## Influence of Particle Size and Temperature on Methane Production From Fique's Bagasse

Influencia del tamaño de partícula y la temperatura sobre la producción de metano a partir del bagazo de fique

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Abstract- The aim of this work was to evaluate the effect of particle size of fique's bagasse (FB) on anaerobic biodegradation and biogas production, by means of co-digestion of this lignoce-Ilulosic substrate using both bovine ruminal fluid and pig manure as inoculums. Anaerobic reactors were incubated by 8 days. Reducing sugar. Volatile Fatty Acid (VFA) and methane productions were measured using three different bagasse particle diameter; 5 mm (bagasse's natural size), 2.36 mm and 0.85 mm. Reduction of bagasse particle size increased reducing sugars formation and improved substrate mass transfer to microbial inoculums. At minor particle size it was favored hydrolytic step and VFA production. Natural particle sizes of bagasse were more difficult to biodegrade than lower ones. In this sense, methane concentration was increased 19% when 0.8 mm particle size was used. Anaerobic fermentation processes were carried out at 25°C and 39°C. Methane production at 25°C, show that these microbial consortia are able to resist temperature changes and transform all products on anaerobic digestion process.

Keywords— Anaerobic digestion, Biogas, Fique´s bagasse, Lignocellulosic waste, Mechanical treatment.

Resumen- El objetivo de este trabajo fue evaluar el efecto del tamaño de partículas del bagazo de fique sobre la producción de biogás empleando como inóculo una mezcla de líquido ruminal con lodo estiércol de cerdo. Los reactores anaerobios fueron incubados durante ocho días. Como variables de respuesta se cuantificó la concentración de azúcares reductores totales, ácidos grasos volátiles y producción de metano, usando tres tamaños diferentes de partícula 5mm (Estado natural del bagazo de fique), 2.36mm y 0.85mm. Durante la fermentación se observó que la reducción del tamaño de partícula del bagazo, incrementó la formación de azúcares reductores, mejorando la transferencia de masa entre el inóculo y el sustrato. El menor tamaño de partícula favoreció la etapa hidrolítica y la producción de ácidos grasos. El bagazo de fique en su estado natural dificulta la biodegradación anaerobia de éste sustrato. En este sentido, la concentración de metano se incrementó un 19% cuando el bagazo se

redujo a 0.8 mm. Los procesos de fermentación anaerobia fueron llevados a cabo a 25°C y a 39°C. La producción de metano a 25°C, demostró que los consorcios microbianos presentes en la mezcla de líquido ruminal y lodo estiércol de cerdo son capaces de resistir los cambios de temperatura y la transformación de todos los productos del proceso de digestión anaerobia.

Palabras clave— Digestión anaeróbica, Biogás, Bagazo de caña de Fique, Residuos lignocelulósicos, Tratamiento mecánico.

## I. INTRODUCTION

Fique 's bagasse (FB) is an agricultural by-product obtained during natural fiber process production, composed by cellulose, hemicellulose, lignin, lipids and proteins [1]. According to its composition, bagasse is considered an important lignocellulosic biomass source [2]. Lignocellulosic wastes are suitable substrates for anaerobic digestion process because of its carbon source [3]. Lignocellulosic biomass digestibility is limited by factors such as cellulose cristallinity, polymerization degree, moisture content, superficial area and lignin content. However, high lignin content in bagasse limits hydrolytic step and bioconversion system [4].

Anaerobic biodegradation of lignocellulosic substrates requires microbial consortia with high hydrolytic and methanogenic activities. Bovine ruminal fluid, active anaerobic sludge, bovine and pig manure have been previously used as biological matrices for anaerobic digestion [5]. A co-

digestion process mixes different inoculums or microbial consortia to improve the anaerobic digestion process. Co-digestion enhances nutrient equilibrium, dilutes toxic components and allows a synergic effect in microorganisms to increase hydrolytic activity [6]. Use of sisal pulp and fish wastes co-digestion increases methane production from 54 to 94% in comparison with other inoculums [7]. Bovine Ruminal Fluid (BRF) is an excellent inoculum for lignocellulosic substrate digestion, because its high cellulosic activity. In addition, if BRF is mixed with methanogenic inoculum, significant biogas yield are achieved [8]. Municipal waste solids mixed with bovine manure or wastewater sludge, increase in almost 20% biogas yields [9]. Temperature is a physical variable that affect microorganism growth and therefore biological reaction rates. Biological reactions (20-40 °C) for methane production from organic matter require more energy than conventional chemical reactions [10].

Pre-treatment processes are designed to decrease cellulose polymerization grade, weak lignin bonds with carbohydrates and increase particle superficial area. This process improves mass transfer process between inoculums and substrate. These processes increase cellulose bioavailability for enzyme biodegradation to monosaccharides. It has been proposed substrate pretreatment based on caustic and/or acid wash, heating and size particle reduction [11].

On the other hand, there is an inverse relationship between substrate particle size and methane yield [12]. For example, size reduction in tomato wastes increases yield values in 23% [13]. Whit sisal residues, hydrolytic activity improves significantly with particle size reduction, generating increasing in methane yields from 0.18 to 0.22 CH4/kg SV [14]. In starch degradation, the best yield value was obtained at substrate particle size of 0.35 mm [15].

