

**Effect of the Inoculum in the Biological Pretreatment of Sorghum Straw for the Production of Biogas Based on Chicken Manure/Efecto del Inóculo en el Pretratamiento Biológico de la Paja de Sorgo para la Producción de Biogas a Partir de Gallinaza**

Alcocer Carrillo María de Jesus<sup>1</sup>, Dra. Ruiz Aguilar Graciela Ma. de la Luz<sup>2</sup> y M. en B.  
Martínez Martínez Juan Humberto<sup>3</sup>

<sup>1</sup>*Ingeniería en Energías Renovables, Instituto Tecnológico Superior de Abasolo (ITESA), Blvd. Cuitzeo de los Naranjos 401, Cuitzeo de los Naranjos, Abasolo, Gto, México, C.P 36976, 462 178 9965.*

<sup>2</sup>*Biotecnologias Para Sustentabilidad, División de Ciencias de la Vida (DICIVA), Campus Irapuato-Salamanca, Universidad de Guanajuato (UG), Ex-Hacienda El Copal, Km. 9 Carretera Irapuato-Silao, Irapuato, Gto, México, C.P. 36500, (462) 62 41889 ext. 1805.*

<sup>3</sup>*Ingeniería en Energías Renovables, Instituto Tecnológico Superior de Abasolo (ITESA), Blvd. Cuitzeo de los Naranjos 401, Cuitzeo de los Naranjos, Abasolo, Gto, México, C.P 36976, 462 601 9032.*

[\*mari\\_susej@live.com.mx\*](mailto:mari_susej@live.com.mx)<sup>1</sup>, [\*graciela@ugto.mx\*](mailto:graciela@ugto.mx)<sup>2</sup>, [\*juan.martinez@tecabasolo.edu.mx\*](mailto:juan.martinez@tecabasolo.edu.mx)<sup>3</sup>

**Resumen:**

En este estudio se analizó el efecto de la cantidad de inóculo (2.5% y 5% p/p) en el pretratamiento biológico con *Trametes versicolor* en la paja de sorgo para la producción de biogás. Lo anterior en un medio sumergido en presencia y ausencia de una solución de glucosa al 1.0% (p/v), como cosustrato. Se operó al 30% del volumen del matraz, en condiciones aerobias. Se analizó la reducción de azúcares en el tiempo 0 y 14 días de incubación, alcanzando una reducción del 90% para ambos inóculos en presencia de glucosa y 40% en ausencia del azúcar. Se analizó el efecto del pretratamiento en la estructura fibrosa de la paja por el método modificado de Van Soet. La paja pretratada se sometió a codigestión anaerobia con excreta de gallina (gallinaza), llevando a cabo la medición de biogás producido durante 75 días. Se cuantificó la concentración de metano por cromatografía de gases. Donde la mayor concentración de metano se detectó en las muestras pretratadas con 2.5% de inoculo en ausencia del cosustrato.

**Palabras clave:** Pretratamiento, Codigestión, Gallinaza, Paja De Sorgo, Biogás.

**Abstract:**

This study focuses on the effect of the quantity of the inoculums (2.5% and 5% m/m) in the biological pretreatment with *Trametes versicolor* in sorghum straw for the production of biogas. The previous was done through submersion in the presence and absence of a glucose solution at 1.0% (m/v), as cosubstrate. 30% of the volume of the Erlenmeyer flask was used, in aerobic conditions. The reduction of sugars was analyzed at time 0 and 14 days of incubation, reaching a 90% reduction for both inoculums in the presence of glucose and 40% in the absence of sugar. The effect of the pretreatment was analyzed in the fibrous structure of the straw by the Van Soet's modified method. The pretreated straw underwent an anaerobic codigestion with chicken droppings (chicken manure), measuring the production of biogas during 75 days. The methane concentration was measured through the chromatography of gases. The highest concentration of methane was detected in samples pretreated with 2.5% of the inoculums in absence of the cosubstrate.

