

Effect of temperature and the Carbon-Nitrogen (C/N) ratio on methane production through anaerobic co-digestion of cattle manure and *Jatropha* seed cake

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Abstract. Anaerobic digestion is a method of agricultural residue transformation used in bioenergy, making these activities energy efficient. However, it can be limited on a larger scale by the availability and diversity of organic residues related to carbon-nitrogen (C/N). Therefore, the anaerobic co-digestion of bovine manure and *Jatropha* seed cake (*Jatropha curcas*) were evaluated, with three different C/N ratios, under two conditions: mesophilic (30°C) and thermophilic (50°C). Biodigesters were mounted with three replicates for each C/N ratio. The highest production of CH₄ was registered after 30 days of processing in the thermophilic condition (C/N 25:1) with 633.95 ± 5.59 mL of CH₄ g⁻¹ VS. In contrast, the lowest production was in the mesophilic condition (C/N 20:1) with 208.66 ± 2.61 mL of CH₄ g⁻¹ VS. The feasibility of co-digestion of agricultural residues in the production of CH₄ as a possible bioenergetic alternative in short periods was demonstrated.

Keywords. Anaerobic digestion, biogas production, agricultural residue, cattle raising residue, biomass use/energy

1. Introduction

The global economy aims to displace fossil fuels to search for renewable energy sources, such as biomass, for clean natural production. Many developing countries have fossil-derived fuels as a primary economic source, which has resulted in overexploitation contributing to the decadence of the environment. This high energy demand warrants a search for alternate and renewable energy sources. According to Sen, Mahalingam, and Sen [1], biogas energy derived from renewable sources can

supplement conventional energy sources while decreasing the import of fossil fuels.

In 2012, Ecuador had an adequate installed power of 4862.4 MW, of which 48.54% were from a renewable source. To sort out the issue applicability of organic residues to obtain a new energy source is the best option to reduce the water bodies pollution due to aggressive oil exploitation. Biogas technology provides an alternative energy source to fossil fuels in many parts of the world by using local resources such as agricultural residues, industrial waste, municipal solid waste, market waste, and animal waste [2]. The vast amounts of waste generated by these activities can be transformed into something useful for rural communities: the new energy carrier: the biogas. According to Pan et al. [3], potential environmental impacts can be eliminated through deploying anaerobic co-digestion of agricultural wastes, thereby obtaining sustainable production and consumption. By recognizing the benefits and disadvantages of using anaerobic co-digestion from various perspectives, the agricultural organic waste can be treated appropriately, and the valuable contents in the organic waste can be recovered or regenerated [4]. Soria & Carvajal [5] confirmed that small biodigesters had been installed primarily in rural zones to avoid sudden blackouts. The agricultural sector provides the source for obtaining fuel with significant potential: organic matter.

Anaerobic digestion is an effective way to transform organic matter as agricultural and livestock waste into biogas, obtaining a bioenergy source, which would make these activities energy efficient. In underdeveloped countries, their economy is based on raw materials: livestock, agriculture, and aquaculture. According to Guarino et al. [6], this anaerobic conversion incorporates

several interdependent, complex sequential, and parallel biological reactions during which the products obtained from one group of microorganisms serve as the substrates for the next, resulting in a transformation of organic matter mainly into a gas mixture of CH₄ and CO₂ with lower concentrations of N₂, H₂, NH₃, and H₂S.

However, the efficiency of this process can be limited on a large scale by the low availability and diversity of organic waste and inadequate carbon-nitrogen (C/N) ratios. Understanding the feedstock properties is very important, as they influence the performance of AD. The feedstock properties directly affect the start-up process, AD process stability, and biogas yield [4]. Rural zones have an unused amount of cattle manure which, as reported by Ministerio del Ambiente, Agua y Transición Ecológica [2], contains the primary source of nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, zinc, copper, and boron, the quality of which depends on the diet received by the animals and on the destination given. In fact, according to Seppälä et al. [8] and Guarino et al. [6], the wide variability of the C/N ratio of manures collected from the very same farm can be observed in a certain period due to different feeding intensities, forage composition, and the hormonal phase of the bovine. In addition, El-Mashad & Zhang [9] mention that when this C/N ratio is too broad, carbon cannot adequately be converted to CH₄, causing not fully achieved production potential. Therefore, co-digestion of substrates with a narrower C/N ratio could help overcome this disadvantage.

