

## **Continuous feeding strategy in the anaerobic digestion of rice husk. Evaluation of biomethane production**

**Elicia Zavala-Murillo, Karolayne Solorzano-Párraga,  
Ricardo J. Baquerizo-Crespo, Yunet Gómez-Salcedo\***

Departamento de Procesos Químicos, Alimentos y Biotecnología, Facultad de Ciencias Matemáticas, Físicas y Químicas, Universidad Técnica de Manabí. Avenida Urbina y Che Guevara, Portoviejo 130105, Ecuador.

(\*) [yunet.gomez@utm.edu.ec](mailto:yunet.gomez@utm.edu.ec)

### **Resumen**

**Estrategia de alimentación continua en la digestión anaerobia de cascarilla de arroz. Evaluación de la producción de biometano.** El presente trabajo evalúa el proceso de digestión anaerobia de cascarilla de arroz mediante alimentaciones consecutivas como alternativa a la operación continua. Se analizó el rendimiento de metano y la cinética del proceso, a una relación inoculum/sustrato (RIS) = 2 y 4, para el residual pretratado. El mayor rendimiento fue  $61,66 \pm 1,44$  NmLCH<sub>4</sub>/gSV, con una RIS = 4. A estas condiciones, posteriormente se realizaron alimentaciones consecutivas. Con esta estrategia el rendimiento aumentó progresivamente hasta 175,58 NmLCH<sub>4</sub>/gSV. Esta estrategia podría ser un paso intermedio entre el proceso discontinuo y continuo, evitando los desafíos de operación y estabilidad de los procesos continuos para este tipo de residuos.

**Palabras claves:** pretratamiento alcalino; bioresiduos; lignina; pretratamiento mecánico; residuos sólidos

### **Abstract**

The present work evaluates the process of anaerobic digestion of rice husks through consecutive feedings as an alternative to continuous operation. The methane yield and the kinetics of the process were analyzed, at a inoculum/substrate ratio (ISR) = 2 and 4, for the pretreated residual. The highest methane yield was 61.66 NmLCH<sub>4</sub>/gVS, with ISR = 4. Thus, consecutive feedings were developed under these conditions. With this approach, methane yield increased progressively until 175.58 NmLCH<sub>4</sub>/gVS. This strategy could be an intermediate step between the discontinuous and continuous process, avoiding the operation and stability challenges of the continuous ones for this type of waste.

**Keywords:** Alkaline pretreatment; Biowaste; Lignin; Mechanical pretreatment; Solid waste

### **Introduction**

Rice is a crop native to the wetter regions of tropical and subtropical Asia. Currently, worldwide rice production exceeds 7.4 million mt, being the second most important cereal in the world concerning its cultivated area, only surpassed by corn<sup>1,2</sup>. In 2020, Ecuadorian rice with shell reached a yield of 5.02 (t/ha), a production of 1546523 tm<sup>3</sup>, and 80.71% of the total rice production in Ecuador coming from the coastal region<sup>4</sup>, with more than 64 million mt of residues<sup>5</sup>. One of the most abundant residues is the husk, a lignocellulosic plant tissue that covers the rice grain<sup>6</sup>. Burning and landfills are the most common practices for the final disposal of rice residue<sup>7</sup>. Even though these practices are low costs for producers, these alternatives cause environmental problems and waste the nutrients in the residues.

The alternatives for the use of rice husk are diverse and include the production of energy and bio-silica<sup>8</sup> and the use as adsorbent material in processes of removal of contaminants<sup>9</sup>. Regarding energy production, anaerobic digestion (AD) is a simple treatment with biogas yields of 0.044 m<sup>3</sup> kg/Vs and

methane of 0.019 m<sup>3</sup> kg/Vs for rice husk in mesophilic conditions (37 °C)<sup>10</sup>. Methane yields are low because cellulose, hemicellulose, and lignin (more than 63.9%<sub>TS</sub>)<sup>11</sup>, compounds with complex molecular structures, which form a tight protective film that hinders the bioavailability of the residual nutrients and constitute a challenge for the implementation of anaerobic digestion of these in continuous operation<sup>12,13</sup>.