**Keywords:** Pretreatment, Codigestion, Chicken Manure, Sorghum straw, Biogas.

## 1. Introduction

The use of lignocellulosic biomass for the production of biogas is not very common due to the complexity of the bonds between hemicelluloses, cellulose, and lignin, which form a barrier that impedes a microbial attack (Zheng et al., 2014). It is, therefore, necessary to apply pretreatments capable of breaking the bonds to have access to the fermentable carbohydrates. The production of methane from lignocellulosic residues consists of two important phases, or stages: pretreatment and hydrolysis, which increase the rate of methane production. The later takes place in a reactor where a consortium of methanogenic anaerobic microorganisms intervenes; these are in charge of degrading the fermentable sugars.

The efficiency of anaerobic digestion depends on different factors such as: temperature, pH, quantity of solids, nutrients, ratio of C:N, time of reaction, among others (Anjum et al., 2016; Mao et al., 2015; Zhang et al., n.d.) and in special cases the structure of the substrates. Recent studies look for ways to increase the rate of methane production through codigestion, since the combination of two or more substrates becomes effective; with the existence of a synergetic relationship between the use of materials like cattle manure which contains methanogenic biological agents, combined with straw, which works as a source of carbon and nitrogen for the bacteria present in cattle manure (Anjum et al., 2016; Mata-Alvarez et al., 2014).

Diverse studies point out that doing a codigestion with lignocellulosic residues implies increasing the hydrolysis efficiency by means of pretreatments. These can be chemical, physical, and biological, however, these have their advantages and disadvantages; one of the most important is the implied cost of their use in a digestibility process.

The chemical pretreatments used to improve the production of methane are done with acids or bases (Zheng et al., 2014). Due to their elevated cost and high toxicity to the environment, mass production is not very profitable. The physical pretreatments, although not involving chemical compounds in the process, require a very elevated demand of energy, especially when referring to mechanical processes. The biological pretreatments take place with microorganisms or enzymes capable of hydrolyzing hemicelluloses efficiently and removing lignin, which means a very low requirement of energy during the process (Mao et al., 2015). One of the most commonly used microorganisms is fungi (Garmaruddy et al., 2011; Ha et al., 2001; Lalak et al., 2016; Müller and Trösch, 1986; Rabemanolontsoa and Saka, 2016; Rouches et al., 2016, n.d.; Sánchez, 2009; Simeng et al., 2015; Wan and Li, 2012; Zhong et al., 2011) the basidiomycete fungi are outstanding primarily because of their mycelial growth which allows them to transport diverse elements of interest to be used as a source of carbon. The fungic degradation is an extracellular production in many of the cases, due to the insolubility of the structural polymers of lignocelluloses. The fungi have two types of systems: hydrolytic system which produces hydrolases capable of degrading polysaccharide and the lignolitic system, which degrades lignin into phenyl rings (Sánchez, 2009).

---

Therefore, in the present work a biological pretreatment with *Trametes versicolor* was studied, in particular the effect of quantity of the inoculum in sorghum straw was analyzed, to later assess the production of biogas based on chicken manure.

## **2. Methods and materials**

### **2.1 Biological pretreatment with *Trametes versicolor***

The fungus used in this research was the strain CDBB-H-1051, provided by the Microbial Collection of the Biotechnology and Bioengineering Department from the Center of Research and Advanced Studies (CINVESTAV for its Spanish acronym), which was re-sown in a PDA medium (agar potato dextrose) and was incubated at 30°C during 10 days. This generates a circular growth on the surface of the medium. Afterwards, from agar it was transferred to a YPG medium (yeast peptone glucose) and placed at 150 rpm during 14 days, later, the grown fungi was filtered for homogenizing and used as inoculums in the treatments with chicken manure.

The sorghum straw was obtained from the agriculture zone belonging to the Division of Life Science Campus Irapuato-Salamanca of the University of Guanajuato (DICIVA-UGTO for its Spanish acronym), which was grounded and sifted in a 0.850 mm screen, followed by a wash with sterile water to remove any possible soil particles present. Then it was dried at 100 °C for 24 hrs. Before using it, it was sterilized in humid heat to eliminate any microorganism that could interfere in the pretreatment (Zhao et al., 2014).

