

# Enhanced Biogas Production through Anaerobic co-Digestion of OFMSW with Maize Cob Waste Pre-Treated with Hydrogen Peroxide

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The enhancement of biogas and CH<sub>4</sub> yields through anaerobic co-digestion (co-AD) of the pre-hydrolysed Organic Fraction of Municipal Solid Waste (hOFMSW) and Maize Cob Waste (MCW) was tested in a lab-scale thermophilic anaerobic reactor. Before the co-AD, MCW was chemically pre-treated with H<sub>2</sub>O<sub>2</sub> at room temperature in alkaline conditions (10 % MCW  $w_{MCW}/v_{alkaline\ water}$ ) with 4 hours contact time, at a ratio of 0.5  $w_{H_2O_2}/w_{MCW}$ . This pre-treatment is the last of a set of pre-treatments catalysed by H<sub>2</sub>O<sub>2</sub> already tested by the authors. The anaerobic reactor was initially fed with hOFMSW obtained from the hydrolysis tank of an industrial AD plant. The hOFMSW was co-digested with the pre-treated MCW and then with non-pre-treated MCW. The co-AD of hOFMSW with pre-treated MCW (CASE 1) increased the biogas yield by 65 % and CH<sub>4</sub> yield by 48 %, when compared to the results obtained with the standalone hOFMSW, while the co-digestion of hOFMSW with non-pre-treated MCW (CASE 2) increased biogas and CH<sub>4</sub> yields by 84 % and 57 %, respectively. Despite of higher yields, the Low Heating value (LHV) and daily energy content (LHV \* Volume of biogas per day) in biogas stream, obtained in CASE 2 were on average 4 % and 1% lower, respectively than in the biogas stream obtained with CASE 1. These results showed that the pre-treatment is recommended before submitting MCW to co-AD and the chemical pre-treatment of MCW with H<sub>2</sub>O<sub>2</sub>, at room temperature, is a promising low-cost way to valorise MCW through co-AD.

## 1. Introduction

Anaerobic Digestion (AD) is a microbiological process able to produce biogas, a renewable fuel suitable for electricity and heat production, as well as for biomethane production (Novosel et al., 2014).

The efficiency of the AD process is affected by the carbon and nitrogen ratio (C:N) in the substrate, whose optimal value is of 25 (Jain et al., 2015). The co-AD of different biomasses allows to achieve the optimal C:N ratio enhancing AD efficiency (Zhang et al., 2013).

The Organic Fraction of Municipal Solid Waste (OFMSW) and Maize Cob Waste (MCW) are suitable for AD. The OFMSW is characterised by a low C:N ratio, due to its high proteins content. Maize is a carbon rich biomass, has low biodegradability and does not contribute significantly to soil fertility; therefore, it can be harvested for other valorisation pathways (Bortoluzzi et al., 2014). MCW is a lignocellulosic biomass mainly composed of cellulose, hemicellulose and lignin. Only cellulose and hemicellulose are fermentable by bacteria after hydrolysis, thus the pre-treatment makes these compounds more accessible and allows some lignin solubilisation before AD. The pre-treatment conditions have to be accurately chosen, because severe conditions may promote metabolic and physiologic inhibitions of the AD process, due to the formation of phenolic compounds or furfural and 5- hydroxymethylfurfurals (5-HMF) (Hendriks and Zeeman, 2009).

The main aim of this work is to test the efficiency of the pre-treatment of MCW at room temperature, in the presence of H<sub>2</sub>O<sub>2</sub>, at the optimized condition of a pH 9.8, 10 %  $w_{MCW}/v_{alkaline\ water}$ , and 4 h of reaction time, for

biogas production during co-AD of hOFMSW. The results obtained were compared with the co-AD of hOFMSW+MCW not submitted to a pre-treatment and the co-AD of hOFMSW with MCW pre-treated with H<sub>2</sub>O<sub>2</sub>, under different conditions previously tested by the authors (Surra et al., 2017). This work contributes to the development of a new pathway to valorise MCW that presently is not valorized.

