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# Effect of Inoculums to Substrate Ratio on Thermophilic Anaerobic Digestion of the Dairy Wastewater

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This work focuses on the biodegradability of the organic pollution load of the dairy industry. The effects of various reports substrate/inoculums (S/I) are considered, respectively for (0), (0.2), (0.4), (0.8) and (1.6) in the thermophilic phase (55 °C). In addition, the monitoring of the variation of COD in all reactors is considered.
The preliminary characterization of substrate (dairy waste), shows that it is rich in volatile matter (88.67%). Which encourages the treatment of the latter by anaerobic biological process.
Similarly, the analysis results of alkalinity, ammonia nitrogen, organic nitrogen and phosphorus after incubation period for the considered organic loading rate (OLR), are below the values that can inhibited the anaerobic digestion process, although pH values (between 7.84 and 8.56) seems to be important.
The cumulative volume of produced biogas increases with respect of the ratio (S / I), where the maximum production of biogas (1,553mL) corresponding to the highest ratio (S / I = 1.6), with a daily average production of 28.26 L / day. This is more than three times the lowest ratio (S / I = 0.2) and twice the average ratio (S / I = 0.4). However, qualitatively the ratio (S / I = 1.6) is less rich in methane (50.7 %) compared to the ratios (S / I = 0.8 and 0.4) which are of the order of 54.5 % and 58.3 %, respectively, but quantitatively, this decrease is largely compensated by the produced methane. Also, the abatement of total COD is considerable (84 %).

## 1. Introduction

Methanization is a biological process which aims at producing, starting from organic waste, a biogas mainly composed of methane and carbon dioxide. Biogas can be valorized by co-generation to produce a renewable energy in the form of electricity and of heat. This technique also induces the production of a residue called digestate.
Sight the importance of this produced biogas from energy point of view (50-75 % methane), several works were published these last years with an aim of determining the quantity of the biogas which can be produced during the degradation of a type of waste given. Several researchers studied the biodegradability of the various types of biodegradable waste through the measurement of the volume of produced biogas, among this waste one can quote: solid fraction of manures ( Mladenovska et al, 2006), organic waste (Hansen et al, 2004 ), sludge produced from wastewater treatment plant (Lin, et al, 1999), municipal solid waste (Erguder et al, 2001), agro-waste (Neves et al 2004) and Castillo (2005), waste of factory of production of olive oil (Fezzani and Bencheikh , 2007).
Others studied the influence of several parameters on the production of biogas, such as the activity of the inoculums, used during the test of biodegrability, as well as the ratio substrate / inoculums (S/I) (Neves et al 2004), and (Raposo et al 2006), the physico-chemical pretreatments applied to waste (Perez et al 2004), granulometry of waste ( Palmowski et al, 2000), and the influence of pH startup.
The experimental program is conducted to study the biodegradability of effluents from the dairy industry through anaerobic digestion process following the effects of different ratio Substrate / inoculums ratio (0; 0.2; 0.4; 0.8 and 1.6) on produced methane as well as the variation of the degradation of the organic COD in the thermophilic phase (55 ° C).

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## 2. Materials and methods

### 2.1 Methodology

Measures of methanogenic potential ((Biochemical Potential methanogenic or BMP) are performed following (Vedrenne et al 2005).

Concretely, 400 mL of a substrate mixture inoculums and nutrient solution are introduced into a 570mL serum bottle. The quantity in substrate is calculated in order to obtain ratios S/I equaling to 1.6, 0.8, 0.4, 0.2 and 0.0 and the compositions are presented in Table 1. All the reactors are maintained in 55 ° C.

With several values of the ratio S/I, the batch reactors are distributed between witnesses containing the inoculum only (the white), and tests which contain the mixture of the inoculum and of the substrate.

These tests are realized in duplicate serum bottle and the results are averaged over the two experimental measurements. After filling, the flasks are sealed with a rubber septum, and their atmosphere is purged with molecular nitrogen (N2). During the incubation period, the Measure of produced biogas volume was performed by liquid displacement (pH = 2). The volume of biogas is measured daily during all the incubation period. The experiment was considered until observation approximately a null produced biogas (49 days).