#### **2.1.1 Fermentation in Liquid State**

The pretreatment took place in a humid form, according to how it's described by Jha et al., (2013), based on the production of biogas for dry and humid fermentation. Furthermore, the pretreatment was done in the presence and absence of a cosubstrate like the glucose solution 1.0% (m/v) to start the degradation of the components of the hay's cellular wall.

The pretreatment took place with percentages of 2.5 and 5.0% of inoculum (m/m), liquid was placed in an Erlenmeyer flask until it reached 30% of its total volume capacity, and a known sample of hay with total solids (ST) less than 10% (Jha et al., 2013; Motte et al., 2013). It was kept in proper temperature conditions and movement for the adequate fungus growth for 14 days. All the pretreatments were repeated three times.

#### **2.1.2 Physical chemical Characteristics of the Pretreatment**

The pretreatment mix was separated into liquid and solid parts: for these the amount of reducing sugars was determined using the most common method (Miller, 1959) and fiber assessment using Van Soest's modified method (Chaves., et al 2002), at times 0 and 14 days respectively.

## **2.2 Anaerobic codigestion**

## 2.2.1 Physical chemical Characteristics of the Substrates and Co digestion Mix

The agricultural residues used in this study were characterized by methods such as: pH determination, Chemical Demand for Oxygen (DQO for its Spanish acronym), Total Solids (ST for its Spanish acronym), total Nitrogen (%N), and organic Carbon (% organic C) based on procedures established by official norms (NOM-AA-034-SCFI-2001, AA-026-SCFI-2010) and standard methods (5220-D, EPA 410.4).

## 2.2.2 Codigestion Process

The study was done in bioreactors operated in batches of 120 ml, with a work volume of 33 ml. The *Trametes versicolor* treated hay was placed. In the bioreactors a mass ratio of chicken manure:water of 1:2 was maintained. All the bioreactors were hermetically sealed. The air was extracted from each bioreactor to insure anaerobic conditions. Then these were incubated for a period of 75 days at 28±1 °C.

## 2.2.3 Measurement of Biogas and Methane.

The biogas was monitored every four days, during the incubation period. The biogas was quantified based on what is reported by Hansen et al., (2004). The concentration of methane was determined in the days of highest production of biogas; each sample was measured by chromatography (Clarus 500,Perkin Elmer).

## 3. Results

### 3.1 Pretreatment

In the results of the biological pretreatment there was a greater decrease of sugar reducers in the medium with glucose at 1% due to a better fungus growth. The reduction reached was between 90 and 91% of reducing sugars. However, sugar release was detected at the end of the incubation period, in the treatments with glucose without the presence of fungi. This can be attributed to some hemicellulose polymers being soluble in water after a prolonged period of time (Devlin, 2000).

The pretreatment with fungi in absence of the cosubstrate had a decrease of reducing sugars of 37 % and 48 %, for a percentage of 2.5 and 5% of inoculum, respectively. In the treatment without glucose and without fungi, the increase was only 1%. Comparing such results, the treatments with glucose at 1% present better results to be used in the anaerobic stage. In spite of this, the rest of the treatments were continued to determine the methane output in the anaerobic phase.

The determination of structural elements (Lignin, hemicellulose, and cellulose) present similar results to those reported by Rouches et al., (2016) using wheat hay in solid state

fermentation, with different glucose quantities and several types of rotting fungi strains, where a repression in the degradation of lignin was observed, due to the quantity of lignin. In the same way the pretreatments with *Trametes versicolor* with glucose as cosubstrate limits the consumption of lignin as a main compound and promotes the consumption of hemicellulose and cellulose, due to the negative influence in the production of delignifying enzymes.

In Figure 1 and 2 the percentages of hemicellulose and cellulose are shown for both pretreatments with and without glucose.

