than the theoretical yield of 350 liter at STP/kg of COD degraded when glucose is used as substrate [22], but the obtained values compare favorably with others that have been reported in studies using similar complex organics as a substrate. The CH₄ yield was fairly high as expected because of the high content of organic matter.

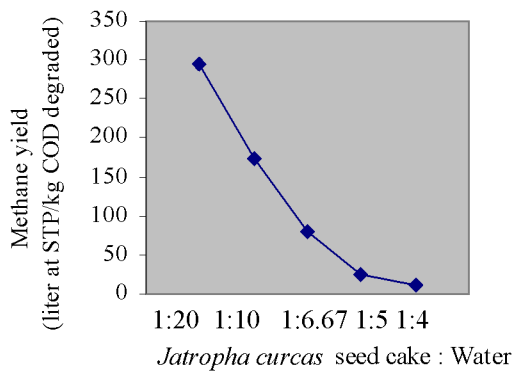


Fig. 9. Methane produced per kilogram of COD degraded during 60 day operation in reactors that contained *Jatropha curcas* seed cake of specified dilutions.

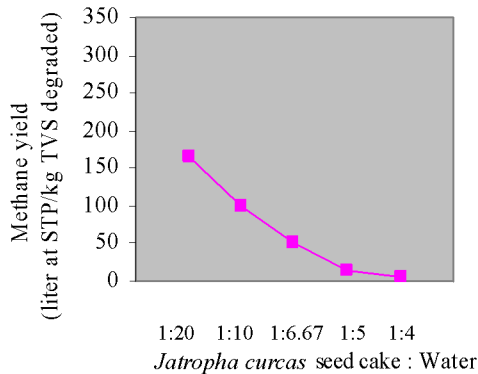


Fig. 10. Methane produced per kilogram of TVS degraded during 60 day operation in reactors that contained *Jatropha curcas* seed cake of specified dilutions.

Compared with the yield of 156 liter/kg seed cake with batch mode in this study, CH₄ yield from banana stem waste [21] and cotton oil cake [23] were 196 and 78 liter/kg of total solid fed to the system, respectively. Singh et al. [13] studied biogas production from *Jatropha curcas* seed cake enriched with digested cattle dung and operated in a semi-continuous mode by two daily feedings; the CH₄ yield obtained from 10% total solid (TS) of *Jatropha curcas* seed cake was higher than that obtained from 15% total solids. From their study, total biogas production recorded was 333 liter/kg seed cake from

the reactors having seed cake at 10% TS, whereas, it was 287 liter/kg seed cake from the reactors having seed cake at 15% TS. Methane in biogas was 66 %.

The reactor performance with *Jatropha curcas* seed cake of specified dilutions is summarized in Table 2.

Table 2. Reactor performance with batch operation from *Jatropha curcas* seed cake of specified dilutions.

Parameters	<i>Jatropha Curcas</i> seed cake: Water				
	1:20	1:10	1:6.7	1:5	1:4
Reactor volume, l	5	5	5	5	5
Initial pH	5.5	5.5	5.5	5.5	5.5
Initial COD, g/l	48.5	100	156	211	223
Final COD, g/l	23.2	49.3	71.7	141	144
% COD removal	52	51	54	33	35
Initial TVS, g/l	55.2	110.4	165.6	200.8	276
Final TVS, g/l	10	23	36.6	74.2	123.9
% TVS removal	82	79	78	63	55
Total gas in 60 days, l	66	85	76	50	46
CH ₄ in 60 days, l	37	43	33	8.4	4
Max. rate of CH ₄ production, l at STP/l-d	7.0	7.5	5.9	1.5	0.85
CH ₄ yield, ml at STP/g COD degraded	296	172	79	24	10
CH ₄ yield, ml at STP/g TVS degraded	164	100	52	13	5.4

This study could contribute to the energy supply of emerging economies in general and remote rural areas in particular. When properly managed, handled and processed, the production of bioenergy may contribute to a decrease in greenhouse gas accumulation in the atmosphere. In many developing countries, high population growth rates, low economic growth, and environmental issues such as deforestation, atmospheric pollution, and water depletion cause serious problems. The provision of adequate and decentralized produced energy is crucial for overall development prospects. A number of options for moving towards sustainable development have been put forward, including biogas. Alternative energy from biogas may prove to be an important fuel in the future.

4 CONCLUSIONS

The results revealed that *Jatropha curcas* seed cake is a good source of biogas production. The appropriate solid content of seed cake for biogas production from a batch process should be a ratio in the range between 1:20 to 1:10 in order to promote rapid CH₄ production and to obtain high CH₄ yield. The highest CH₄ yield was observed from the reactor that contained a slurry of *Jatropha curcas* seed cake at the dilution of 1:20. When operating at this ratio, the CH₄ production was approximately 156 liter at STP/ kg of *Jatropha curcas* seed cake added to the reactor, which indicated that 6.4 kg of *Jatropha curcas* seed cake is needed to produce 1 m³ of pure CH₄. Nearly similar CH₄ volumes were produced at dilutions of 1:10 at much higher solids content, thus the transformation of *Jatropha curcas* seed cake to CH₄ was most efficient at the highest dilution tested.

