

# Evaluation of the biodegradability of a heat-treated anaerobic inoculum

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**Abstract:** The citrus industry produces a considerable amount of solid and liquid waste, which are mostly wasted. Anaerobic digestion of sewage sludge is an attractive option as it can produce fuels in the form of methane gas. However, the presence of complex organic compounds and pathogenic microorganisms significantly hinders the effectiveness of this process. The objective of this work is the selection of a source of inoculum free of pathogenic microorganisms by means of a thermal pretreatment and in this way is conducive to the treatment of residual sludge from the Citrus industry. The inoculum was taken from a geomembrane reactor which uses cattle manure as a substrate. 6 biodegradability kinetics were performed, varying the substrate-inoculum ratio, three of them with inoculum without pretreatment and another three with treatment, all under mesophilic conditions. SV parameters, pH, and biogas production were determined.

Keywords: Anaerobic digestion, Anaerobic inoculum, residual sludge, biodegradability

#### Introduction

The citrus industry is characterized by high water consumption during the production process, which has to be treated by physical or chemical processes, generating residual sludge (Coelho et al., 2011). Most of the sewage sludge has incorrect final disposal, producing high levels of contamination. Previously, sludge disposal has been carried out through traditional methods, such as incineration, landfilling, or confinement. However, this does not reduce the environmental impact, and in some cases the laws are not complied with, leading to other options such as disposal by biological methods, that is, aerobic composting and anaerobic digestion. (Semblante et al., 2015; Chang et al., 2011).

Anaerobic digestion is influenced by different factors, where the substrate-inoculum ratio (S/I) is a key factor for optimizing the process. Parra-Orobio et al., (2015). They mention that it is necessary to use a viable bacterial culture that contains a wide spectrum of microorganisms since the inoculum reproduces until it reaches a microbial population large enough to put the anaerobic digestion process into optimal operation. However, the presence of complex organic compounds, pathogenic microorganisms, extracellular polymeric substances, and various inhibitory compounds considerably hinder the efficiency of the anaerobic digestion process of sewage sludge (Anjum et al., 2016). By applying heat treatment, a sludge rich in available nutrients can be generated, which can be used more easily in anaerobic processes (Yamaguchi et al., 2006). Thermal treatment allows the solubilization of organic matter and facilitates the hydrolysis phase, which is the limiting rate (Coelho et al., 2011).

#### **Materials and Methods**

# Origin of the inoculum

The anaerobic inoculum without treatment (AIWT) was taken from a geomembrane biodigester, which works with a substrate of cattle excreta. Subsequently, samples were taken and placed in sealed Erlenmeyer flasks, to later take them to a thermostatic bath for one hour at a constant temperature and pressure of 90 ° C and 1 ATM respectively. Rodríguez de la luz (2018) shows that these heat pretreatment conditions inactivate pathogenic microorganisms such as salmonella spp and fecal coliforms.

# Regeneration of methanogenic activity

Once the Pretreated Anaerobic Inoculum (PAI) was obtained, a reactivation of the methanogenic activity was carried out so that the bacterial community was not affected after the thermal pretreatment, for the PAI was fed with a simple substrate that was Glucose, being an easy to digest substrate, achieved that the bacterial community could reproduce



quickly and for this, the PAI was placed in Erlenmeyer flasks of 500 mL, it was isolated from oxygen so that the medium was in anaerobic conditions, it was placed in constant stirring and at a temperature of  $35 \pm 2$  ° C.

### Biodegradability tests

To evaluate biodegradability, 6 kinetics were mounted, all using citrus residual sludge substrate, but the Substrate-Inoculum ratio (S/I) was varied. On the other hand, 3 residence periods were evaluated, as shown in Table 1. From kinetics 1 to 3 are with AIWT and from 4 to 6 with PAI.

For these tests, the samples were placed in fully sealed 500 mL Erlenmeyer flasks, placed on an orbital shaker and inside a room at a controlled temperature of  $35 \pm 2$  ° C, SV was determined by the standard method 2540 G SM, pH using a CONDUCTRONIC pc 18 potentiometer and Biogas production was measured by means of water displacement

Relationship S/I (%)	10 (S) - 90 (I)	30 (S) - 70 (I)	50 (S) - 50 (I)
Docidon so timo	10	10	10
Residence time	15	15	15
(days)	20	20	20

Table 1. Formulation of treatments

To evaluate the best conditions for the development of the pretreated anaerobic inoculum, a 32 factorial experimental design was applied, using the statistical analysis software NCSS 2007

## **Results and Discussion**

## Biodegradability tests

The behavior for AIWT is shown from kinetics 1 to 4, where it can be observed that there is a slight increase in SV content, but as time passes it stabilizes, this is similar to that presented by ZahidGaur and Suthar (2017).