## 2. Material and Methods

### 2.1 Feedstock Characterization

The hOFMSW was collected from the hydrolysis tank of an industrial Portuguese AD plant, located in Lisbon. This waste is produced in canteens, restaurants and malls. It was stored at 4 °C in glass bottles until use. In contrast, MCW was collected from a local Portuguese farmer located in Coruche, in Lisbon surroundings. It was air-dried up to a moisture content of 13 % w/w, grounded (2-4 mm) and stored at 4 °C until use. The characterisation of the hOFMSW was performed in duplicate samples and included: Total Solids (TS), Ashes and Volatile Solids (VS) (method APHA 2540:2005); total Chemical Oxygen Demand (tCOD) and soluble Chemical Oxygen Demand (sCOD) (method APHA 5220 B:2005); Total Kjeldal Nitrogen (TKN) (method ISO 5663:1984), Ammonium Nitrogen (NH<sub>4</sub>-N) (method ISO 5664:1984) and Organic Nitrogen (o-N) (TKN-NH<sub>4</sub>-N); phosphorus (P) (method ISO 6878:2004); Elemental Analysis (EA); and Volatile Fatty Acids (VFAs) – (Acetic Acid (AA), Formic Acid (FA), Propionic Acid (PA) and Butyric Acid (BA)). EA was performed on the dried OFMSW (2 h at 105 ± 1 °C) in a Thermo Finnigan Elemental Analyzer - CE Instruments, model Flash EA 1112 (CHNS). VFAs were analysed with a HPLC system (Dionex ICS3000, USA).

The characterisation of MCW included: moisture content, TS, Ashes and VS, TKN, NH<sub>4</sub>-N, o-N, P, EA, lignin, cellulose and hemicellulose. The analytical methodologies were the same as described for the hOFMSW. Lignin, cellulose and hemicellulose contents were assessed according to Goering and Van Soest (1970).

### 2.2 Pre-Treatments of MCW

In order to remove lignin and solubilise part of the hemicellulose and cellulose into digestible sugars, MCW was submitted to chemical pre-treatments at room temperature in the presence of H<sub>2</sub>O<sub>2</sub>, at a 0.5 w<sub>H<sub>2</sub>O<sub>2</sub></sub>/w<sub>MCW</sub> ratio, pH of 9.8 and with 10 % w<sub>MCW</sub>/v<sub>alkaline water</sub> and 4 hours of reaction time (Pre3). This pre-treatment was compared pre-treatments Pre1 and Pre2 previously tested (Surra et al., 2017) and shown in Table 1.

*Table 1: Pre- treatment conditions applied to MCW before co-AD with the OFMSW. Apart from the mentionend conditions, all the assays were submitted to a 0.5 H<sub>2</sub>O<sub>2</sub>/MCW (w/w) ratio at pH 9.8.*

Assay	Catalyst	T (°C)	t (h)	Reference
hOFMSW+Pre1	2 % MCW (w <sub>MCW</sub> /v <sub>alkaline water</sub> )	23	4	Surra et al. (2017)
hOFMSW+Pre2	10 % MCW (w <sub>MCW</sub> /v <sub>alkaline water</sub> )	23	72	Surra et al. (2017)
hOFMSW+Pre3	10 % MCW (w <sub>MCW</sub> /v <sub>alkaline water</sub> )	23	4	This work

The Pre3 was performed without stirring, by mixing 1 g of ground MCW and 40 mL of catalyst solution. The samples were filtered in Gooch crucibles under low vacuum (Vacuubrand GMBH, Germany). The remaining solid fraction was dried at 105 °C, during 2 h, and submitted to lignin, cellulose and hemicellulose quantifications. The liquid fraction was analysed in duplicate for monomeric sugars and inhibitors (phenolic compounds, furfural and 5-HMF). Monomeric sugars and phenolic compounds concentrations were determined through HPLC (ICS 3000 DIONEX). Furfural and 5-HMF were assessed by HPLC (Thermo Surveyor and Dionex ICS3000). The efficiency of the pre-treatment was calculated through the assessment of lignin, cellulose, and hemicellulose and monosaccharides solubilisations, as well as phenolic compounds, furfural and 5-HMF production. The results were statistically analysed (ANOVA; p-value=0.05). Lignin, cellulose and hemicellulose solubilisations were calculated according to Eq(1):

$$\alpha (\%) = 1 - \frac{\alpha \text{ after pre - treatment}}{\alpha \text{ in MCW}} \times 100 \quad (1)$$

Where  $\alpha$  stands for the total removal percentage of lignin, cellulose and hemicellulose.

