ANAEROBIC DIGESTION EXPERIMENT USING *CYNARA CARDUNCULUS* L. STALKS.

Oliveira I.D.B.[1], Diberardino S.[2], Gominho J.[1], Duarte E.[1]
[(1)] Instituto Superior de Agronomia (ISA-UTL); [(2)] Laboratório Nacional de Energia e Geologia, I.P.
Instituto Superior de Agronomia, TU Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal. Tel: +351 21 3653000. ivomdb@gmail.com or eduarte@isa.utl.pt

1 INTRODUCTION

Anaerobic digestion is an industrial process applied to organic wastes treatment with several environmental and energetic advantages over other forms of treatment and specially when is integrated in the agriculture sector (Möller, 2009; Prochnow, 2009; Chynoweth, 1987). In addition to the organic wastes treatment there is an emerging interest in the production of biomethane as a biocombustible through anaerobic digestion of biomass and / or energy crops (IEA, 2010; Chanakya, 2009; CONCAWE, 2008; Tilche, 2008; Yadvika, 2004; Gunaseelan, 1997; Chynoweth, 1987). *Cynara cardunculus* L. or commonly known as cardoon is a perennial herb or herbaceous crop native to Mediterranean region, grown since ancient times as a wild plant or as vegetable using intensive management techniques (Ortega, 2007; Fernández, 2006). The average biomass annual production varies from 15 to 20 tons of biomass/ha depending on soil and rainfall with 11% of moisture content and the following biomass partitioning: 40 % stalks, 25% leaves and 35 % capitula (Gominho, 2001 and 2008). Different studies have been shown the high potential of this plant as energy crop: the aerial biomass used as a solid biofuel and the oil from seeds used for the production of biodiesel (Fernández, 2006). However, the interest in their use in the production of biomethane has never been investigated. Different studies show that the addition of biomass or energy crops to the anaerobic digestion of cattle dung or the anaerobic digestion of energy crops residues with the addition of partially digested cattle dung or sewage digested sludge enhanced biogas production and methane yield (Chanakya, 2009; Yadvika, 2004). The goal of the research performed on *Cynara cardunculus* L. was to increase the knowledge about the use of this promising industrial crop for biogas production in Mediterranean countries or countries with similar edapho-climate conditions.

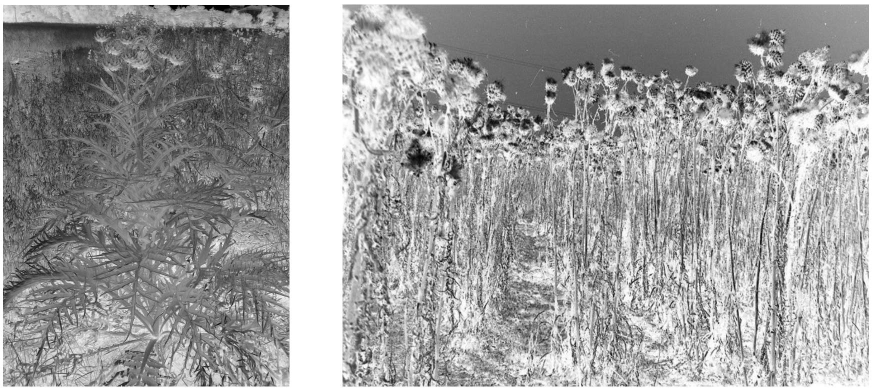

FIGURE 1 ***Cynara cardunculus*** **L. or cardoon aspect during vegetative cycle (left) and at the end of the cycle (right)**

2 MATERIALS AND METHODS

2.1 Substrate and Inoculums

The *Cynara cardunculus* L. used in these studies was collected from a pedagogic field (BioEnergISA) built at ISA campus (Instituto Superior de Agronomia, Lisboa) in October 2008. The leaves and the capitula were removed and only the stalks were used. The stalks were grounded, sieve and a 40-60 Mesh fractions of the material was stored in a dry place at environment temperature. This material is designed as substrate (***S***). In the anaerobic digestion experiment two different substrates were used a substrate submitted to a pre-treatment and a untreated substrate. The pre-treated substrate was submitted to a thermal pre-treatment using a 160ºC and a 12.5 % of solids during 30 min.

It was weighed 30 g of 40-60 Mesh fractions of *Cynara* stalks and 240 ml of distilled water were placed into small inox steel reactors using a solid/water ratio of 1/8. The inox steel reactors were tightly closed and placed in a rotate oil heated bath (160ºC) for 30 min.