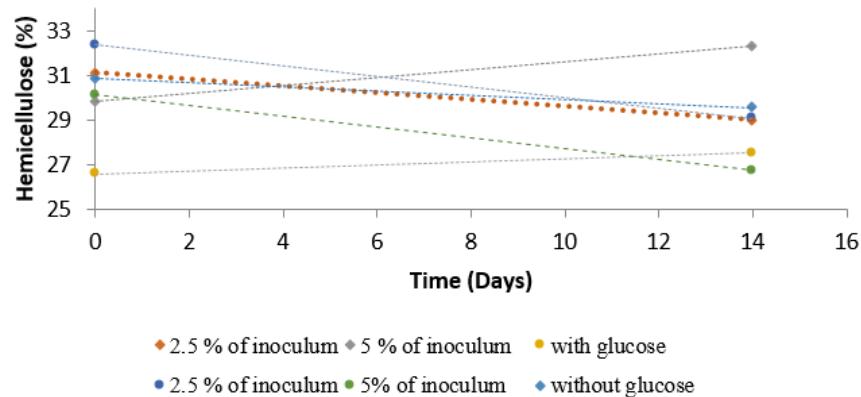


Figure 1. Presence of hemicellulose in *Trametes versicolor* pretreated sorghum straw.

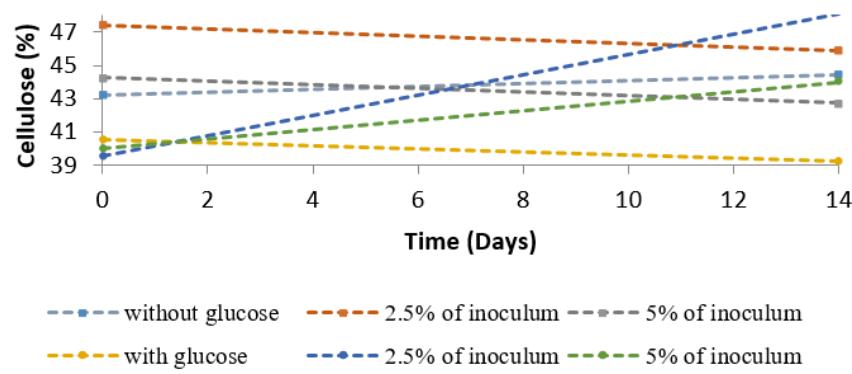


Figure 2. Pretreatment with, and without glucose as co substrate.

In Figure 3 the lignin behavior is shown in both media, with and without glucose. It indicates a light increase in lignin in the pretreatments with glucose due to a decrease in the ligninolytic activity of the fungi which causes the consumption of hemicellulose and cellulose. In the pretreatments without glucose good results were obtained in lignin elimination.

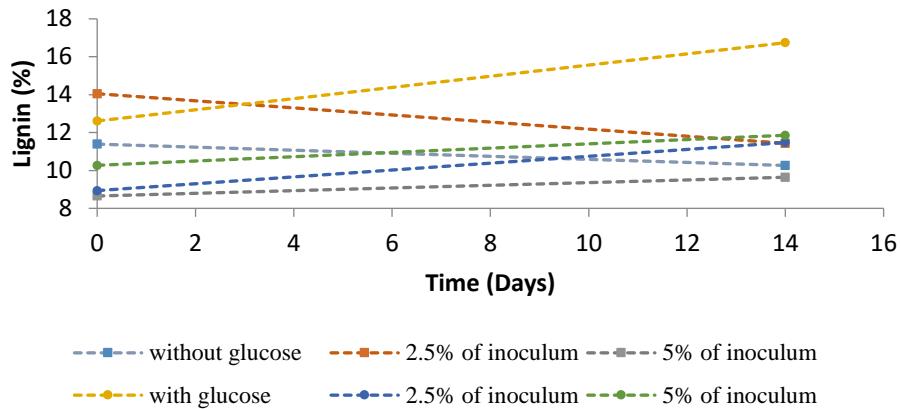


Figure 3. Percentage of lignin in sorghum straw pretreated with *Trametes versicolor*.

### 3.2 Anaerobic Co Digestion

#### 3.2.1 Characteristics of substrates and co digestion mix

The physical-chemical analysis of interest for the substrates of chicken and cow manures were determined, the last one was used as a positive control (Table 1). The characteristics of the co digestion mix for the anaerobic stage is shown in table 2.