The implementation of technologies for AD requires the evaluation of operating conditions and regimes. Biochemical methane potential (BMP) assays are the first link to evaluate AD through the study of the anaerobic biodegradability of a substrate or mixture of co-substrates, methane yield, and analysis of the kinetics of the process<sup>14</sup>. The determination of the stability of the process and organic load rate achievable in a continuous operation system requires another type of experimentation characterized by long periods. Consecutive feeding is an alternative method to predict the expected performance of a process, with results like the operation of semicontinuous reactors<sup>15</sup>. The operation of reactors in consecutive feeds could constitute an intermediate step between

the batch process and continuous operation, avoiding the design, operational, and stability challenges of continuous processes<sup>16</sup>. Thus, the present work evaluates the process of anaerobic digestion of rice husk in the long term through consecutive feedings as an alternative mode of operation.

## Materials and Methods

### Substrate

The rice husk (RH) came from the Santa Mónica mill, Rocafuerte city, Manabí province. The characterization of RH was in terms of total solids (TS), volatile solids (VS)<sup>17</sup>, and elemental composition (carbon, hydrogen, sulfur, oxygen, and nitrogen)<sup>18</sup>. The average particle size used granulometric analysis by sieving the sample and determination of the size distribution. The calculation of the particle diameter considered a surface equal to the mean of the set of particles ( $D_{pm}$ ), estimated as (1)<sup>19</sup>:

$$D_{pm} = \frac{1}{\sum \frac{\Delta X_i}{D_{p_i}}} \quad (1)$$

Where:  $\Delta X_i$  is the remaining mass fraction in the sieve corresponding to that size interval concerning the total amount of sieved RH, and  $D_{p_i}$  is the arithmetic mean particle diameter [mm] in each fraction, according to the minimum and maximum sizes of the sieve analyzed.

### Rice husk pretreatment

RH pretreatments were mechanical ( $RH_M$ ) and mechanical-alkaline ( $RH_{PT_M}$ ). The mechanical pretreatment used a 2800 W electric mill for 2 minutes to obtain ground RH with a particle size of 0.15 mm<sup>13</sup>. The alkaline pretreatment used a solution of sodium hydroxide 3% (on a dry basis) in contact with the biomass for 60 minutes and a temperature of 120 °C<sup>20</sup>. The  $RH_{PT_M}$  samples entered an oven at 120 °C for 6 hours to remove moisture. The evaluation of the pretreatments used the contents of lignin<sup>21</sup> and cellulose<sup>22</sup> in RH. Another required parameter was the hemicellulose content from the holocellulose<sup>23</sup> estimated as (2)-(3):

$$\% = \frac{\text{dry residue weight [g]}}{\text{original sample weight free of extracts [g]}} * 100 \quad (2)$$

$$\% \text{ hemicellulose} = \text{holocellulose} - \text{cellulose} \quad (3)$$

### Inoculum

The inoculum was a mixture of bovine manure and water in equal parts, filtered to eliminate coarse solids that remained at room temperature, under anaerobic conditions, and degassed for 30 days. The characterization of the inoculum was in TS and VS<sup>17</sup>.

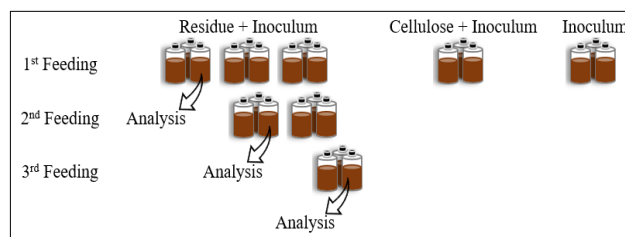
### Biochemical methane potential assays