In Colombia, fique 's industry produces 20,800 kg of residual wastes (bagasse and juice) per seeded hectare; these are delivered to grounds and water streams causing environmental pollution problems [16].

A possible solution of fique 's bagasse environmental pollution problem is the utilization as substrate for biogas production. However, its high lignin content requires a specific treatment. For this reason, the aim of this research was to evaluate the effect of fique's bagasse particle size and temperature on methane production during anaerobic biodegradation of this substrate.

## **II. MATERIALS AND METHODS**

#### A. Substrate

Fique 's Bagasse was obtained as a sub-product during natural fiber process production from Fique Industry. Bagasse samples were conserved in cooled containers during transportation and analysis. FB was sun dried at environmental conditions for 36 hours. Natural particle size diameter of FB was 5 mm. During pretreatment, FB was ground in a Willey-Mil's equipment to achieve particle size diameter of 0.85 and 2.36 mm. Different parameters such as: pH, lignin, cellulose, hemicelluloses and Acid Detergent Fiber (ADF) content, Total Solids (TS) and Volatile Solids (VS) were determined according to Standard Methods for Examination of Water and Wastewater [17].

#### **B.** Inoculas

Bovine Ruminal Fluid (RF) from urban slaughterhouse and Pig Manure (PM) from municipal pig farms were used as inoculums for digestion in a 1:1 ratio v/v. Inoculum composition used in anaerobic digesters is presented in Table I.

INOCULUM CHARACTERIZATION (RF-PM)			
Parameter	RF-PM		
рН	8		
TS (%)	43.7		
VS (%)	23.6		
Alkalinity (mgCaCO3/L) Volatile Fatty Acid – VFA (mg/L)	3100 7200		

#### TABLE I INOCULUM CHARACTERIZATION (RF-PM)

### C. Experimental Design

Anaerobic fermentation experiments were carried out using fique's bagasse with particle sizes of 5, 2.36 and 0.85 mm. Reactors were incubated for 8 days at 25 and  $39 \pm 2$  °C containing an operational volume of 350 ml. Hydrolytic activity, pH, VFA and methane percentage were considered as response variables. Total Reducing Sugar (TRS) concentration was determined according to Dinitrosalicylic Acid Method – DNS, using a GENESYS 20 Thermo Spectronics Spectrophotometer [18]. VFA concentration was quantified by titration method [19]. Methane percentage was determined with a PGD-IR (model Status Scientific Controls) infrared gas detector.

Experimental results were analyzed with Stat-Graphics plus 5.1, StatPoint Inc. (Virginia, EE.UU) Software. The Fisher's test (F) was used to verify data statistical significance between results.

### **III. RESULTS AND DISCUSSION**

According to the Fique's bagasse characteristics, this substrate is considered a lignocellulosic waste, for this reason the results are compared with another investigations where this type of matrix is evaluated.

## A. Effect of mechanical treatment on FB lignocellulosic structure.

In Table II, pH, TS and VS values from FB is not affected by particle size diameter reduction. TS and VS concentrations from FB were suitable for start-up anaerobic digestion process according with other studies [20]. Additionally, lignin content is decreased proportionally with reduction of waste particle size. These results suggest that smaller size particles improve mass transfer between inoculums and substrate. On the other hand, reduction of particle size decreases ADF concentration and improve FB digestibility.

Parameter	FB 5mm	FB 2.36 mm	FB 0.85 mm
pН	4	4	4
TS(%)	93.1	92	92.7
VS(%)	89.2	88.8	88.6
Cellulose(%)	41.8	23.4	18.7
Hemicellulose(%)	22.1	24.8	27.1
Lignin(%)	16.6	15.5	6.81
ADF(%)	64.6	44.8	44.7

TABLE II COMPOSITION OF THE FIQUE'S BAGASSE

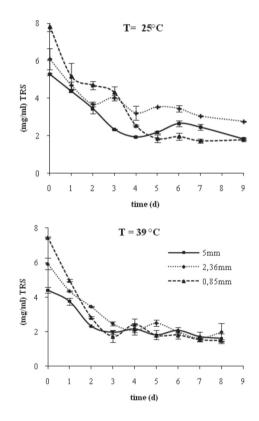
# B. Efecto of temperature and mechanical treatment on hydrolytic stage

In Fig. 1, it is observed that smaller particle size increase TRS concentration. Hydrolytic activity, defined as total reducing sugar consumption rate, was only observed until day 4.

Hydrolytic activity for particles size of 0.85, 2.36 and 5 mm at 39 °C were 1.93, 1.04 and 0.96 (mg/ml TRS/d), respectively. Experiments carried out at 25 °C achieved hydrolytic activity of 1.55,

1.20 and 0.87, respectively, at the same particle size. These results indicate that mechanical treatment improves strongly the hydrolytic activity of microbial consortia at the initial stage. This effect can be attributed because the increase of the superficial area at smaller particle size, allowing a better interaction between substrate and inoculums [21], [22]. These results are correlated with changes on lignocelullosic structure during FB mechanical treatment (see Table I). On the other hand, temperature affects hydrolytic activity being higher at 39 °C than at 25 °C; because enzymatic activity of microorganisms present an optimal temperature of 37 °C  $\pm$  2 °C [23].

