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# Influence of inoculums/substrate ratios (ISRs) on the mesophilic anaerobic digestion of slaughterhouse waste in batch mode: Process stability and biogas production

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## Abstract

Batch digestion experiments were conducted to determine the effects of inoculums/substrate ratio of three different ratios (ISRs) 0.3, 0.5 and 1. The substrate chosen in this study was slaughterhouse waste from Adrar city (south west of Algeria). The inoculation was made by sludge issued from wastewater treatment plant. During this study, several parameters of anaerobic digestion were measured, such as pH, VFA, AT and volume of the produced biogas. pH variation and VFA/TA values have shown high stability of anaerobic digestion process.

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# 1. Introduction

Industrial processes generate waste varying in composition and quantity at various stages, and disposal of such waste has become more and more difficult, particularly in view of stringent environmental pollution control standards stipulated by statutory authorities. With rapid depletion of conventional energy sources, the need to find

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an alternative, preferably renewable, source of energy from waste is becoming increasingly important for the sustainable development [1].

It is important to evaluate appropriate techniques and/or technologies for effective energy recovery from waste. Anaerobic digestion is considered as the one of the best treatment method for organic fraction of the segregated waste. Anaerobic digestion process consists of hydrolysis, acidogenesis, acetogenesis, and methanogenesis stages, and each stage is a function of metabolic condition of the various microorganisms [2].

Anaerobic digestion technologies ensure recovery of energy in the form of biogas, which is a clean fuel as compared to other conventional solid or liquid fuels. Among the many biological treatment methods experimented so far, anaerobic digestion possesses several advantages such as low energy requirement, low sludge production, low nutrient requirements and possibility of operation at high organic loading rate at a relatively low hydraulic retention time [3].

For the optimization of the anaerobic digestion process, the selection of inoculum source and the inoculum to substrate (ISRs) ratio are the important operational parameters for the assessment of anaerobic biodegradability of organic wastes [3].

A study of the anaerobic digestion of the solid waste generated from slaughterhouse was conducted at mesophilic temperature (35 °C) in batch mode. A laboratory scale multi-reactor system was used to compare the stability of anaerobic digestion process and biogas production at three different ratios (ISRs) 0.3, 0.5 and 1.

#### Nomenclature

ISRs Inoculum/Substrate Ratios VFA Volatile Fatty Acids TA Total Alkalinity TS Total Solid VS Volatile Solids

#### 2. Materials and methods

## 2.1. Substrate and inoculum:

The substrate used was a mixture of slaughterhouse waste collected from slaughterhouse of Adrar city. The digesters inoculation was carried out using sludge issued from wastewater treatment plant of Adrar city. Substrate and inoculums characteristics were summarized in table 1.

#### 2.2. Anaerobic digestion tests

The experiments were carried out in 1L serum bottles with rubber caps of appropriate size. Three different inoculum/substrate ratios (ISRs)0.3, 0.5 and 1, which were achieved by keeping a constant inoculum concentration (30 gVS/L) and varying the substrate concentration. The bottles were kept at 35 °C [4] in a temperature controlled water-bath and inverted twice per day manually. The biogas volume produced in the digesters was determined by connecting the bottles to a water-filled manometer and measuring the water displacement at the beginning of each sampling event. Volumetric gas production was normalized to standard temperature and pressure conditions (25°C and 101.325 kPa) using the perfect gas law [5]. All the experiments were carried out in triplicate and the results were expressed as means. The experience was conducted until a small production of biogas was observed.

## 2.3. Analytical methods

The pH was measured using ph-meter HANNA 8141. The Volatile Fatty Acids (VFA) and the total alkalinity (TA) were recognized as a good working parameters of the anaerobic digestion [6], their concentrations has been achieved according to the method described by [7]. Total solid (TS) was determined by keeping a known mass (Ws)

of sample in the oven at 105°C for 24 h and measuring the new mass (Wdm) after heating at this temperature. The TS content was calculated from the expression (Wdm x 100)/Ws. The dried samples were then ignited at 550°C for 12 h and the sample weight (Wash) was measured. The percentage of volatile solids (VS) was determined by the expression ((Wdm-Wash) 100)/Wdm [8, 9].