The biomethanation tests (BPM) were performed in triplicates in 250 mL bottles, with a working volume of 150 mL at a temperature of 35±0.5 °C. The methane measurement method was

liquid displacement using a 15% NaOH solution<sup>24</sup>. The agitation of the reactors was manual before measuring the volume of methane produced<sup>25</sup>. The assay included positive control reactors (with cellulose as substrate) to evaluate the inoculum performance and blank reactors (only with inoculum) to neglect the endogenous methane production. The normalization of the methane yield used standard temperature and pressure (273 K and 1 atm)<sup>24</sup>. The preliminary study determined the influence of the inoculum/substrate ratio (ISR) (2:1 and 4:1) and particle size (RH and  $RH_M$ ) on the biomethanation potential of the residual. Additionally, the study included one trial with the best result of ISR and the combination of pretreatments ( $RH_{PT_M}$ ). The analysis of response variable results was by analysis with a confidence level of 95.0%.

### Consecutive feedings

The consecutive feed test used nine reactors (Figure 1) fed with the same ISR. In addition, there were blank and positive control reactors.



**Fig. 1:** Experimental design of the consecutive feedings assay.

The second and third feedings began when daily methane production was < 1% of cumulative volume for three consecutive days<sup>25</sup>. In addition, the ratio of volatile fatty acids [mg Ac. Acetic/L]/total inorganic carbonate [mg CaCO<sub>3</sub>/L] (FOS/TAC) was a monitored variable in the analyzed reactors<sup>26</sup>, as well as the solid content and pH.

### Kinetic assessment

Kinetic models show that dynamics of methane production are influenced by a) the growth rate of microorganisms, b) the relationship between microbial growth rate, and substrate utilization c) the effect of substrate on microorganisms. The modified Gompertz (Eq. 4) and Chapman models (Eq. 5) were used to estimate the methane production rate, the lag phase, and the methane production potential<sup>27</sup>.

$$B = B_o * \exp \left( -\exp \left[ \frac{u_m e}{B_o} (\lambda - t) + 1 \right] \right) \quad (4)$$

$$B = B_o [1 - \exp(-bt)]^c \quad (5)$$

Where: B is the cumulative methane production over time (m<sup>3</sup>/kg SV), B<sub>o</sub> is the maximum cumulative methane production (m<sup>3</sup>/kg SV), u<sub>m</sub> is the maximum methane production rate (m<sup>3</sup>/kg SV\*d), λ is the adaptation time of the inoculum to the substrate (d), t is the incubation time (d), and b and c are model parameters. The statistical criteria for model adjustment were the coefficient of determination (R<sup>2</sup>) and the root means square error (RMSE).

## Results

### Characterization of the inoculum, RH, RH<sub>M</sub>, and RH<sub>PT-M</sub>

Table 1 reports the characterization of the RH and the inoculum used in the first experimental run. The variety of rice influences the composition of the husk. However, results from other authors corroborate that the residual has a low nitrogen content (0.82 - 0.45%) compared to the high carbon content (34.05 - 31.95%)<sup>25</sup>.

**Table 1.** RH and inoculum characterization.

Parameter	RH	Inoculum
TS [%]	92.15 ± 0.06	2.94 ± 0.18
VS [%TS]	78.02 ± 0.14	66.62 ± 0.35
N [%TS]	0.32 ± 0.01	-
C [%TS]	36.30 ± 0.20	-
H [%TS]	6.18 ± 0.03	-
S [%TS]	0.16 ± 0.00	-
O [%TS]	34.26 ± 0.24	-
C/N ratio	113.43 ± 3.36	-
C/H ratio	5.87 ± 0.01	-

The results correspond to a residual with low humidity and high VS content and are consistent with the literature (TS= 89.41%; VS= 74.01%)<sup>26</sup>. Regarding the pretreatments (Table 2) RH<sub>M</sub> and RH<sub>PT-M</sub> affected the content of lignin, cellulose, and hemicellulose.

**Table 2.** Lignin, cellulose, and hemicellulose content of RH after pretreatments.

Pretreatment	Lignin [%TS]	Cellulose [%TS]	Hemicellulose [%TS]
RH	33.01 ± 3.83 <sup>b</sup>	66.25 ± 0.07 <sup>a</sup>	18.161 ± 0.40 <sup>b</sup>
RH <sub>M</sub>	26.91 ± 1.61 <sup>a</sup>	78.03 ± 2.23 <sup>c</sup>	5.04 ± 2.11 <sup>a</sup>
RH <sub>PT-M</sub>	21.27 ± 2.73 <sup>a</sup>	71.56 ± 2.28 <sup>b</sup>	3.706 ± 0.67 <sup>a</sup>

a, b, and c: denote significant differences between groups.