## 2.3 Anaerobic Digestion Assays

The AD assays were carried out in a 2.1 L lab-scale stirred reactor (New Brunswick Scientific, NY, USA) under thermophilic condition ( $50 \pm 2$  °C), at pH of 8.0. The hOFMSW used is the same as of the industrial-scale digester. The lab-scale anaerobic digester was started up with hOFMSW at an Organic Load Rate (OLR) of 2.16 g VS/(L.d), a hydraulic retention time (HRT) of 21 d, until the biogas production was stable. The pre-treated MCW under condition Pre3, and the non pre-treated MCW were co-digested with the hOFMSW at an OLR of 2.48 g VS/(L.d) (+15 % OLR). The biogas composition, removal percentages of TS, VS, tCOD, and sCOD, and TKN, NH<sub>4</sub>-N, o-N, and VFAs were assessed in duplicate to check reproducibility. Biogas and CH<sub>4</sub> yields were calculated according to Eq(2):

$$\eta_{\alpha} = \frac{V_{\alpha}}{OLR \times V_{inflow}} \quad (2)$$

where  $\eta_{\alpha}$  stands for biogas or CH<sub>4</sub>,  $V_{\alpha}$  is the volume of  $\eta_{\alpha}$  produced (L),  $OLR$  is the Organic Load Rate (gVS/L.d), and  $V_{inflow}$  is the volume of substrate fed per day (L/d).

The removals of VS, TS, tCOD, and sCOD were assessed through Eq(3):

$$\eta_{i,removal}(\%) = \frac{C_{i,inflow} - C_{i,digestate}}{C_{i,inflow}} \times 100 \quad (3)$$

Where  $C_i$  is the concentration of solids (g/L) or COD (mg O<sub>2</sub>/L) either in the inflow or in the digestate.

The biogas composition was determined by gas chromatography (Varian 430-GC) according to ASTM D 1946, and by a portable GFM 410 Analyser (GASdata, UK). The Low Heating Value (LHV) is calculated according to IPCC (2006) (Eq. 4):

$$LHV(MJ/m^3) = \sum_{i=1}^n HHV_i x_i - (0.212H - 0.0245M - 0.008Y) \quad (4)$$

where  $HHV_i$  is the High Heating Value of the component  $i$  (MJ/m<sup>3</sup>),  $x_i$  is the volume fraction (% v/v) of the component  $i$  in the biogas stream, and  $H$ ,  $M$  and  $Y$  are the volume percentages of H<sub>2</sub>, moisture and O<sub>2</sub>, respectively.

TKN, NH<sub>4</sub>-N, o-N, VFAs and EA were performed according to methodologies described in section 2.2.

Experimental data were compared with the results obtained in the AD of the standalone hOFMSW, co-AD of hOFMSW+Pre1 and co-AD of hOFMSW+Pre2 (Surra et al., 2017).

## 3. Results and discussion

### 3.1 Effect of the Pre-treatments

Table 2 shows that during Pre2 (3 d reaction time), the solubilizations of cellulose, lignin and hemicellulose were always higher than 60 % w/w. In Pre1 and Pre3 (4 h reaction time), lignin, hemicellulose and cellulose removals did not exceed 5.0 % w/w (Pre3), 5.6 % w/w (Pre3) and 11.3 % w/w (Pre1), respectively. This confirms that reaction time affects significantly lignin removal (Banerjee et al., 2011). Although Gould (1985) claimed that very low lignin can be solubilised at pH lower than 11, the significant lignin removal, obtained during Pre2 at pH of 9.8, suggests that reaction time has a key role in biomass depolymerisation. At pH of 9.8, and constant reaction time (4 h), the increase of MCW concentration from 2 % w<sub>MCW</sub>/V<sub>alkaline water</sub> (Pre1) to 10 % w<sub>MCW</sub>/V<sub>alkaline water</sub> (Pre3) allowed only slight lignin solubilisation changing from 0 % w/w in Pre1 to 5 % w/w in Pre3.

