Influence of inoculum, particle size and inoculum-substrate ratio on CH_4 production from *Ulex* sp.

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ABSTRACT

The performance of Anaerobic Digestion (AD) of solids wastes is affected by several factors. Most of them are related to each other. Currently, publish studies about AD only care about the individual influence of these variables, discarding possible interaction. A response surface experimental design was used to determine the most important variables and possible interactions – influence of inoculum type (anaerobic suspended sludge and granular sludge), *Ulex* sp. particle diameter (dp) and inoculum to substrate ratio (ISR) – on the Biochemical Methane Potential (BMP) and the maximum initial methane production rate (*k*). BMP and *k* of *Ulex* sp. varied between 153-308 L CH_4 kg^{-1} VS and 14-49 L CH_4 kg^{-1} VS d^{-1}, respectively. Higher ISR and a mixture of granular and suspended sludge had a positive effect on the biodegradability of waste. A dp of 1.85 mm were defined as the optimal condition to simultaneously maximize the BMP and *k*.

KEYWORDS

Anaerobic Digestion; Biochemical Methane Potential; Biodegradability Rate; Factorial Experimental Design; *Ulex* sp.

INTRODUCTION

Shrubland areas, rich in biomass, occupy 1.9 million hectares in the Portuguese territory (AFN, 2011) and close to 1 million hectares in the region of Galicia (Spain) (Núñez-Regueira et al., 2004). These areas are increasing rapidly due to the abandonment of agricultural land, which is rapidly becoming shrubland. *Ulex* sp. (also known as gorse, furze or whin) is an evergreen shrub in the family *Fabaceae*, which creates an extreme fire hazard due to its oily, highly flammable foliage and seeds. Lignocellulosic biomass from forests, due to its widespread availability, at relatively low cost, and because it does not compete with food crops is an option as alternative and renewable energy source.

Anaerobic digestion (AD) could be a potential treatment for biodegradable solids wastes. It consist in the organic recycling as it provides renewable energy, namely biogas (Borjesson et al., 2014). Eiroa and co-workers (2012) determined the biomethane potential of *Ulex* sp. in 160 L CH_4 kg^{-1} VS. The performance of AD, in term of methane produced, depends of several factors, including the C:N ratio, particle size, pH,

inoculum to substrate ratio (ISR), macro and micronutrients, inhibitory or toxic compounds and dry matter content (Mata-Alvarez et al., 2000). Usually, possible interactions are not considered. In most cases, the effects are studied independently. A design of experiments is an efficient statistical analysis that optimize the factors that are interrelated (Gilmour, 2006).

This work aims at determine the influence of inoculum type, substrate particle diameter (dp) and ISR, on the Biochemical Methane Potential (BMP) and methane production rate of *Ulex* sp. A response surface experimental design was used to statistically determine the most important variables and possible interactions.

METHODS

Inoculum and substrate. Biodegradability assays were performed using three different Inocula (Table 1): anaerobic suspended sludge (S) from a municipal WWTP, anaerobic granular sludge (G) from a brewery industry, and a mixture (M) of the two previous inocula (50:50% v/v). Inocula were characterised in terms of total and volatile solids (TS and VS, respectively) content. The specific methanogenic activity (SMA) was determined according to described in Costa et al. (2012b). Ulex sp. (Table 1) was collected in a shrubland in the North of Portugal and dried at room temperature. It was characterised in terms of solids, chemical oxygen demand (COD) and nitrogen. Then, gorse was crushed in an industrial mill into 3 different fractions: dp of 0.5 mm, 2.25 mm, and 4 mm.

Table 1.

Anaerobic biodegradability assays. Anaerobic biodegradability batch assays, following a response surface methodology design of experiments, were used to determine the BMP and methane production rate from *Ulex* sp., with different dp, digested with different inocula, with variation of ISR.

Response surface design of experiments. A Central Composite Face Centred (CCFC) was used. The effect of two numeric factors, dp of *Ulex* sp. and ISR, and one categorical factor, inoculum type (S, M and G), were studied on two response variables, BMP and maximum initial methane production rate (*k*), using a response surface methodology. The levels used in the anaerobic biodegradability assays are shown in Table 2. The design matrix of the experiments and their statistical analysis were made by means of the software package Design-Expert ® (Stat-Ease, Inc., Minneapolis, USA). The experiments were randomly performed. BMP and *k* data were processed for Eq. (1), including the analysis of variance to obtain the interaction between the process variables and the responses. The *p-values* of the parameters estimation were used to validate the model, where *p-value* ≤ 0.05 indicated significant model terms.