### 2.2 Analytical methods

Liquid phase characterization was undertaken before and after anaerobic digestion period through the determination of pH, total solids (TS), total volatile solids (TVS), Alkalinity (TA ) and total alkalinity (TAC ), chemical oxygen demand (COD), ammonia nitrogen ($NH4^+$ ), total Nitrogen (NTK) and total phosphorus (Pt) according to Standard Methods ( APHA, et al , 1998 ) pH was determined using a pH-meter (Jenway 3510 PH meter) and methane in the biogas was analyzed by a gas chromatograph (Arlo Erba strumentazione 4300 (fugueur,120 DFL) with a flame ionization, equipped with stainless steel column (4m long, 3mm outer diameter). The injector, detector and oven temperatures were 40, 80 and 120 ◦ C, respectively, where a 1mL gas sample was injected into the chromatograph using Helium as a gas carrier.

### 2.3 Substrate Composition in each reactor

The quantity in substrate is calculated in order to obtain ratios S/I equaling to 1.6, 0.8, 0.4, 0.2 and 0.0 and the compositions are presented in Table 1.

*Table 1: Substrate composition*

| | Inoculum | | Substrate | | Substrate/Inoculum |
|---|---|---|---|---|---|
| Reactors | TVS (g/L) | Mass(g) | TVS (g/L) | Mass(g) | S/I |
| OLR0 | 22.88 | 10.29 | 0.00 | 0.00 | 0.0 |
| OLR1 | 22.88 | 10.29 | 36.61 | 8.35 | 1.6 |
| OLR2 | 22.88 | 10.29 | 18.31 | 4.20 | 0.8 |
| OLR3 | 22.88 | 10.29 | 9.15 | 2.12 | 0.4 |
| OLR4 | 22.88 | 10.29 | 4.58 | 1.06 | 0.2 |

### 2.4 Substrate and inoculums characterization

The physicochemical main features of sludge and dairy waste was deferred in Table.2

*Table 2: Characteristics of substrate and inoculums*

| Parameter | Substrate | Sludge |
|---|---|---|
| pH | 5.68 | 8.19 |
| $NH_4^+$ (mg/L) | 87.26 | 95.62 |
| NTK (mg/L) | 122.49 | 143.64 |
| Soluble COD (mg/L) | 67,605.63 | 50,704.22 |
| Total COD (mg/L) | 106,382.98 | 53,191.49 |
| Total Phosphor (mg/L) | 171.20 | 22.79 |
| TS (g/L) | 25.80 | 36.28 |
| TVS (g/L) | 22.88 | 16.34 |

Table 2, above presents the characteristic substrate (dairy waste) and inoculums (sludge). According to the table, the substrate pH is slightly acidic but it is slightly basic for the inoculums. Concerning ammonia and organic nitrogen, the table shows that their concentrations are high for inoculums compared to substrate.

## 3. Results and discussion

### 3.1 Cumulated biogas volume obtained from substrate

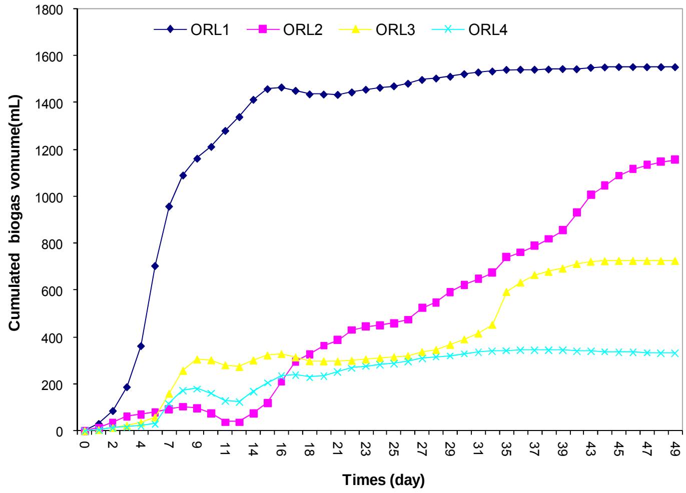


*Figure1: Cumulated biogas volume of dairy waste (T=55 ° C)*

The cumulated produced biogas from organic fraction of dairy waste, in thermophilic phase (T = 55 ° C) was presented in Figure.1. It should be noted that the biogas production of dairy waste was calculated after eliminating the inoculum effect. The production is maintained until the 49$^{th}$ day.