The lignin, cellulose, and hemicellulose content of RH differs from that reported in the literature, depending on the type of rice, harvest time, and production method, among other factors. The average particle size of RH was 0.21 mm, while RH<sub>M</sub> presented an average particle size of 0.17 mm. The pretreatments decreased the lignin content and increased the cellulose content due to the modification of the material structure and the increase in the surface area available for mass transfer. The efficiency of alkaline pretreatments is higher in lignin removal than in removing acetyl groups from hemicelluloses or cellulose solubilization<sup>28</sup>.

### Influence of ISR and particle size

Although the theoretical yield of the residual reaches 334 mLCH<sub>4</sub>/gVS<sup>29</sup> and the average reported in the literature is 50 mLCH<sub>4</sub>/gVS, the methane yield of the RH for both ISR was low (<35 mLCH<sub>4</sub>/gVS) (Table 3). The analysis of variance decomposed the variability of B<sub>0</sub> in contributions of the factors (pretreatment and ISR) with significant effects on methane yield (p-value<0.05).

**Table 3.** Kinetic models for cumulative methane production.

Kinetic model		ISR 2	ISR 2	ISR 4	ISR 4	ISR 4
		RH	RH <sub>M</sub>	RH	RH <sub>M</sub>	RH <sub>PT-M</sub>
Chapman	B <sub>0</sub>	22.21 ± 4.28	23.52 ± 0.10	34.94 ± 4.05	52.42 ± 4.08	69.03 ± 5.17
		0.15 ± 0.09	0.20 ± 0.09	0.12 ± 0.05	0.07 ± 0.05	0.06 ± 0.00
	20.09 ± 5.10	35.33 ± 4.34	13.53 ± 4.73	3.20 ± 1.39	1.24 ± 0.05	
	R <sup>2</sup>	0.97	0.98	0.97	0.96	0.98
	RMSE	0.88	0.77	1.34	2.47	2.62
Modified Gompertz	B <sub>0</sub>	20.92 ± 2.51	23.35 ± 0.11	33.19 ± 1.93	58.64 ± 10.21	61.66 ± 1.44
		1.24 ± 0.47	1.84 ± 0.70	1.74 ± 0.37	2.19 ± 0.46	2.86 ± 0.02
	9.81 ± 2.87	8.66 ± 4.49	9.57 ± 5.38	7.12 ± 1.08	0.16 ± 0.01	
	R <sup>2</sup>	0.97	0.98	0.97	0.96	0.98
	RMSE	0.87	0.74	1.36	2.44	2.78

The mechanical treatment decreased the lignin content and increased methane production by 50.02% in the assay with the highest ISR. Likewise, complementing the mechanical pretreatment with the alkaline pretreatment increased methane production by 97.56% due to the breakdown of the lignin protective layer around the hemicellulose and cellulose chains<sup>30</sup>.

The reduction in size releases cellular compounds and increases the available contact surface, increasing the bioavailability of the residual<sup>30</sup>. The application of mechanical pretreatment to rice straw residues (D<sub>p</sub>m = 20 mm), to obtain particles with sizes between 0.15- and 0.075 mm, increases methane production by up to 80%<sup>13</sup>. Likewise, the literature reports the advantages of alkaline pretreatment with 71% increases in biogas production<sup>31</sup>.