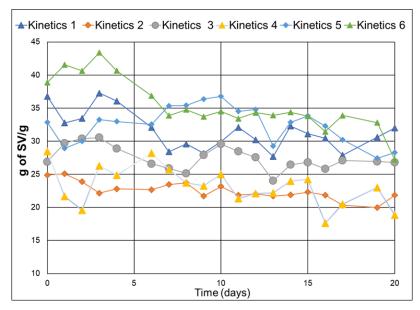


Figure 1. SV content in biodegradability kinetics.

For the PAI, the behavior is similar to that presented by Wang et al. (2010) and Li et al. (2017) who evaluated the effect of thermal pretreatment on the removal of SV (Figure 1), managing to observe an increase in their removal, similar to that presented in this work.



In Figure 2 the pH is shown, it is observed that there is a tendency to remain within the ranges of neutrality, however, a slight increase is notable as time progresses, this is similar to that presented by Luis-Garcez (2015) who evaluated the codigestion of bovine excreta with residues from the citrus industry, obtaining a slightly elevated pH as time passes, this without causing inhibition in the process.

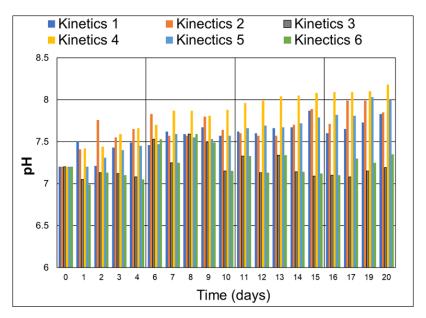


Figure 2. PH profile in biodegradability kinetics

the results showed that the total biogas production from the digestion of citrus residual sludge. It is superior for the kinetics used by the IAP, similar to that obtained by Roa-Rosas et al. (2015) who used cattle excreta as inoculum for the anaerobic digestion process in the same way. However, there is no information on studies using this heat pretreated manure inoculum.

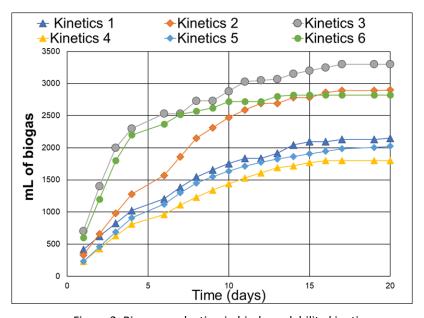


Figure 3. Biogas production in biodegradability kinetics

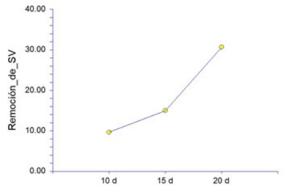


## Statistical analysis of the biodegradability of PAI

The Analysis of Variance shows that the residence time has a greater influence on the biodegradability kinetics. Regarding the inoculum-substrate relationship, it is shown that experiments with a proportion of 90% IAP have better removals.

Variation source	DF	Sum of squares	Mean square	F-Ratio	Probability level	(Alpha = 0.05)
Residence time	2	714.89	357.45	67.73	0.000823*	0.997
Percentage of PAI	2	176.22	88.11	16.69	0.011445*	0.924
S	4	21.11	528			
Total (adjusted)	8	912.22				
Total	9					

Table 2. Analysis of variance



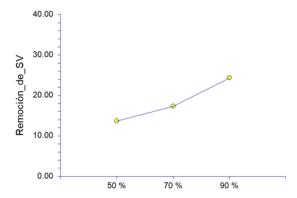


Figure 4. Removal Stockings Residence time

Figure 5. Removal Stockings Percentage of PAI

#### **Conclusions**

The possibility of treating Citrus Residual Sludge with a heat-treated inoculum by anaerobic digestion was demonstrated. Obtained values of the potential in biogas production (above 2,500 mL of biogas). On the other hand, it was shown that the kinetics of PAI removed a higher content of S.V. being the kinetics 90 PAI - 10 LR the one that managed to achieve the highest removal. In the same way, it was shown that stage 3 after 15 days has a greater removal. For all the above, the results are encouraging and an alternative for the use of this technology on a larger scale for energy purposes.

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