Table 3 shows that in Pre2 and Pre3 glucose and fructose solubilizations were on average 4 times higher than in Pre1. These results suggested that higher MCW concentration (10 % w<sub>MCW</sub>/V<sub>alkaline water</sub> during Pre2 and Pre3) originates higher sugar solubilisation in the liquid phase and that keeping constant the pH (9.8), MCW concentration is more important than the reaction time for sugar release.

In all the samples, neither furfural nor 5-HMF were detected (DL furfural: <0.1 mg/L; DL 5-HMF: <0.5 mg/L). Table 4 shows the concentrations of phenolic compounds after pre-treatment: p-Coumaric acid (p-CA) and Ferulic Acid (FA) were the main phenolic compounds produced, followed by minor concentrations of Caffeic (CA), Chlorogenic (Cl) and Protocatechuic (CT) acids. The samples submitted to Pre2 (3 d reaction time), showed an average concentration of 9.12 mg/L p-CA and 10.2 mg/L FA. During Pre3, 6.0 mg/L p-CA, 2.4 mg/L FA, 3.5 mg/L CA, 1.8 mg/L Cl, and 2.4 mg/L CT were quantified. All concentrations were below those considered critical for the AD process according to the available literature (Akassou et al., 2010).

Table 2: Cellulose, lignin and hemicellulose solubilizations of MCW (pH 9.8).

Assay	Cellulose (% w/w)	Lignin (% w/w)	Hemicellulose (% w/w)	t (h)
Pre1	11.3	0.0	0.0	4
Pre2	63.4	68.6	61.9	72
Pre3	0.0	5.0	5.6	4

Table 3: Concentration of monomeric sugars in the pre-treated liquid phase.

Assay	Glucose (mg/L)	Fructose (mg/L)	Arabinose (mg/L)	Xilose (mg/L)	Galactose (mg/L)	t (h)
Pre1	148	181	<1.2	<1.7	<5.9	4
Pre2	928	846	<1.2	<1.7	<5.9	72
Pre3	653	710	19.0	34.0	3.0	4

Table 4: Concentration of phenolic compounds in the pre-treated liquid phase.

Assay	p-CA (mg/L)	FA (mg/L)	CA (mg/L)	Cl (mg/L)	CT (mg/L)
hOFMSW +Pre1	1.08	0.35	< 0.85	< 0.35	< 1.10
hOFMSW +Pre2	9.12	10.21	< 0.85	< 0.35	< 1.10
hOFMSW +Pre3	6.00	2.40	3.50	1.80	2.40

### 3.2 Co-Anaerobic Digestion Assays

TS and VS removals did not show any significant difference (ANOVA,  $p=0.05$ ) in co-AD assays (Table 5), with average removals of 51.9 %TS and 63.7 %VS. The highest tCOD and sCOD removals were observed during AD with hOFMSW alone (80.1% and 74.6%, respectively) and co-AD of hOFMSW with non-pre-treated MCW (72.3 % and 74.6 %). The percentages of tCOD removals agree with literature data (Lopez et al., 2016).

Table 5: Removal percentages of TS, VS, tCOD and sCOD during the AD and co-AD experiments.

Assay	TS (% w/v)	VS (% w/v)	tCQO (% w/v)	sCQO (% w/v)
hOFMSW	48.4	67.7	80.1	72.3
hOFMSW +Pre1	52.9	63.3	60.9	66.9
hOFMSW +Pre2	46.1	58.6	77.4	51.1
hOFMSW+Pre3	56.5	63.1	73.3	70.6
hOFMSW+MCW	55.8	65.5	74.6	74.4

The comparison between the ratios of  $\text{NH}_4\text{-N/TKN}$  and  $\text{o-N/TKN}$  show a significant reduction of  $\text{o-N}$  during the AD with hOFMSW and the co-AD with hOFMSW+Pre1 and hOFMSW+Pre3, demonstrating that protein degradation occurred efficiently.  $\text{o-N}$  reduction was lower during co-AD with hOFMSW+Pre2 and mostly absent with co-AD with non-pre-treated MCW (Figure 1).