Substrate	N%	ST (g/L)	% C.org.	% MO	% Ashes	DQO (mg/L)
Cow Manure	2.2±0.1	24.5±0.9	24.8±0.0	42.8±0.0	57.2±0.0	604±39.5
Chicken manure	4.1±0.2	26.7±3.9	30.5±1.8	52.6±3.2	47.4±3.2	738±0.7

Table 1. Characteristics of the substrates.

M	DQO(mg/L)		ST(g/L)		%N		%C organic	
<i>Without glucose</i>								
Without Fungi	T <sub>0</sub> 738±70	T <sub>75</sub> 805±33	T <sub>0</sub> 41±5	T <sub>75</sub> 66±9	T <sub>0</sub> 3.0±0	T <sub>75</sub> 0.05±0	T <sub>0</sub> 36±2	T <sub>75</sub> 19±0.8
Inoculum 2.5%	660±42	814±14	35±3	76±6	3.1±0.1	0.03±0	36±3	22±0.7
Inoculum 5%	674±42	764±65	33±5	69±9	3.9±0.4	0.02±0	37±1	25±4
<i>With glucose</i>								
Without inoculum	701±0.7	754±26	39±4	60±6	3.5±0.2	0.06±0	37±0.4	22±1
Inoculum 2.5%	683±111	811±17	38±12	78±3	3.6±0.4	0±0	33±2	25±4
Inoculum 5%	639±12	794±21	32±5	81±4	2.1±±0.8	0.46±0	33±4	28±4
Pos. Control	603±39	399±25	30±2	44±2	3.0±1.4	0.04±0	46±1	38±1

T<sub>0</sub>= Initial Time T<sub>75</sub>= Final Time M= Sample

Table 2. Characteristics of the co digestion mix.

### 3.3 Measurement of biogas and methane

In Figures 4 and 5 the evolution of the production of biogas with and without glucose can be observed. There is a greater production in the 2.5% fungi pretreated hay reaching its maximum point between 45 and 50 days, with a biogas production of 50 and 60ml.

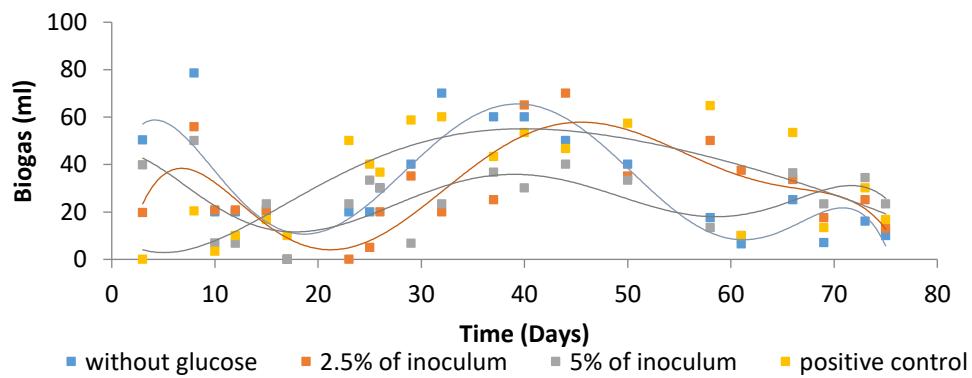


Figure 4. Measurement of biogas with sorghum straw pretreated without glucose.