As inoculums (***I***) was used digested sewage sludge from a Waste Water Treatment Plant (WWTP, ETAR de Chelas, Lisboa). The sewage sludge was freshly collected using conventional cleaned plastic containers of 5L during the start-up of the anaerobic digestion experiment.

2.2 Experimental procedure

Laboratory experiments were carried out using three batch reactors with a working volume of 2000 ml and were operated using mesophilic temperature of 37 ºC within a incubation time of 32 days. The temperature was maintained using a water-heating jacket, in which the water was circulated through a water heater. The protocol experiment was based on Gunaseelan (1995). In these experiments three reactors were used B_0 containing *inoculum*, B_1 containing *inoculum* and pre-treated substrate and B_2 containing *inoculum* and untreated substrate. Reactors content were mixed everyday for 10 min by a mechanical stirrer. The experiment was conducted using the following protocol:

TABLE 1 **Protocol used for the experiment**

Reactors	**H_2O addition** (ml)	**Substrate concentration (S)** (g VS l^{-1})	**Inoculum (I)** (ml)	**I/S ratio** (ml **I**/g VS **S**)
B_0 (control)	462	NA	1538	NA
B_1 (pre-treated substrate) & B_2 (untreated substrate)	462	28.7	1538	116.1

Note: NA, not applicable

The high volume of *inoculum* adopted was based on the experimental procedure adopted in Gunaseelan (1995) to maintain a constante pH.

2.3 Analytical methods

The chemical composition of the whole stalks fractions was determined using Tappi standard methods in relation to ash, moisture content, extractives, lignin and polysaccharides as described in (Gominho, 2001). *Cynara* stalks were also characterized in terms of Total solids, Volatile solids, Total Organic Carbon (TOC), Minerals (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Mn^{2+}, Cu^{+}, Zn^{2+}, Fe^{2+} and P^{5+}), Nitrogen ($N\text{-}NH_4^{+}$, N_K and N_{total}) and pH according to Standard Methods.
During anaerobic digestion experiment different measurements were applied volatile solids and total solids reduction, biogas accumulation, methane content and pH.

Biogas accumulation was measured according the gas-liquid displacement method using as biogas holders 1l and 2l cylinders. Biogas was collected using the SGE GAV-200 MK ll system and the samples were storage in a refrigerator at -4ºC. The methane content was determined at weekly intervals using Variant-3800 gas chromatograph with a thermal conductivity detector with helium as the carrier gas at 99.9995 %. The injector, column and detector temperatures were 60ºC, 50ºC and 150ºC respectively. The methane yield was calculated by subtracting the amount of methane produced by the control from the methane production of each reactor and dividing the difference by the mass of VS in the substrate fed to the reactor.

The following formula was used to estimate the VS reduction:

$$\text{VS reduction (\%)} = 100\,(M_0-(M_n-M_i))/M_0,$$

where M_0 is the mass of VS in *Cynara* added to the reactors (g); M_n is the mass of VS in the reactor at the end of fermentation (g); and M_i is the mass of VS in the *inoculums* reactor B_0 (control) at the end of fermentation (g).

3 RESULTS AND DISCUSSION

3.1 Substrate Composition

Among the constituents of *Cynara* stalks C/N and C/P ratios are the most practical and important parameters for anaerobic digestion. The high correspondent values indicated that this substrate has to be co-digested with another component (Mshandete, 2004). The digested sewage sludge used as inoculums has better nitrogen content. In this way was decided to use an I/S ratio of 116.1 wish corresponded to C/N ratio of 14. Also in terms of polysaccharides content it’s possible to say that *Cynara* stalks present 35% and 21% of cellulose and hemicelluloses respectively (Table 2). In terms of extractives value there is a 7% difference between the ones already published (Gominho, 2001 and 2008; Fernández, 2006). This difference could be related to the fact that after stalks harvest they were left on the field for one month and had possibly occurred lixiviation of extractives.