The crop of *Jatropha curcas* has fast growth, resistance to drought, easy propagation, and high oil content [10]. Nonetheless, much hull waste is generated in *Jatropha* seed oil extraction. Its direct incorporation into the soil is considered inefficient in providing value due to its unfavorable physicochemical characteristics (high pH, EC, and phenolic content) [11]. Despite that, the crude oil obtained by mechanical extraction and refining of the seed complies with most of the parameters established by the German standard DIN 51605 that oils must meet for their use as raw material to obtain biodiesel [12].

Accordingly, pine nut residue (*Jatropha curcas*) has been recently used as an alternative to producing biofuels because of the hard shell that presents around 70% of the total weight of the grain [8]. This seedcake is made by removing the oil from the seed chemically or mechanically. The *Jatropha* pressed cake contains toxic materials, which disqualifies it from animal feed or natural fertilizer [13].

For example, project K007 in Galapagos Island ensures the sustainability of the diesel replacement used for electricity generation on Floreana Island with vegetable oil from the agro-industrial development of the 12,700 km of *J. curcas* existing as live fences in the Manabí province [14].

This innovative technological project has already implemented a hybrid system that integrates: dual thermal generators, photovoltaic panels, and an energy storage system (batteries). Among the agro-industrial achievements is the structuring of the *Jatropha* value chain, the improvement of life quality, the establishment of two cooperatives of popular and solidary economy, the

implementation of 2016's agricultural expansion plan, and training in field schools [15].

The co-digestion of different matrixes: agroindustrial and urban wastewater, sludge from domestic water treatment plants, animal excrements (cattle, pigs, chickens, horses), plant biomass, algae, and microalgae, has been evaluated with mixed results. Thus Álvarez et al. [16] performed the co-digestion of pre-treated cyanobacterial biomass (*Arthrospira platensis*) and raw cattle manure in a mesophilic regime (30°C) and with a C: N ratio of 19: 1, they reached a methane yield of 482.54 ± 8.27 mL of CH₄ g⁻¹ volatile solid (VS).

Kethobile et al. [15] used the torrefaction method to transform solid products into thermal energy. The treated *Jatropha* seed cake had the highest carbon and hydrogen content and the lowest oxygen level at evaluated torrefaction temperature levels. Still, at 250°C, it showed the potential to be a solid fuel source. These results determine the feasibility of its implementation in future evaluations.

The main objective of this research was to evaluate the viability of the energy conversion of the mixture of cattle manure and *Jatropha* seed cake by anaerobic co-digestion. In mesophilic, thermophilic regimes with three different carbon-nitrogen ratios (20: 1, 25: 1, 30:1). This research was suitable for producing biofuel in anaerobic digesters to low-cost substrates rich in organic carbon, such as *Jatropha* seed cake.

2. Materials and Methods

2.1 *Jatropha* seed cake obtaining

To obtain the *Jatropha* seed cake, the mechanical extraction of the crude oil was carried out by hydraulic pressing of the seeds, which had previously been debarked, with a hydraulic press of 6.9 x 10⁴ kPa. The principle of this type of extraction is based on the application of pressure (mechanical stress) on a particular area in the seed, breaking the cell structure due to the presence of forces that eliminate the interstitial spaces between the seed structures, as a result of the mechanical effect occurs the extraction of oil [18]. The performance of the yield of the oil extracted from the seed exposed to pressure exerted by the piston was analyzed, obtaining 16.05 ± 2.26 g oil 100 g⁻¹ seed. The *Jatropha* seed cake was collected and piled in plastic bags, which were contained at 4°C for 15 hours approximately. The sample residue was ground in a food grinder and dried in a force ventilation oven at 60°C. The dry mass was then mixed, and small portions were taken to determine Volatile solid (VS), total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP); the rest was conditioned in the plastic bag and kept under conservation (4°C) until the beginning of the incubation.

2.2 *Cattle manure collection and characterization*

The raw cattle manure was collected at the El Algarrobo farm in the Engabao commune (2°36'37'' S; 80°26'12'' W) of the General Villamil Playas canton of Guayas province, Ecuador, during February, March, and April 2020 (rainy season), and immediately transferred to the Laboratorio de Biotecnología Microbiana (Lab-Biotem

S.A.) Guayaquil, Guayas, Ecuador. Raw cattle manure was sieved (5mm) to separate large particles, weighed, and then determined the content of VS, TP, TN, and TOC.