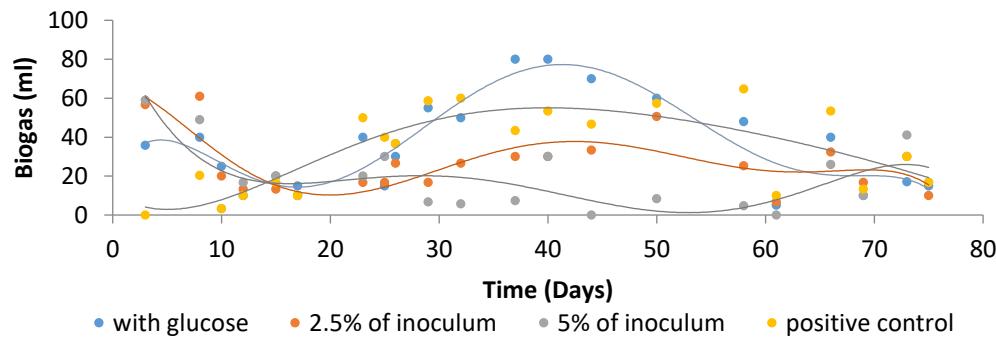


Figure 5. Measurement of biogas with sorghum straw pretreated with glucose.

The concentration of methane in the reactors with hay without glucose with 2.5 and 5.0% inoculum, where the maximum concentration of methane was in days 65 and 68. The reactors with pretreated hay with the co substrate maintained a linear gas release in the co digestion with 2.5% inoculum. The reaction time in the media with 5% inoculum was late due to the instability of the pH in the co digestion mix yielding inhibiting compounds. In Figure 6 and 7 such behavior can be observed.

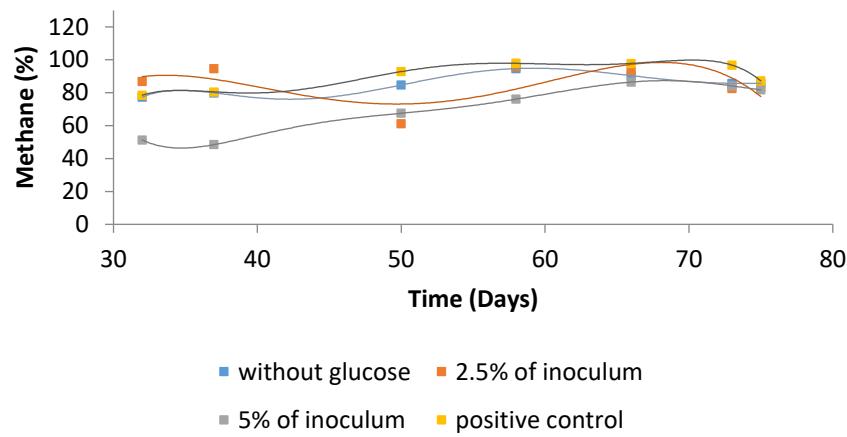


Figure 6. Concentration of methane in media without glucose.

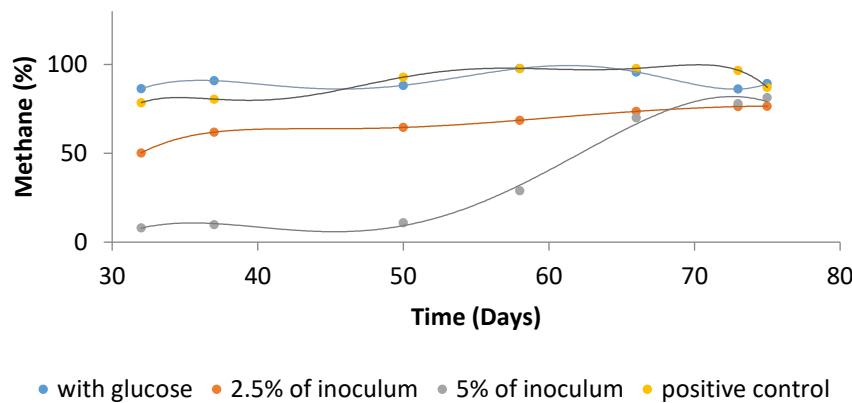


Figure 7. Concentration of methane in media with glucose as co substrate.

#### 4. Conclusions

As a result of the experiment, it can be concluded that glucose as a co substrate in the pretreatments done on sorghum hay have a negative effect on the degradation of lignin in the pretreatment, due to the excessive consumption of carbohydrates which yield a low or null ligninolytic activity.

For the production of biogas there is greater stability with hay pretreated with 2.5% inoculum in comparison to bioreactors with 5% inoculum. This can be attributed to an inhibition for substrate, which can be corrected with follow up analysis techniques to specify the possible inhibiting factors.