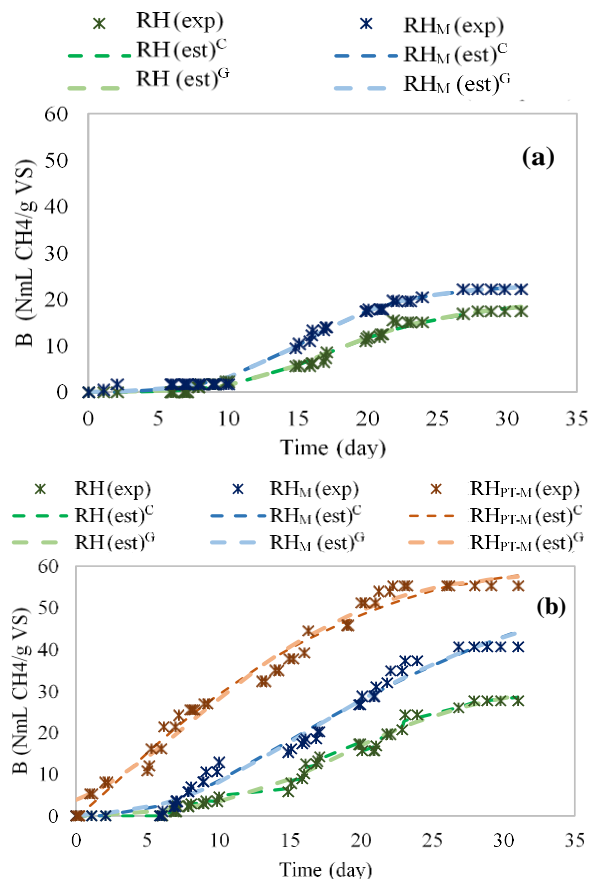
Regarding the ISR, this variable influences the kinetics of the process<sup>32</sup>. The ISR 4 increased the methane yield and the production speed, associated with an improvement in the biodegradability of the biomass (Figure 2). In addition, the anaerobic treatment of RH<sub>PT-M</sub> presented an inoculum adaptation time of 0.16 d, in contrast to the other variants, with times between 7.12 and 9.81 d.

### Consecutive feedings

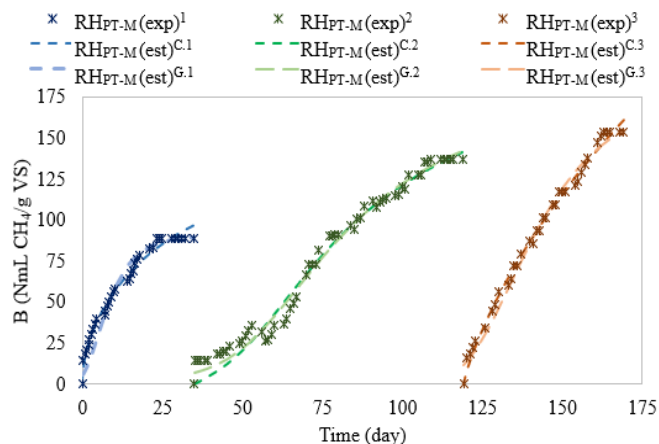
The possibility of treating a higher amount of matter in a smaller reactor is an advantage of the continuous mode of operation over the batch one. However, the methane yields observed in continuous digesters with rice crop residues are low and the recommended organic loading rates (OLR) are also low (up to 1 g<sub>VS</sub>/L day)<sup>33</sup>. Consecutive feeding is an approach between the batch and continuous operating modes. The consecutive feeding uses the feed for the batch mode set concerning ISR and is opposed to continuous feeding defined by the OLR, which indicates the flow of substrate fed per unit of reactor working volume.

The inoculum (VS=54.69 %TS, pH= 7.8) used in the assay was a blend of the inoculum used in the BMP (without AD) and the

liquid fraction obtained after the BMP. The results (Fig. 3) indicate a progressive increase in methane production. Each feeding considered the reset of the cumulative methane production curve.



**Fig. 2:** Cumulative methane production. (a)  $ISR=2$  y (b)  $ISR=4$ . (exp): experimental data (est)<sup>C</sup>: Chapman model; (est)<sup>G</sup>: Modified Gompertz model.