We notice that the maximum production of biogas increases from 333 mL for OLR4 (S / I = 0.2), to 728 mL for OLR3 (S / I = 0.4), 1,157 mL for OLR2 (S / I = 0.8), and finally to the value of 1,553mL for OLR1 (S / I =1.6). These results are similar to those of (Buendia et al 2008) and (Chen et Hashimoto 1996), indicating an increasing biogas production with respect of S/I ratio.

### 3.2 Cumulated methane and carbon dioxide obtained from dairy waste

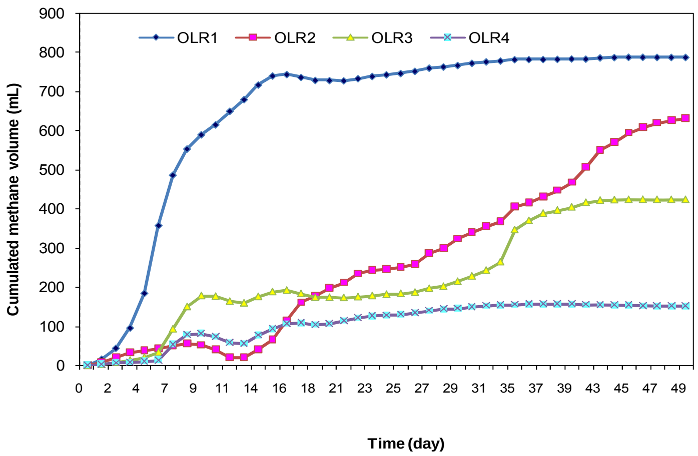


*Figure 2: Cumulated CH4 volume (T=55 ° C)*

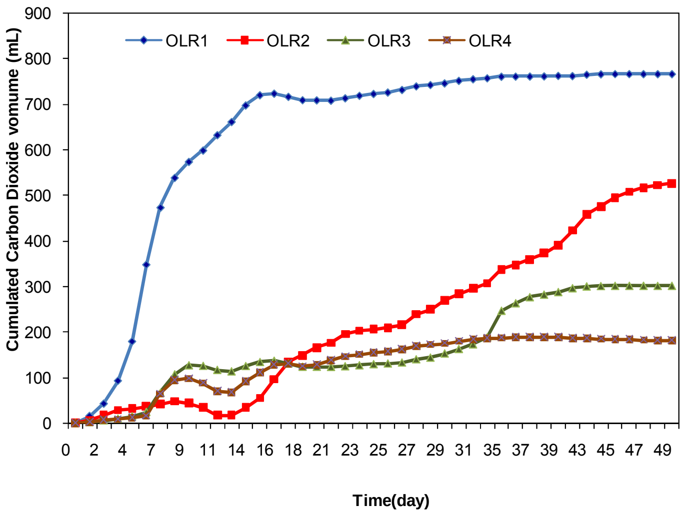


*Figure 3: Cumulated $CO_2$ volume (T=55 ° C)*

The composition of produced biogas anaerobic digestion process is a very significant parameter for the control and the monitoring of this process. Indeed a consequent production of biogas reflects the good operation of the digester.
Figure 2, represents the cumulated volume of produced methane during the incubation period in thermophilic phase, the peoduced volume of methane is lies between 150 mL for OLR4 and 787 mL for OLR1. This cumulated volume increases respectively with the ratio (S/I).
In the same way for the produced volume of carbon dioxide, see Figure 3.This increase can be explained by the increase in concentration of the micro-organisms in the liquid phase.

**3.3 Biogas Composition**

Figure 4, represents the average composition of biogas expressed as a percentage of methane and carbon dioxide.