#### Acknowledgments

The authors wish to thank the technical assistance of Engineer Jaqueline Alfaro Negrete and the student Engineer Samuel Celaya Herrera during the making of the present work.

#### References

- Anjum, M., Khalid, A., Mahmood, T., Aziz, I., 2016. Anaerobic co-digestion of catering waste with partially pretreated lignocellulosic crop residues. *J. Clean. Prod.* 117, 56–63. doi:10.1016/j.jclepro.2015.11.061
- Devlin, T.M., 2000. *Bioquímica: Libro de Texto con Aplicaciones Clínicas*. Reverte.
- Garmaroody, E.R., Resalati, H., Fardim, P., Hosseini, S.Z., Rahnama, K., Saraeeyan, A.R., Mirshokraee, S.A., 2011. The effects of fungi pre-treatment of poplar chips on the kraft fiber properties. *Bioresour. Technol.* 102, 4165–4170. doi:10.1016/j.biortech.2010.12.054
- Ha, H.-C., Honda, Y., Watanabe, T., Kuwahara, M., 2001. Production of manganese peroxidase by pellet culture of the lignin-degrading basidiomycete, *Pleurotus ostreatus*. *Appl. Microbiol. Biotechnol.* 55, 704–711. doi:10.1007/s002530100653

---

Hansen, T.L., Schmidt, J.E., Angelidaki, I., Marca, E., Jansen, J. la C., Mosbæk, H., Christensen, T.H., 2004. Method for determination of methane potentials of solid organic waste. *Waste Manag.* 24, 393–400. doi:10.1016/j.wasman.2003.09.009

Jha, A.K., Li, J., Zhang, L., Ban, Q., Jin, Y., 2013. Comparison between Wet and Dry Anaerobic Digestions of Cow Dung under Mesophilic and Thermophilic Conditions. *Adv. Water Resour. Prot. Adv. Water Resour. Prot.* 1.

Lalak, J., Kasprzycka, A., Martyniak, D., Tys, J., 2016. Effect of biological pretreatment of *Agropyron elongatum* "BAMAR" on biogas production by anaerobic digestion. *Bioresour. Technol.* 200, 194–200. doi:10.1016/j.biortech.2015.10.022

Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Review on research achievements of biogas from anaerobic digestion. *Renew. Sustain. Energy Rev.* 45, 540–555. doi:10.1016/j.rser.2015.02.032

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S., 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renew. Sustain. Energy Rev.* 36, 412–427. doi:10.1016/j.rser.2014.04.039

Miller, G.L., 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Anal. Chem.* 31, 426–428. doi:10.1021/ac60147a030

Motte, J.-C., Trably, E., Escudé, R., Hamelin, J., Steyer, J.-P., Bernet, N., Delgenes, J.-P., Dumas, C., 2013. Total solids content: a key parameter of metabolic pathways in dry anaerobic digestion. *Biotechnol. Biofuels* 6, 164. doi:10.1186/1754-6834-6-164

Müller, H.W., Trösch, W., 1986. Screening of white-rot fungi for biological pretreatment of wheat straw for biogas production. *Appl. Microbiol. Biotechnol.* 24, 180–185. doi:10.1007/BF00938793

Rabemananjara, H., Saka, S., 2016. Various pretreatments of lignocellulosics. *Bioresour. Technol., Pretreatment of Biomass* 199, 83–91. doi:10.1016/j.biortech.2015.08.029

Rouches, E., Herpoël-Gimbert, I., Steyer, J.P., Carrere, H., 2016. Improvement of anaerobic degradation by white-rot fungi pretreatment of lignocellulosic biomass: A review. *Renew. Sustain. Energy Rev.* 59, 179–198. doi:10.1016/j.rser.2015.12.317

Rouches, E., Zhou, S., Steyer, J.P., Carrere, H., n.d. White-Rot Fungi pretreatment of lignocellulosic biomass for anaerobic digestion: Impact of glucose supplementation. *Process Biochem.* doi:10.1016/j.procbio.2016.02.003