**Fig. 3.** Cumulative methane production in the consecutive feeding assay. (exp): experimental data; (est)<sup>C</sup>: Chapman model; (est)<sup>G</sup>: Modified Gompertz model; 1, 2 and 3 correspond to the first, second, and third feeding, respectively

The liquid fraction of the BMP digestate blended with fresh inoculum improved the degradation of  $RH_{PT-M}$  concerning the preliminary BMP assay, increasing 46.57 % the methane yield. The increase in methane production from the second

feed compared to the first was 78.41%, and the increase in the third feed compared to the second was lower (15.81%). The third feed increased methane production by 184% concerning BMP.

The Chapman and Gompertz models (Table 4) fit satisfactorily. The consecutive feeding test limited the stationary phase of methane production due to the decision criteria adopted to carry out each feeding, causing the Chapman model to overestimate the methane yield even though the fit was satisfactory ( $RMSE < 7.54$ ). The Chapman model requires an experimentation time that prolongs the stationary stage of methane production to improve the assessment of  $B_0$ . However, using both models was pertinent due to the reported kinetic parameters of interest.

**Table 4.** Methane production during the consecutive feeding assay.

Kinetic model	Time (d)			
	0 - 35	36 - 119	120 - 169	
Chapman	$B_0$	$140.15 \pm 4.56$	$167.22 \pm 8.71$	$248 \pm 5.53$
	B	$0.02 \pm 0.01$	$0.04 \pm 0.01$	$0.00 \pm 0.01$
	C	$0.58 \pm 1.73$	$3.80 \pm 2.42$	$0.80 \pm 0.01$
	$R^2$	0.97	0.97	0.99
	RMSE	3.94	7.54	4.05
Modified Gompertz	$B_0$	$90.38 \pm 11.54$	$161.25 \pm 10.92$	$175.58 \pm 10.58$
	$\mu_m$	$6.27 \pm 2.02$	$2.72 \pm 0.80$	$4.19 \pm 0.81$
	$\Lambda$	0.00	$8.48 \pm 6.72$	0.00
	$R^2$	0.92	0.98	0.98
	RMSE	7.10	6.48	5.14

The continuous regimen carries the risk of instability and inhibition due to the accumulation of intermediate compounds as volatile fatty acids (VFA) in the system. Monitoring control variables such as solids content and FOS/TAC allows for predicting issues in the stability of the process. Therefore, the assay considered the analysis of three reactors at the end of the processing time of each feeding (TS, VS, FOS/TAC, and pH) (Table 5).

**Table 5.** Physicochemical characterization of the reactor effluent.

Residual	TS (%)	VS (%TS)	pH	FOS/TAC
$RH_{PT-M}$ (1era)	$4.00 \pm 0.02$	$58.98 \pm 0.55$	8.34	0.15
$RH_{PT-M}$ (2da)	$4.07 \pm 0.03$	$58.63 \pm 0.09$	8.50	0.13
$RH_{PT-M}$ (3da)	$4.18 \pm 0.08$	$58.46 \pm 0.65$	8.39	0.21

The pH of the reactors was slightly above 8.0 (with an optimal range between 7.0 and 7.8<sup>34</sup> without this leading to a stress condition causing system failure. High pH refers to buffering capacity in the reactors and low FOS/TAC values. Even though the optimum ratio of FOS/TAC varies with the type of substrate and load of organic rate, the interval observed in the reactors (0.13-0.21) indicates that the system did not accumulate VFA that limit or inhibit the activity of methanogens. Likewise, the VS content did not present significant variations at the end of each feeding, confirming that there was no accumulation of solids in the system.

The analysis of these variables (pH, FOS/TAC, VS), the reduction of the latency time, and the increase in the value of the biogas production rate concerning the preliminary assay indicate that the strategy of consecutive feedings induced the adaptation microbial.

## Conclusions

The investigation demonstrated that the consecutive feeding strategy applies to RH treatment with progressive increases in methane yield and decreasing methane production time. The FOS/TAC, pH, and VS parameters established that the process was stable. Using pretreatments in schemes of anaerobic digestion of agricultural residuals implies an additional complexity. However, using mechanical and alkaline pretreatments for RH increases methane yield due to lignin solubilization. The results suggest that consecutive feeding is an alternative to consider in the projection of the application of anaerobic digestion of RH.

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