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \beta_{ijk} X_i X_j X_k \qquad (1)$$

Where Y_i indicates the predicted response variable; β_0 is the constant coefficient; β_i is the coefficient of the X_i; β_{ij} and $\beta_{ijk}$are the interaction coefficients; and X_i, X_j, X_k are the independent variables.

Table 2

Experimental procedure. Biodegradability assays were performed according to the directives defined in Angelidaki et al. (2009). All batch tests were performed in triplicate at 37 ºC. Blank (only with inoculum) and control (with microcrystalline cellulose (50μm)) assays were also performed. BMP was determined per unit of volatile solids (VS) of *Ulex* sp. added to each vial. The blank was used to discount the methane produced by the residual substrate present in the inoculum. Methane yield (B_0) was defined as the amount of CH_4 produced during the assays in relation to the theoretical BMP (Costa et al., 2012a). Methane accumulated in the vials' headspace was measured by gas chromatography using a gas tight syringe to sample 500 μL. Methane production was corrected for standard temperature and pressure (STP) conditions (0°C and 1 atm).

Analytical Methods. Ammonium (NH_4^+), Total Kjeldahl nitrogen (TKN), total solids (TS), and VS were measured according to standard methods (APHA, 1998). Total and soluble COD were determined using standard kits (Hach Lange, Germany). The methane content of biogas was analysed with GC (Chrompack 9000), as described in Costa et al (2012a). The GC was equipped with a FID and Carbowax 20M (80-120 mesh) column (2 m x 2 mm). Nitrogen was used as carrier gas (30 mL min^{-1}). The detector, injector, and oven temperatures were 35, 110, and 220ºC, respectively. Volatile Fatty Acids (VFAs) were determined by Jasco HPLC (Tokyo, Japan), using a Metacarb column maintained at 60°C and with UV/VIS detection at 210 nm. The mobile phase was sulfuric acid (5 mM) fed at a rate of 0.6 mL min^{-1}. Crotonic acid was used as internal standard.

RESULTS AND DISCUSSION

Effect on the biochemical methane potential. Avicel (microcrystalline cellulose) had methane yields of 93±2, 98±2 and 96±4 %, respectively for S, M and G. Although the SAA of S and M sludge were very low, they were capable of effectively biodegrade the substrate and convert it to CH4. The best results were 308 L CH_4 kg^{-1} VS with G, dp=0.5 mm, ISR=4 and with M, dp=2.25 mm, ISR=4. B_0 varied between 31 and 62%, confirming that a significant portion of the available substrate is not being converted to CH_4. A statistical analysis was carried out to quantify which factors and respective interactions have more influence on the BMP' determination. All variables were considered significant (p-value<0.05) ($A=C>B^2>B>C^2$) (Eq. 1), and no interactions were considered significant.

$$BMP=126.8+32.6A+30.7B+16.8C-7.9B^2+4.2C^2 \quad \text{(Eq. 1)}$$

The response surface of the specific methane production from the digestion of *Ulex* sp. in different conditions is shown in a three dimensional graph in Figure 1a-b-c. Decreasing the ISR from 4 to 1 caused 35-40% decrease in the BMP. This trend is shown in the response surface in Figure 1b-c. The BMP is the maximum methane that can be produced by a given substrate. Therefore, it should be independent on the inoculum type, source, activity, concentration, etc., while only the rate should change. However the BMP determined with S were 12-23% lower than the ones determined using G as inoculum (Figure 1a-b), considering a similar time frame. BMP increased with higher % of G, although with lower variation compared with the ISR effect (Figure 1b). Eventually, by extending the time frame, the BMP differences between each assay

would decrease or even be eliminated. However, it would be impossible to extend the retention time over 40 days due to economic and operational reasons in real operations. A concave surface was observed in Figure1a-c, with the optimal dp near the middle level tested.