Sánchez, C., 2009. Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnol. Adv.* 27, 185–194. doi:10.1016/j.biotechadv.2008.11.001

Simeng, Z., Sacha, G., Isabelle, H.-G., Marie-Noëlle, R., 2015. A PCR-based method to quantify fungal growth during pretreatment of lignocellulosic biomass. *J. Microbiol. Methods* 115, 67–70. doi:10.1016/j.mimet.2015.05.024

Wan, C., Li, Y., 2012. Fungal pretreatment of lignocellulosic biomass. *Biotechnol. Adv., Special issue on ACB 2011* 30, 1447–1457. doi:10.1016/j.biotechadv.2012.03.003

*Zhang, Z., Zhang, G., Li, W., Li, C., Xu, G., n.d. Enhanced biogas production from sorghum stem by co-digestion with cow manure. Int. J. Hydrot. Energy. doi:10.1016/j.ijhydene.2016.02.042*

*Zhao, J., Ge, X., Vasco-Correa, J., Li, Y., 2014. Fungal pretreatment of unsterilized yard trimmings for enhanced methane production by solid-state anaerobic digestion. Bioresour. Technol. 158, 248–252. doi:10.1016/j.biortech.2014.02.029*

*Zheng, Y., Zhao, J., Xu, F., Li, Y., 2014. Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog. Energy Combust. Sci. 42, 35–53. doi:10.1016/j.pecs.2014.01.001*

*Zhong, W., Zhang, Z., Luo, Y., Sun, S., Qiao, W., Xiao, M., 2011. Effect of biological pretreatments in enhancing corn straw biogas production. Bioresour. Technol. 102, 11177–11182. doi:10.1016/j.biortech.2011.09.077*

### **Semblanzas de los autores**

*María de Jesús Alcocer Carrillo.* Estudiante de la carrera de Ingeniería en Energías Renovables por parte del Instituto Tecnológico Superior de Abasolo (ITESA), con especialidad en Biocombustibles en la línea de producción biológica de biogás.

*Graciela Ma. de la Luz Ruiz Aguilar.* Postdoctoral Research Assistant (2001-2002). Department of Civil and Environmental Engineering. University of Iowa. Iowa City, Iowa, USA, Doctora en Ciencias, Especialidad Biotecnología Ambiental 6 de Julio del 2001, Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México, D.F., México., Maestra en Ciencias, Especialidad Biotecnología Ambiental, 8 de Enero de 1997, Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México, D.F., México., Ingeniera Bioquímica, Especialidad Productos Naturales, 12 Marzo de 1993, Departamento de Bioquímica, Instituto Tecnológico de Celaya, Celaya, Gto., México, Profesor de Tiempo Completo, Asociado C (2004-a la fecha). Departamento de Ciencias Ambientales, División de Ciencias de la Vida (antes Instituto de Ciencias Agrícolas, ICA), Campus Irapuato-Salamanca, Universidad de Guanajuato. Irapuato, Guanajuato, México, Línea de investigación: Uso Eficiente, Manejo y Rehabilitación de Suelo y Agua.

*Juan Humberto Martínez Martínez.* Doctorado por titular en Ciencias en Ingeniería Química por la División d Ciencias Naturales y Exactas (DCNE), Campus Guanajuato de la Universidad de Guanajuato, Maestro en Biociencias por la División de Ciencias de la Vida (DICIVA), Campus Irapuato-Salamanca de la Universidad de Guanajuato, e Ingeniero Mecánico por la División de Ingenierías (DICIS), Campus Irapuato-Salamanca de la Universidad de Guanajuato; ha desarrollado proyectos en diseño, construcción e implementación de sistemas de producción de biogás en el sector ganadero, agrícola y agroindustrial. Actualmente Profesor de Tiempo Completo en el Instituto Tecnológico Superior de Abasolo (ITESA), adscrito a la carrera de Ingeniería en Energías Renovables. Sus líneas de investigación están enfocadas a la producción

---

biológica de biogás, producción biológica de hidrógeno y diseño de biorreactores para la producción de biocombustibles.