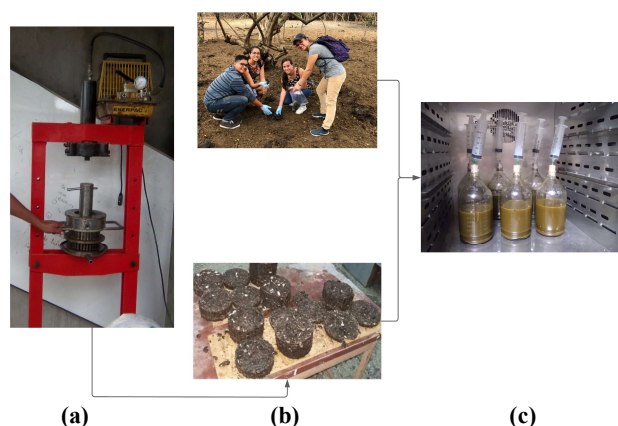


Figure 1. Flow diagram of obtaining the *Jatropha* seed cake by mechanical extraction and its anaerobic co-digestion: (a) Hydraulic pressing; (b) Collect of *Jatropha* seed cake and raw cattle manure; (c) Anaerobic co-digestion of *Jatropha* seed cake and raw cattle manure in a mesophilic and thermophilic regime (30-50°C).

2.3 Experimental Anaerobic Digestion setup

Anaerobic co-digestion was performed in batch mode biodigesters, following the Biochemical Methane Potential (BMP) methodology to evaluate the potential methane yield in *Jatropha* seed cake and cattle manure. The biodigesters consisted of 1000 mL glass bottles; each bottle contained a final concentration of substrates *Jatropha* seed cake (JSC) + cattle manure (CM) 7,7 g VS L⁻¹. The anaerobic digestion experiment was set up as a randomized design, with three replicates and treatments corresponding to 20:1, 25:1, and 30:1 C/N ratios performed in thermophilic (50°C) mesophilic (30°C) conditions, resulting in nine experimentations for each thermal state.

Finally, the biodigesters were filled up to 700 mL with distilled water, and nitrogen gas was purged for five minutes to maintain the anaerobic condition in the biodigester. Then the biodigester was sealed and incubated at 30°C and 50°C separately. The methane concentration was monitored and measured daily for 40 days. Similarly, the anaerobic digestion conditions described above of CM were performed independently.

Table 1. Matrix of the Experimental design used in the co-digestion of *Jatropha* seed cake and Cattle manure.

Operation mode	JSC %	CM %	C/N	g VS L ⁻¹
Batch	70	30	20:1	7.7
Batch	65	35	25:1	7.7
Batch	50	50	30:1	7.7

JSC: *Jatropha* seed cake, CM: cattle manure, C/N: Carbon-Nitrogen ratio, VS: volatile solids.

2.4 Analytical methods

The TOC contained in the JSC and CM residue was analyzed by the chromic acid oxidation method proposed by Degtjareff [19] and modified by Walkey and Black [20], respectively. The TN and TP were measured with a persulfate digestion method designed by D'Elia et al.

[21] and the vanadate-molybdate methods [22], respectively, with a Water Testing and Color Measurement Photometer MD 600 (Lovibond®, Dortmund, North Rhine-Westphalia, Germany). VS was determined according to Standard Methods 2540 G. Methane yield was determined with a Digital Handheld Gas Leakage Detector model 7889 (E-instruments® Langhorne, PA, USA). All analytics were done in triplicate.

2.5 Theoretical energy potential

The estimation of the energy potential of each treatment was determined, assuming that 1m³ of methane has a calorific value of 36 MJ. Therefore, 1m³ of CH₄ will produce 10 kWh, considering an electrical conversion efficiency of 35% [23].

2.6 Statistical Analyses

The graphs and statistical analyses were performed with the GraphPad PRISM version 9.2.0 (GraphPad Software, Inc.®, San Diego, CA, USA). The results were analyzed using the variance (ANOVA double and one-way) parametric test and the Tukey test post hoc with a significance level of $p \leq 0.05$.