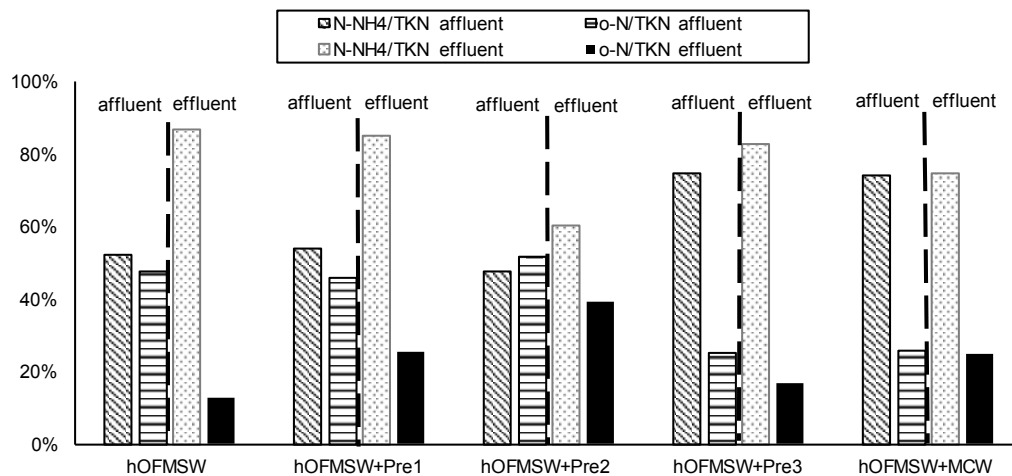


Figure 1: Comparison of  $\text{NH}_4\text{-N}$  and  $\text{o-N}$  contents over TKN in the effluents.

EA showed that the C:N ratio in the affluent increased with co-AD from a value of 9.46 in the AD assay with hOFMSW to 9.66, 12.53, 21.21, 12.57 in the co-AD assays with hOFMSW+Pre1, hOFMSW+Pre2, hOFMSW+Pre3 and with hOFMSW+MCW, respectively. The C:N ratio observed in the co-AD hOFMSW+Pre3 is quite close to the optimal value of 25. The increase of C:N ratio during the co-AD reflects in the increase of biogas and methane yields (Figure 2), confirming that MCW, if properly pre-treated, can balance the C demand for enhanced co-AD.

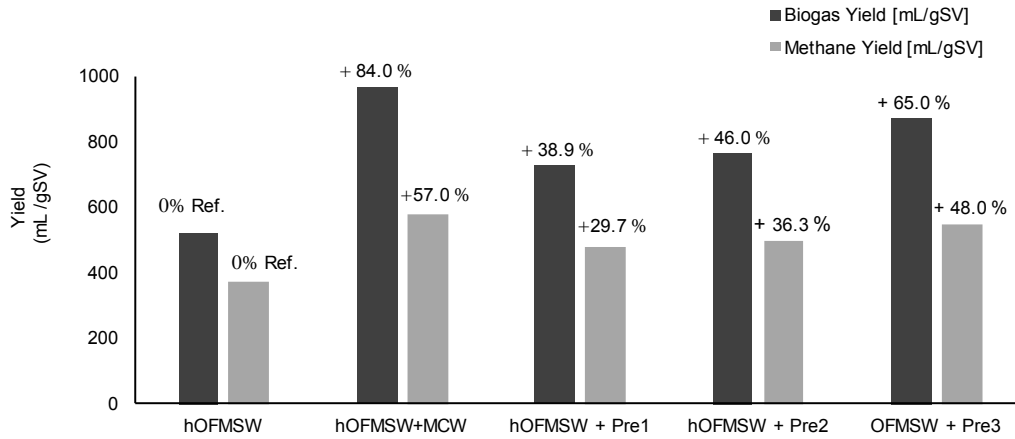


Figure 2: Biogas and methane yields during AD and co-AD experiments.

The methane content in biogas streams did not show any significant difference (ANOVA,  $p < 0.05$ ) during the AD of hOFMSW alone and the co-AD's of hOFMSW+Pre1 and OFMSW+Pre2, with an average value of 66.5 % v/v. A slight decrease in methane content was observed in the biogas streams obtained during co-AD of hOFMSW+Pre3 (63.1 % v/v) and hOFMSW+MCW (60.1 % v/v). During these two latter assays, it was observed the most favourable average biogas production (4,830 mL/d and 5,017 mL/d, respectively) (Table 6). The co-digestion of OFMSW+Pre3 increased biogas and methane yields by 65 % and by 48 % and co-digestion of hOFMSW with non-pre-treated MCW by 84 % and by 57 %, respectively, when compared to the hOFMSW alone (Figure 2). Despite the highest yield, and similar biogas volume produced during these two co-digestion assays, the biogas stream obtained during co-AD of OFMSW+MCW has a LHV on average 4 % lower than the LHV obtained in the co-AD of OFMSW+Pre3 (Table 6).