According to the model defined, the maximum BMP for *Ulex* sp. would be obtained using granular sludge as inoculum (A=1), an ISR of 4 g $VS_{inoculum}$ $g^{-1}VS_{substrate}$ and dp=1.95 mm (upper corner in Figure 1b, and highest point in Figure 1c). In those conditions the BMP would be 324 L CH_4 kg^{-1} VS (B_0=65%). This result increased 100% the BMP achieve with Eiroa et al. (2012).

Effect on the biodegradability rate. The maximum initial biodegradability rate (L CH_4 kg^{-1} VS d^{-1}) was determined as the slope of the initial specific CH_4 production. *Ulex* sp. had a *k* from 14.1 to 49.2 L CH_4 kg^{-1} VS d^{-1}. Lower p-value correspond to the most significant parameters, thus the model should consider: A=C=A^2=B^2>AC>B>C^2>BC (Eq. 2).

$$k=7.6+36.2A+4.2B+5.1C-1.4AC-0.25BC-27.8A^2-0.9B^2+0.5C^2 \quad \text{(Eq. 2)}$$

Figure 1d-e-f represent the maximum initial methane production rate of *Ulex* sp. digested with different conditions (variations of dp, ISR and inoculum). The best *k* (49.2 L CH_4 kg^{-1} VS d^{-1}) would be obtained with a mixture of S and G (45:55%, v/v), dp=1.80 mm and ISR=4. ISR had an effect almost linear. *k* increases with the ISR increase (Figure 1e-f), since with less substrate the biodegradation will be faster. *k* increases when adding G to the inoculum until it reaches a mixture containing 55% of G and 45% of S (Figure 1d-e). Although the G is more active and therefore capable of convert the metabolites into CH_4, maybe the higher surface area of the suspended sludge is favourable for the initial hydrolysis of the residue. The structured layers of granular sludge shield the methanogenic microorganisms inside the granules, making it less susceptible to inhibitions, but simultaneously hamper the recalcitrant residue access. Also, the particle size shows a concave surface with the optimum diameter at 1.80 mm, although the differences are not high (Figures 1d-f).

Figure 1

CONCLUSIONS

(i) BMP of *Ulex* sp. varied between 153-308 L CH_4 kg^{-1} VS. Higher BMP values were obtained with higher ISR and granular sludge as inoculum. (ii) *k* varied between 14-49 L CH_4 kg^{-1} VS d^{-1}. A mixture of granular and suspended sludge and a high ISR had a positive effect on the initial biodegradability rate. (iii) No inhibitions were observed and the hydrolysis was the limiting step. (iv) An inoculum consisting in 55% G and 45% S (v/v), an ISR $\geq$ 4 g $VS_{inoculum}$ g^{-1} $VS_{substrate}$, and dp=1.85 mm were defined as the optimal condition to simultaneously maximize the BMP and *k*.

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Table 1. Solids content, specific acetoclastic activity (SAA), and hydrogenotrophic methanogenic activity (SHMA) of inocula used in anaerobic biodegradability assays. *Ulex* sp. characterisation.

Parameter (Units)	**S**	**G**	**M**
TS (g L^{-1})	75.0±9.4	90.4±1.0	76.5±3.1
VS (g L^{-1})	44.2±4.3	83.2±1.0	55.7±2.7
SAA (mL $CH_{4@STP}$ g^{-1} VS d^{-1})	<10	130±15	24±8
SHMA (mL $CH_{4@STP}$ g^{-1} VS d^{-1})	134±18	357±14	285±11
			***Ulex* sp.**
TS (g g^{-1}_{Ulex})			0.68±0.02
VS (g g^{-1}_{Ulex})			0.66±0.02
COD (g g^{-1}_{Ulex})			0.94±0.05
N-Kjeldahl (g N g^{-1}_{Ulex})			0.0163±0.0002
Ammonium (g N-NH_4^+ g^{-1}_{Ulex})			0.0080±0.0021

Table 2. Factors and respective levels in the experiments design of Central Composite Face Centred.