3. Results

3.1 Content of VS, TN, TP, TOC, Mesophilic, and Thermophilic Co-Digestion (JSC and CM)

The content of VS, TN, TP, and TOC of the JSC and CM substrates submitted to anaerobic co-digestion was, for JSC: 68.98 ± 3.03% for the VS, 39.91 ± 6.26 mg g⁻¹, 18.44 ± 2.81 mg g⁻¹, and 422.88 ± 22.48 mg g⁻¹ for the TN, TP, and TOC, respectively. For CM, the VS was 70.24 ± 8.71%, and 5.67 ± 1.23 mg g⁻¹, 1.77 ± 0.24 mg g⁻¹, and 273.99 ± 15.22 mg g⁻¹ for the TN, TP, and TOC, respectively (Table 2).

After 28 days of processing, the highest CH₄ production rate in the biodigesters submitted to mesophilic conditions (30°C) occurred in the 30:1 C/N ratio obtained 425.45 ± 17.78 mL CH₄ g⁻¹ VS. In contrast, the lowest production was observed in the 20:1 C/N ratio with 208.66 ± 2.61 mL CH₄ g⁻¹ VS. The methane yield in the 25:1 C/N ratio was 412.54 ± 18.99 mL CH₄ g⁻¹ VS. Thermophilic conditions (50°C) presented the highest production in 25:1 C/N ratio with 633.95 ± 5.59 mL CH₄ g⁻¹ VS. The lowest output for the 30:1 C/N ratio with 524.60 ± 12.41 mL CH₄ g⁻¹ VS. The methane yield resulted from the 20:1 C/N ratio was 570.70 ± 12.78 mL CH₄ g⁻¹ VS.

Table 2. Composition of the substrates used in the anaerobic co-digestion under two thermal regimes (30, 50°C).

Component	<i>Jatropha</i> seed cake	Cattle manure
TOC (mg g ⁻¹)	422.88 ± 22.48	273.99 ± 15.22
TN (mg g ⁻¹)	39.91 ± 6.26	5.67 ± 1.23
TP (mg g ⁻¹)	18.44 ± 2.81	1.77 ± 0.24
VS (%)	68.98 ± 3.03	70.24 ± 8.71
C/N	10.60	48.32

TOC: total organic carbon, TN: total nitrogen, TP: total phosphorus, VS: volatile solid, C/N: carbon-nitrogen ratio

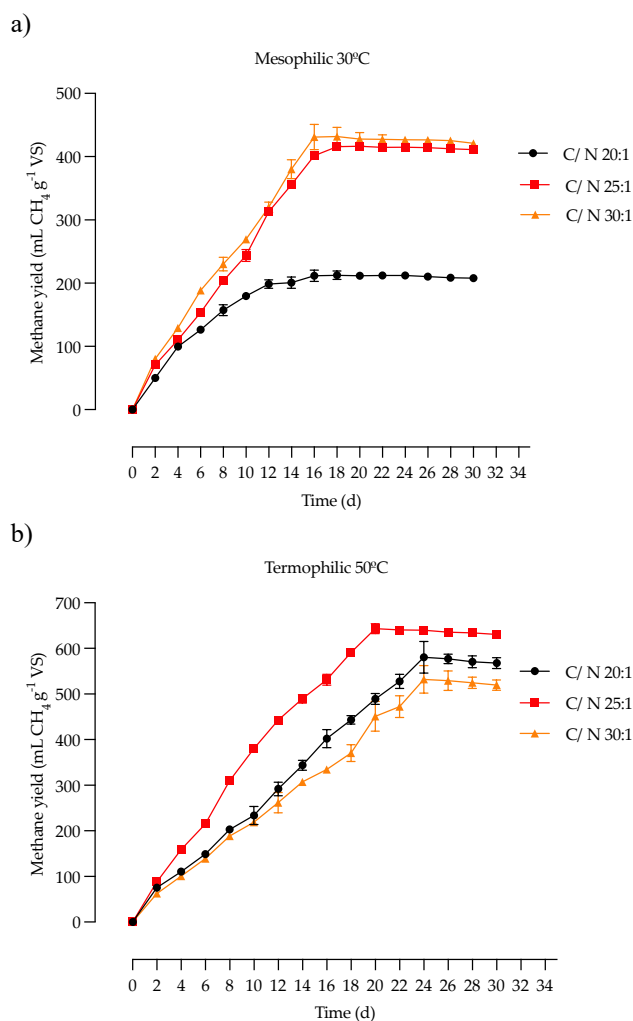


Figure 2. Methane yields were obtained in three ratios C: N (20:1, 25:1, 30:1) in the co-digestion of *Jatropha* seed cake and Cattle manure, under a) mesophilic regimen of 30°C and b) thermophilic regime of 50°C.