Table 6: Average biogas composition during co-AD experiments (STP).

Assay	CH <sub>4</sub> (% v/v)	CO <sub>2</sub> (% v/v)	O <sub>2</sub> (% v/v)	N <sub>2</sub> (% v/v)	H <sub>2</sub> S (ppm)	CO (ppm)	H <sub>2</sub> (ppm)	Biogas (mL/d)	LHV (MJ/m <sup>3</sup> )
hOFMSW	66.1	32.4	0.00	0.80	970	109	155	2,389	22.4
hOFMSW+Pre1	66.5	32.6	0.00	1.20	737	76.0	70.0	3,787	22.5
hOFMSW+Pre2	66.8	32.2	0.00	0.50	722	5.50	51.0	3,941	22.6
hOFMSW+Pre3	63.0	36.9	0.00	-	783	10.0	115	4,830	21.3
hOFMSW+MCW	60.3	39.6	0.00	-	820	7.00	90.0	5,017	20.4

Aiming to assess the process stability, NH<sub>4</sub>-N and VFAs were quantified in the digestate (Table 7).

Table 7: NH<sub>4</sub>-N and VFAs concentrations quantified in the digestate and threshold limits.

Assay	hOFMSW (mg/L)	hOFMSW+ Pre1 (mg/L)	hOFMSW+ Pre2(mg/L)	hOFMSW+ Pre3 (mg/L)	hOFMSW+ MCW (mg/L)	Threshold (mg/L)	Ref.
NH <sub>4</sub> -N	2,113	1,952	2,171	2,097	2,544	2,500	Kayhanian, 1994
AA	243	719	1,844	2,154	2,512	4,000	Drosg, 2013
PA	19.5	67.3	876	<10.0	<10.0	900	Wang et al., 2009
BA	69.0	106	412	51.0	<11.5	1,800	Wang et al., 2009
tVFAs	289	834	3,132	2,205	2,512	4,000	Drosg, 2013

The NH<sub>4</sub>-N concentration during co-AD of hOFMSW+MCW (2,544 mg/L) exceeded the threshold for AD, which can cause ammonia accumulation, thus inhibiting acetotrophic methanogens (Table 7). The concentrations of the Acetic acid increased during all co-digestion assays, whilst a significant reduction of Propionic and Butyric acid concentrations occurred during co-AD with hOFMSW+Pre3 and hOFMSW+MCW.

Co-AD of hOFMSW+MCW, that produced the highest biogas and methane yields, showed concentrations of NH<sub>4</sub>-N and VFAs closer to the thresholds and higher than the concentrations observed in co-AD with hOFMSW+Pre3.

#### 4. Conclusions

The results showed that the chemical pre-treatment catalyzed by H<sub>2</sub>O<sub>2</sub>, at pH 9.8, 4h of reaction time, and at room temperature (Pre3) is a promising and low energy demanding pre-treatment applicable to MCW to allow its co-AD with hOFMSW. The reaction time of Pre2 produced inhibitors that affected the efficiency of co-AD process. The co-digestion of OFMSW with pre-treated MCW increased the biogas yield by 65 % and CH<sub>4</sub> yield by 48 %, when compared to the results obtained using OFMSW alone. The co-digestion of hOFMSW with non-pre-treated MCW increased biogas and CH<sub>4</sub> yields by 84 % and 57 %, respectively. Despite the higher yields, the LHV and daily energy content (LHV \* Volume of biogas per day) of the biogas obtained in the co-AD of hOFMSW with non-pre-treated MCW were on average 4 % and 1% lower, respectively than the LHV obtained with co-AD of hOFMSW+Pre3. These results allow concluding that a pre-treatment is recommended before submitting MCW to AD and that co-digestion of hOFMSW with pre-treated MCW allows a significant enhancement of biogas and methane yields.

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