

## Influence of storage time and temperature on the methanogenic activity of anaerobic sludge from the ikiam biodigester plant

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### Abstract

Anaerobic digestion is widely used in wastewater treatment, generating biogas and a reusable effluent. These results depend on the quality of the inoculum, whose preservation outside the biodigester is critical, since improper storage affects its composition. This study evaluated the effect of storage time (7, 14, and 21 days) and temperature (4, 25, and 37 °C) on an inoculum from the tubular biodigester at Amazon Regional University Ikiam, which is fed with domestic wastewater. Physicochemical parameters and specific methanogenic activity were monitored. Results show that at 4 °C the inoculum remained stable for up to 21 days, whereas at 25 °C and 37 °C it lost biological capacity after the first week due to faster degradation. It was concluded that conservation at 4 °C is the most suitable strategy for preserving the inoculum.

Keywords: Specific Methanogenic Activity (SMA), anaerobic digestion, inoculum.

### Introduction

Anaerobic digestion is a widely used technology for wastewater treatment, which biologically processes organic waste using specific systems. One model of such systems is the plastic tubular biodigester, which, due to its easy installation and low cost, is particularly suitable for developing countries and rural regions (Martí & Cipriano, 2012). The implementation of a biodigester provides an effective solution for managing organic waste, resulting in the production of biogas — primarily composed of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>)

— and an effluent that can be utilized as fertilizer (Atem et al., 2008).

For efficient anaerobic digestion, several factors must be considered: the substrate, temperature, retention time, and especially the inoculum (Soto et al., 1993). The inoculum consists of aggregates of anaerobic microorganisms inside the biodigester. These microbial communities are responsible for degrading complex organic matter and transforming polluting waste into useful resources (biogas and fertilizer). Both products depend largely on inoculum quality, making it critical to know the storage conditions necessary to preserve their efficiency (Meegoda et al., 2018).

It is common to collect and store inoculum from operating biodigesters because they contain active microbial communities and are thus considered high-quality inocula. However, storing inoculum may affect its microbial activity, compromising its ability to convert substrate into methane. The impact of storage is evaluated through specific methanogenic activity (SMA) tests, which determine the inoculum's capacity to transform substrates into biogas (Astals et al., 2020).

In South America, particularly in the Amazon region, the variation of inoculum activity under different storage conditions has not been evaluated. This creates a limitation in designing effective strategies to preserve inoculum activity in pilot plants and rural projects (Martí et al., 2017).

Considering this limitation, the objective of this research is to evaluate the effect of storage time and temperature on the physicochemical parameters and specific methanogenic activity of the inoculum. To this end, the specific methanogenic activity of fresh inoculum and inoculum stored under different combinations of temperature and time was quantified to determine the condition that best preserves its physicochemical parameters and methanogenic activity.

## **Methodology**

The inoculum used came from a tubular biodigester fed with domestic wastewater and part of the wastewater treatment plant at Amazon Regional University Ikiam, located in Tena, Ecuador. The biodigester operates at  $25 \pm 3^{\circ}\text{C}$ , with a volume of  $5 \text{ m}^3$  and a hydraulic retention time of approximately 12 hours. The inoculum was stored in 1 L amber bottles under anaerobic conditions at  $4^{\circ}\text{C}$ ,  $25^{\circ}\text{C}$ , and  $37^{\circ}\text{C}$  for 7, 14, and 21 days. Characterization was carried out on the fresh inoculum and during the three-week storage period. The physicochemical parameters monitored included volatile solids by gravimetric methods and pH by potentiometric methods (APHA, AWWA, & WEF, 2017).

For the specific methanogenic activity (SMA) assays, serum bottles (120 mL) were prepared in triplicate, each containing 60 mL of inoculum and the amount of substrate (sodium acetate) required to achieve an inoculum-to-substrate ratio of 5 (based on volatile solids) (Astals et al., 2020). The bottles were incubated at  $37 \pm 1^\circ\text{C}$  for three days, and biogas production was measured every 24 hours using the water displacement method with a 0.02 M NaOH solution (Astals et al., 2015; Yoo, Han & Wee, 2013). The experimental design used was a  $3^2$  factorial design, with storage temperature and time as independent factors and methane production as the dependent variable.

## Results and Discussion

The results (Figure 1) show that at temperatures of  $25^\circ\text{C}$  and  $37^\circ\text{C}$ , degradation processes and acid accumulation accelerated, causing sharp pH drops and faster losses of volatile solids compared to the  $4^\circ\text{C}$  condition, where changes occurred more slowly.

In the specific methanogenic activity (SMA) tests, a progressive decrease was observed, with a more pronounced decline at higher storage temperatures. At  $4^\circ\text{C}$ , the reduction was mild (about 13%); at room temperature, it was greater (~27%); and at  $37^\circ\text{C}$ , the loss was substantial (~50%) compared to the fresh inoculum (Astals et al., 2020).

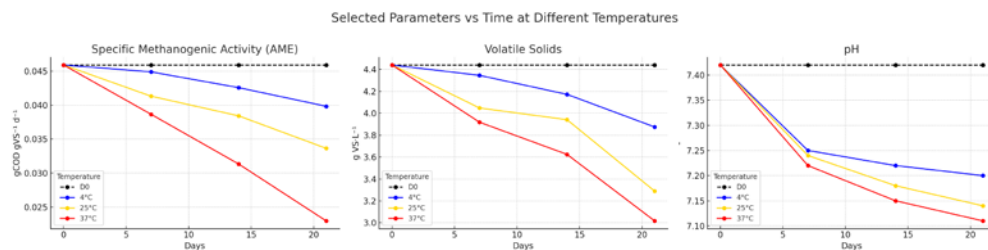


Figure 1. SMA and parameters as a function of storage time at different temperatures. Black: fresh inoculum; blue:  $4^\circ\text{C}$ ; yellow:  $25^\circ\text{C}$ ; red:  $37^\circ\text{C}$ .

The reduction in volatile solids indicates degradation and partial solubilization of the biodegradable organic fraction of the sludge (Atem et al., 2008). The pH drop results from an imbalance caused by excessive acidogenesis and insufficient methanogenesis, leading to the accumulation of acidic compounds in the medium that may inhibit the activity of methanogenic archaea (Parra et al., 2014).

## Conclusions

Overall, the results show that although the inoculum retains some activity during storage, it progressively loses stability and methanogenic capacity over time. Storage at  $4^\circ\text{C}$  yielded the

best results, preserving the inoculum's methanogenic viability and parameters. As storage time and temperature increased, biological activity was significantly compromised.

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