Factor (Units)	**Real values of coded levels**		
	-1	**0**	**+1**
A: Inoculum (*not applicable*)	S	M	G
B: dp (mm)	0.5	2.25	4
C: ISR (g $VS_{inoculum}$ g^{-1} $VS_{substrate}$)	1	2.5	4

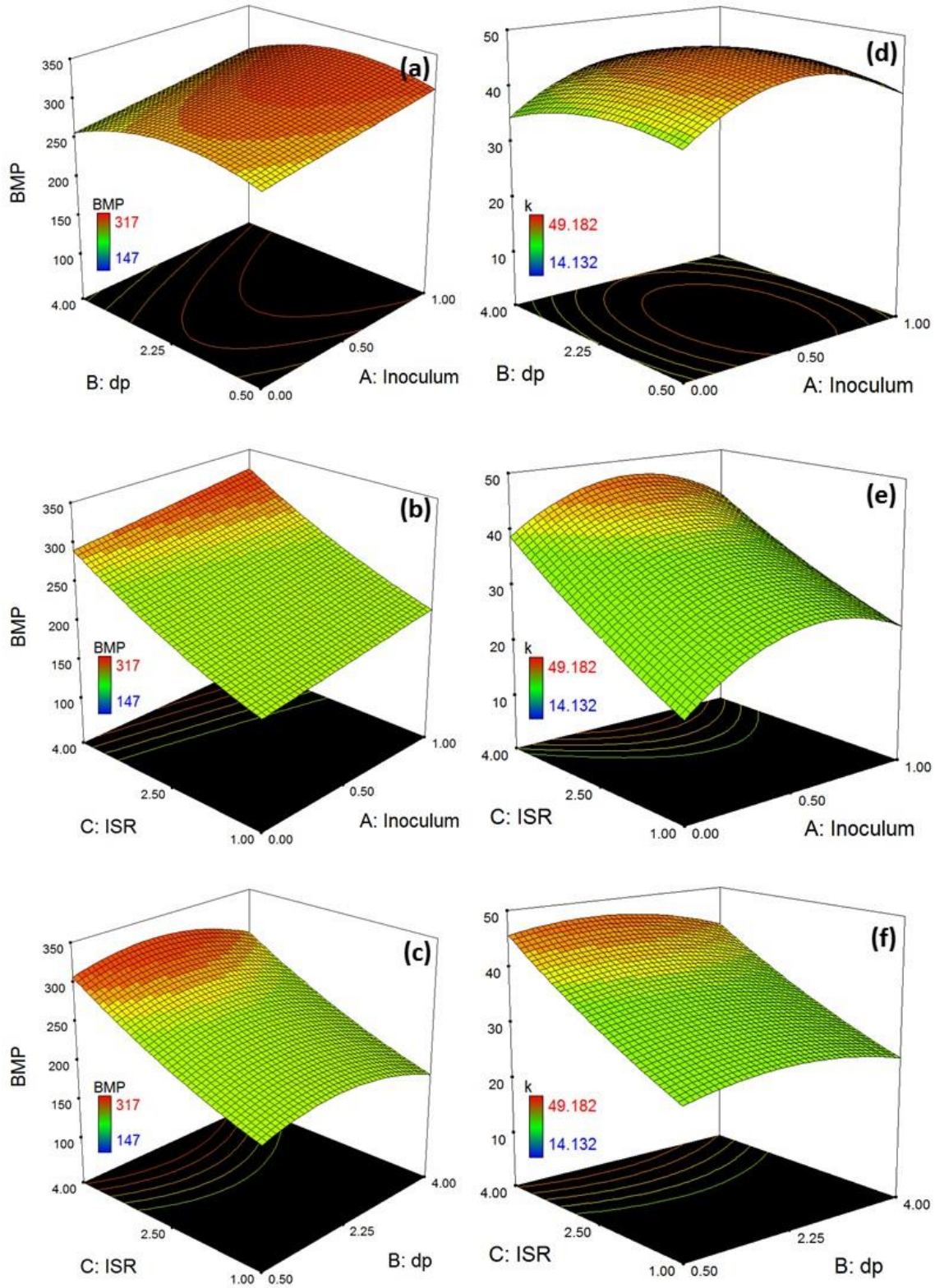

Figure 1: (a-b-c) Response surface of the BMP (L CH_4 kg^{-1} VS) as a function of: (a) inoculum and dp (ISR=4); (b) Inoculum and ISR (dp=1.95mm); and (c) dp and IRS (granular sludge). (d-e-f) Response surface of the maximum initial biodegradability rate (*k*) (L CH_4 kg^{-1} VS d^{-1}) as a function of: (d) inoculum and dp (ISR=4); (e) Inoculum and ISR (dp=1.80mm); and (f) dp and IRS (granular sludge).