## 3. Results and discussions

ble 1.Substrate and inoculums characteristics.		
	Substrate (%)	Inoculum (%)
Volatile Solid (VS)	18.96	8.21
Total Solid (TS)	91.97	52.51

#### 3.1. Process stability

PH is a very interesting indicator in the stabilization process it shows us the good behavior of the anaerobic digestion [10]. Anaerobic processes are strongly influenced by changes in pH. The anaerobic digestion takes place optimally near neutrality pH 7 with optimal value between 6.5 and 7.5 [11].



Fig. 1.pH variation.

As can be seen in Fig. 1, the pH evolution, for all ratios (ISRs), can be divided into three main parts. The first part lasted 10 days and characterized by a drop in pH from 7.5 to a minimum value of 6.5. During the anaerobic acid-phase stage of complex organic substrates, mainly constituted by carbohydrates, proteins and lipids, these are converted basically to volatile fatty acids (VFA) and to a lesser extent to other low molecular weight compounds [12].

In the second part, the pH values increased. This increase is due to the fatty acids consumption by bacteria acetogenesis [13] to produce the biogas precursors. And finally, the third part from the  $22^{nd}$  day, during which we noted a pH values stabilization between 6.8 and 7.6. It is explained by the stabilization process of anaerobic digestion and it reached of all the anaerobic digestion stages. These results were noted by [14,15] in their researches on anaerobic digestion of organic waste.



Fig. 2.VFA variation.

Numerous observations related to anaerobic digestion suggest that volatile fatty acids (VFA), as one of the most important parameters for the accurate control of anaerobic digestion, have a direct correlation with the digester performance. As can be seen in Fig. 2 VFA values increase to 1.44 (gacetic acid /l) for 0.3 and 0.5 (ISRs). And to 1.08 (gacetic acid /l) for 1(ISRs). This phase of VFA production corresponds to "hydrolysis acidogenesis" phase [16]. After this increase, a decrease of VFA was observed which is due to VFA consumption by bacteria which correspond to "acetogenesis" phase.

Fig. 3illustrates the relationship that may exist between the VFA and the pH during anaerobic digestion. We can note a reverse in a case compared another form. So the variation of the two parameters is inversely proportional.





c) Fig. 3.pH VFA variation: a): 0.3 (ISRs), b): 0.5 (ISRs) and c): 1 (ISRs)

The reactional medium has a high rate of alkalinity that allowing it to ensure a good anaerobic digestion operation. As can be seen in fig. 4, the  $12^{th}$  day is characterized by an increase in the TA values. This increase is followed by a decrease and stabilization of the TA values. The increase in pH from the  $12^{th}$  day is due to the medium buffering capacity (TAC =  $2.5(gCaCO_3/1)$  and consumption of volatile fatty acids by bacteria.



Fig. 4.TA variation

The VFA/TA ratio can be used as a measure of process stability [17]: when this ratio is lower than 0.5(equiv. acetic acid/equiv. CaCO<sub>3</sub>). Fig. 5 illustrates the VFA /TA ratio values, and shows that the ratio was during the whole experience period less than 0.5. This indicates the feasibility of the process even with the significant production of VFA whose presence may inhibit the anaerobic digestion process [18]. Several authors consider that the control of VFA, pH and TA is necessary for a good operation of the anaerobic digestion [19].



Fig. 5.VFA/TA Ratio

#### 3.2. Biogas production

The experiments lasted for 47 days. Fig. 6 shows the cumulative biogas production of slaughterhouse waste as a function of time at different (ISRs) ratios. As can be seen, the biogas production increased as the (ISRs) value decreased [20]. The cumulative biogas production after 47 days of digestion for the different 1, 0.5 and 0.3 (ISRs) was 504, 856 and 864 mL, respectively. Raposo and al. and Shujuan and al. [12, 21] published that the substrates amount contributed substantially in increasing the amount of produced biogas.



Fig. 6.Biogascumulative volume

#### 4. Conclusion

The stability and progress of the reaction from solid substrate to biogas as an end product was monitored by measuring the pH, volatile fatty acids-total alkalinity and (VFA/TA) ratio. The results obtained demonstrated that in the ratios range (ISRs), the pH ranged from 7 to 8 and this parameter was always stable during the anaerobic digestion process. In addition, within the above (ISRs) range the VFA/TA ratios were always lower than the failure limit values (0.5), which demonstrated the high stability of the anaerobic digestion process of this substrate at mesophilic temperature. The biogas production increased as the (ISRs) value decreased. The production of VFA has not inhibited the anaerobic digestion process; this is due to the buffering capacity of the medium.

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