Table 3. Maximum theoretical production and electrical potential of the methane yield obtained in the co-digestion of *Jatropha* seed cake and Cattle manure under 30°C and 50°C.

	Ratio C:N	Maximum production (mL CH ₄ g ⁻¹ VS)	Theoretical production (m ³ kg ⁻¹ VS)	Calorific value (MJ kg ⁻¹ VS)	Electricity Potential (kWh kg ⁻¹ VS)
Mesophilic regime of 30°C	20:1	208.7	0.21	7.51	2.1
	25:1	412.6	0.41	14.86	4.1
	30:1	425.5	0.43	15.32	4.3
Thermophilic regime of 50°C	20:1	570.7	0.57	20.44	5.7
	25:1	633.9	0.63	22.69	6.3
	30:1	524.6	0.52	18.71	5.2

4. Discussion

The *Jatropha* seed cake (JSC) has an interesting biochemical and structural composition (proteins between 50-60%, availability of macro-micro nutrients), making it an ideal substrate for anaerobic digestion processes.

The thermophilic condition is an essential factor in co-digestion systems because it allows greater availability of nutrients and accelerates microbial metabolism.

Ewunie, Morken & Yigezu [24] showed that in the co-digestion of JSC and crude glycerol by applying thermal Pretreatment processes and operating the fermenters in a mesophilic regime of 37°C, the methane yield increases by 40% to 353.90 mL g⁻¹ VS compared to control; however, in the present investigation, the maximum methane yields were reached in the thermophilic regime (50°C) with 64% more CH₄ production than the mesophilic regime (30°C). It should be noted that the values mentioned show favorable results with the use of cattle manure as a co-substrate for cultivation compared to other raw materials, with values of up to 60% more CH₄ production.

Other factors, such as substrate type, concentration, pH, temperature, stirring, and bacteria seeding, can influence biogas generation. CO₂ and other gases reduce the biogas quality of its applications. Therefore, the gas must be treated before applications such as transportation fuels or natural gas network injection. Secondary gases are removed by physical or chemical washing and membrane separation. The choice of purification technology depends on the composition and quality of the gas and the available resources [25].

On the other hand, the theoretical production of methane evidences the theoretical capacity of CH₄ generation of the substrates as a potential value that can be transformed into energetic elements. Suhartini, Lestari & Nurika [26] show that food residues and solid residues/waste tofu are substrates that achieve a theoretical yield of 0,356 y 0,347 m³ kg⁻¹ VS, with 100% and 50:50 proportions, respectively. In the present research, a maximum of 0,63 m³ kg⁻¹ VS when using 65% JSC and 35% CM represents 80% higher methane production per kilogram of volatile solids than the previously mentioned substrates.

In addition, the implementation of large-scale anaerobic co-digestion systems can lead to the goals of integrated waste management, addressing waste reduction, and utilization of renewable energy. The selection of microbial consortia in the improvement of the overall treatment efficiency and biogas yield, plus the optimization of the digestion performance, will increase value-added products [5], reduce environmental footprint, and supports local and national economies, including the reduction of operating costs of co-digestion mode as compared to mono-digested systems [27].

5. Conclusion

Jatropha seed cake and cattle manure are substrates with a high potential for transformation into energetic compounds such as methane as a viable alternative for biogas production in anaerobic co-digestion systems. Within this process, the thermophilic regime (50 C) and a 25:1 C: N ratio allow a higher yield of methane in *batch* digestion systems, which leads to an increase in electrical potential.

Implementing the biogas energy derived from renewable sources will reduce small spills in the diesel unloading operations, affecting the ecosystem. The

initiation of similar K007 projects broadcasts a different approach to traditional biofuels (ethanol and biodiesel) since it takes advantage of the living *Jatropha* fences in Manabí, which are traditionally used only as property separation boundaries and do not have any economic use.

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