TABLE 2 **Composition of the dry Cynara stalks**

Constituent	% of TS
Volatile Solids (VS)	95.7
Total Organic carbon (TOC)	36.6
Total Nitrogen (N_{total})	0.30
Kjeldahl Nitrogen (N_K)	0.30
$N\text{-}NH_4^+$	0.02
Minerals (Ca^{2+}, Na^+, Mg^{2+}, K^+, Mn^{2+}, Cu^+, Zn^{2+}, Fe^{2+} and P^{5+})	2.7; 1.7; 0.3; 0.5; 0.0; 0.01 and 0.1
C/N	126
C/P	305
Extractives	6.4
Total Lignin	20.49
Total Polysaccharides:	
Monosacharides composition	**% of Sugars**
Glucose	35.2
Xylose	16.7
Manose	1.4
Arabinose	1.3
Galactose	1.4
Rhamnose	0.6

3.2 Anaerobic digestion experiment

The results achieved in terms of cumulative methane yield and VS reduction are in accordance with other energy crops (Prochnow, 2009; Gunaseelan, 1997 e 2008; Chynoweth, 1987). However, during the first days of the experiment there were gas leaks that affected indirectly cumulative methane yield determination which contributed to an underestimation of this parameter. Notwithstanding results show that *Cynara* stalks are a good substrate for biogas and methane production with 53% CH_4 content. Assuming that *Cynara* stalks have a cumulative methane yield between 0.3-0.4 l CH_4/g VS_{added}, (Figure 1 and Table 3) a crop productivity of 15 ton/ha which 40% are stalks (Gominho, 2001 & 2008) and 9.97 kWh/m^3 heating value of biogas (Ahrens, 2004) it is possible to achieve an energy production between 42.90 – 57.2 MWh/ha with the use of Biogas.

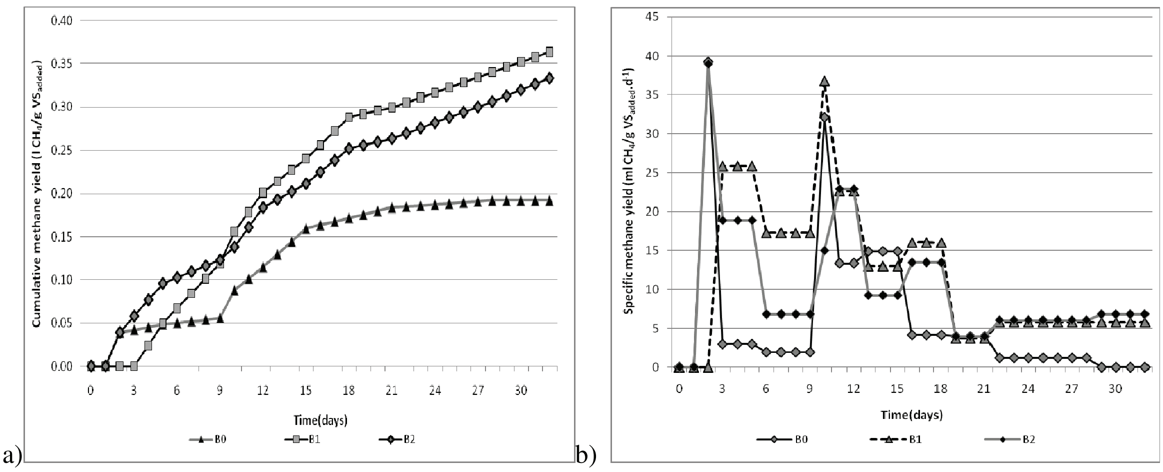

FIGURE 2 **a) Cumulative biogas production during the anaerobic digestion experiment (B0-Control; B1-containing pre-treated substrate; B2- containing untreated substrate); b) Daily biogas production during the anaerobic digestion experiment (B_0-Control; B_1- containing pre-treated substrate; B_2- containing untreated substrate)**

TABLE 3 **Results of the Anaerobic digestion experiment**

Reactors	pH_0 (substrate)	pH_i(Mixture)	pH_f	VS reduction (%)	Specific Biogas Yield (l/g VS_{added})*	Specific Methane yield (l/g VS_{added})*
B_0	NA	6.88	7.28	NA	0.19	0.19
B_1	4.44	6.91	7.25	56.15	0.68	0.36
B_2	6.25	7.09	7.30	41.69	0.63	0.33

*There was one unpredicted leak gas in both reactors during the first 7 days which affected biogas quantification; Note: NA, not applicable

4 CONCLUSIONS

Cynara cardunculus L. is already a promising energy crop for Bioenergy field in Mediterranean countries. The results achieved show that *Cynara* stalks are a good substrate for biogas and methane production in Mediterranean countries. *Cynara* stalks have a specific methane yield that could possibly be higher than 0.3 l CH4/g VS_{added} depending on the pre-treatment applied. However, there is still work to be done in terms of choosing the most suitable pre-treatment for Mediterranean countries. There is also an interest in using the all crop material (stalks, leaves and seeds) for methane production (Gunaseelan, 2008) or combining two processes Biodiesel and Biogas production through co-digestion glycerol and de-oiled cake with other substrates.

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