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REFERENCES

- [1] J. Raynal, J.P. Delgenes, R. Moletta, "Two-phase anaerobic digestion of solid waste by a multiple liquefaction reactors process" *Bioresource Technology*, 65:97-103,1988.
- [2] B. Goel, D.C. Pant, V. Kishore, "Two-phase anaerobic digestion of spent tea leaves for biogas and manure generation", *Bioresource Technology*, 80:153-156, 2001.
- [3] B. K. Ahring, "Perspectives for anaerobic digestion", *Advances in Biochemistry Engineering/Biotechnology*, Springer-Verlag Berlin Heidelberg, 81:1-30, 2003.
- [4] M. Corral, Z. Samani, A. Hanson, G. Smith, P. Funk, H. Yu, J. Longworth "Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure", *Bioresource Technology*, 99:8288-8293, 2008.
- [5] S. Leao, I. Bishop, D Evans, "Spatial-temporal model for demand and allocation of waste landfills in growing urban regions", *Computers, Environment and Urban Systems*, 28: 353-385, 2004.
- [6] B. Schmook, P.L. Seralta, "J. curcas: distribution and uses in the Yucatan Peninsula of Mexico", In Gubitz G.M., Mittelbach M., & Trabi M. eds., *Biofuels and industrial products from Jatropha curcas*, pp. 53-57, DBV Graz.,1997.
- [7] J. Heller, "Physic Nut. *Jatropha curcas* L. Promoting the Conservation and use of Underutilized and Neglected Crops. I". Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, Rome. 1996.
- [8] M.J. Herrera, P. Siddhuraju, G. Francis, G. Davila-Ortiz, K. Becker, "Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico" *Food Chemistry*, 96:80-89, 2006.
- [9] W. M.J Achtena, L.Verchothb, Y.J. Frankenc, E. Mathijdsd, V.P. Singhe, R. Aertsa, B. Muysa, "Jatropha bio-diesel production and use", *Biomass and Bioenergy*, 32:1063-1084, 2008.
- [10] G.M. Gubitz, M. Mittelbach, M. Trabi, "Exploitation of the tropical oil seed plant *Jatropha curcas* L." *Bioresource Technology* 67:73-82, 1999.
- [11] N. Mahanta, A. Gupta, S.K. Khare, "Production of protease and lipase by solvent tolerant *Pseudomonas aeruginosa* PseA in solid-state fermentation using *Jatropha curcas* seed cake as substrate" *Bioresource Technology* 99:1729-1735, 2008.
- [12] G.M. Gubitz, M. Mittelbach, M. Trabi, "Biofuels and industrial production from *Jatropha curcas*", *Jatropha 97 Symposium*. 23-27 February 1997. Managua, Nicaragua, Published by Dbv-Verlag für die Technische Universität Graz, Graz, Austria, 263 pp., 1997.
- [13] R.N. Singh, D.K. Vyas, N.S.L. Srivastava, M. Narra. "SPRERI experience on holistic approach to utilize all parts of *Jatropha curcas* fruit for energy", *Renewable Energy* 33:1868-1873, 2008.
- [14] N. Sinbuathong, B. Sillapacharoenkul, R. Khun-Anake, D. Watts "Optimum organic loading rate for semi-continuous operation of an anaerobic process for biogas production from *Jatropha curcas* seed cake", *International Journal of Global Warming*, 2:179-188, 2010.
- [15] G. V. Nallathambi. "Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from *Jatropha curcas* on dry lands". *Biomass and bioenergy*, 33:589-596, 2009.
- [16] P. Buurman, B.V. Lagen, E.J. Velthorst, Manual for Soil and Water Analysis. Backhuys Publishers, Leiden. pp.14, 1996.
- [17] A. Walkley, "A critical examination of rapid method for determination organic carbon in soils - effect of variations in digestions conditions and of inorganic soil constituents" *Soil Science* 63: 251-264, 1947.
- [18] Soil Science Society of America, Inc. American Society of Agronomy, Inc. "Soil testing and Plant Analysis. No. 3. Methods of soil Analysis : Chemical Methods. Part 3", Madison, Wisconsin, USA . pp. 1001, D.L. Sparks editor Bartels J.M. Managing editor 1996.
- [19] G.E. Rayment, F.R. Higginson, "Australian Laboratory Handbook of soil and water chemical methods" Inkata Press Melbourne Sydney. pp 71, 1992.
- [20] APHA, AWWA, WEF. "Standard methods for the examination of water and wastewater" 18th ed. USA.,1992.
- [21] V.C. Kalia, V. Snonakya, N. Raizada " Anaerobic digestion of banana stem waste", *Bioresource Technology* 73:191-193, 2000.
- [22] G. Tchobanoglous, F. L. Burton, " Wastewater Engineering Treatment, Disposal, and Reuse", Revised from Metcalf & Eddy Inc. 3rd ed. McGraw Hill Inc., Singapore, 1991.
- [23] A. Isci, G.N. Demirer "Biogas production potential from cotton wastes", *Renewable Energy* 32:750-757, 2007.