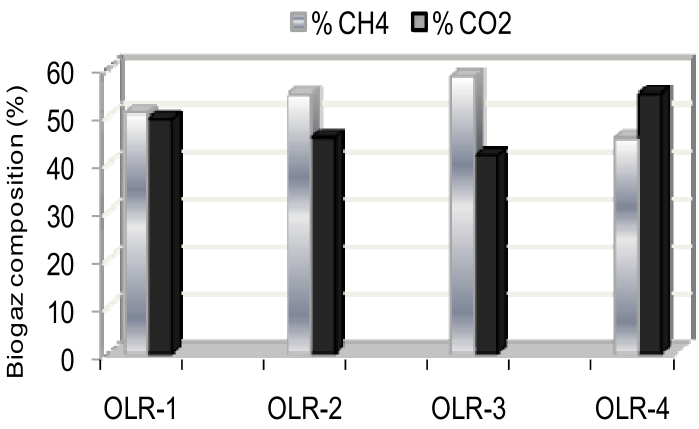


*Figure 4: Biogas composition, (T=55 ° C)*

This figure shows clearly that, during the incubation period the average percentage of methane in the produced biogas of the four OLRs tested was: 50.7 %, 54.5 %, 58.3 % and 45,4 % corresponding to OLR1(S/I=1.6), OLR2(S/I=0.8), OLR3(S/I=0.4) and OLR4(S/I=0.2), respectively .
From a qualitative point, the high percentage of methane is obtained for ratio S/I = 0.8 and is less rich for the highest ratio S/I = 1.6. However, from a quantitative point, this lack is largely compensated by the increase in the production.
Generally, a percentage of methane ranging between 50 and 80 % remains acceptable in anaerobic digestion process.

### 3.4 Kinetics of total COD yield with time

The chemical oxygen demand represents the total amount of the pollution load in the substrate. Figure 5 shows that the variation of COD with time, for different ratios. It is shown that there has been a considerable reduction in COD compared to those obtained prior to digestion, with a yield of 84.97 %.

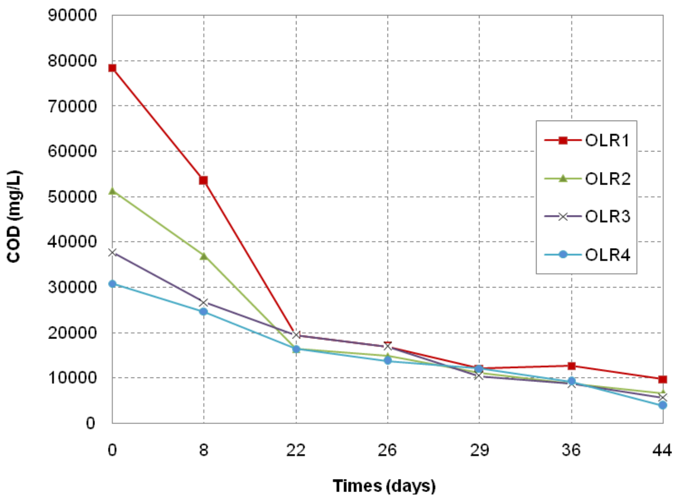


*Figure 5: Variation of COD with time for different tested OLR*

## 4. Conclusion

In the light of the results obtained, one can conclude that the effect of the substrate ratio S/I on anaerobic digestion of the dairy waste is very important in order to obtain optimal removal.
In thermophilic phase, the cumulated biogas volume increased with respect to (S/I) ratio. The maximum biogas volume produced is of 1,553 mL for OLR1 (the highest ratio S/I=1.6). Whereas volume of produced biogas for the smallest S/I ratio (S/I=0.2) is five times lesser than the later one and half as for S/I ratio equals to 0.4. This cumulated volume increases respectively with the ratio (S/I).
Qualitatively, the produced biogas is less rich in methane (50.7 %) for the highest ratio (S/I=1.6) compared to the ratios tested, S/I=0.8 and 0.4, which are about 54.5 % and 58.3 % respectively. However, quantitatively, this fall is largely compensated by the increase in the biogas production.
Moreover, the abatement of total COD is considerable (84%).

### Notation

| | |
|---|---|
| OLR | organic loading rate |
| COD | chemical oxygen demand |
| TS | total solid |
| TVS | total volatile solid |
| BMP | biological methane production |
| S/I | ratio TVSsubstrat/TVSinoculum |

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