Fig. 1. EFFECT OF PARTICLE SIZE AND TEMPERATURE (25°C Y 39°C) DURING HYDROLYTIC STAGE OF FB ANAEROBIC DIGESTION.



# C. Effect of temperature and mechanical treatment on VFA production.

Fig. 2 and 3 show that during Anaerobic Digestion of Fique's Bagasse (ADFB), pH values were maintained in a range between 7 and 8.5. These pH range favor growth and metabolic activity of microbial consortia. The biological behavior can be related to VFA variations. Size particle reduction increases hydrolytic activity, producing increases in VFA concentrations. VFA produced during acidogenic stage were no affected with temperature variations. However, VFA consumption rate was faster at 39°C.

Fig. 2. EFFECT OF PARTICLE SIZE ON PH VARIATIONS AND VFA PRODUCTION AT 25  $^{\circ}\mathrm{C}$  DURING ADFB

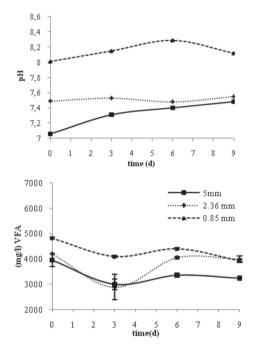
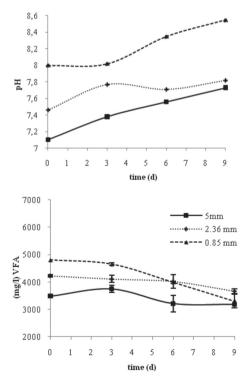


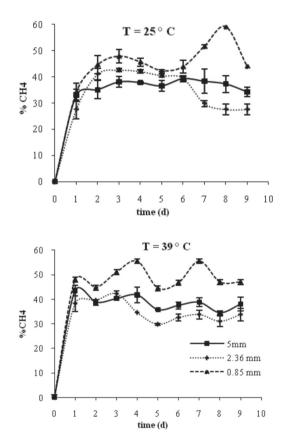
Fig. 3. EFFECT OF PARTICLE SIZE ON PH VARIATIONS AND VFA PRODUCTION AT 39 °C DURING ADFB



## D. Effect of temperature and mechanical treatment on methane production during FB anaerobic biodigestion.

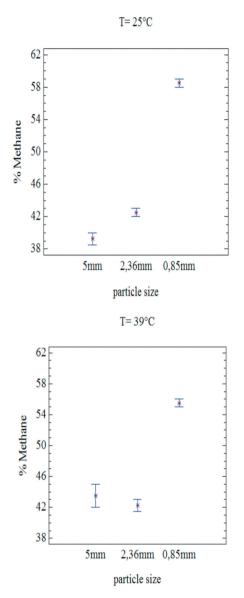
Fig. 4 shows that it is possible bioreactor startups for anaerobic biodigestion using 3 different FB particle sizes (5, 2.36 and 0.85 mm) at 25 and 39°C. These results can be explained in terms of equilibrium between methanogenic bacteria, acid consumer bacteria and inoculum adaptation to FB substrate. Higher methane production was obtained with smaller FB particle size. At 25 °C, best results of methane production were achieved at 8th day. At 39 °C, the higher value was achieved at 4th day.

Fig. 4 EFFECT OF PARTICLE SIZE AND TEMPERATURE (25 °C Y 39 °C) ON METHANE PRODUCTION DURING ADFB



In Fig. 5 are depicted statistical analyses of FB particle size effect on methane production at two different temperatures. The probability function (P) of Fisher test was 0.0004 and 0.0046 for methane production at 25 °C and 39 °C, respectively showing that there are significant differences between each experiment (IC 95%).

Fig. 5. STATISTICAL ANALYSES OF FB PARTICLE SIZE EFFECT ON ADFB



Finally, these results are according to studies carried out by Palmowski and Müller. These authors have demonstrated that reduction in particle sizes improves anaerobic digestion of substrate, due to increases in superficial areas of available substrates for microbial metabolism [24].

## **IV. CONCLUSIONS**

Particle-size reduction has been the most commonly used factor to describe the increase in substrate surface area resulting from a pre-treatment [25]. Pretreatment proved to be suitable for applications at full-scale biogas plants, increasing the methane yield of fique's bagasse by up to 19%.

Reduction of FB particle size affects the lignocellulosic structure, probably by cellulose crystallinity decrease, improving mass transfer substrate/ inoculums. Mechanical treatment influenced on everyone of anaerobic digestion stage, because hydrolytic activity are higher at smaller FB particle sizes, and produced higher VFA values and methane yields.

Additionally, mixture of bovine ruminal fluid and pig manure were able to degrade and adapt to this lignocellulosic substrate (Fique`s Bagasse) working efficiently at mild temperature conditions.

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