Effect of three pretreatment techniques on the chemical composition and on the methane yields of Opuntia ficus-indica (prickly pear) biomass

Effect of three pretreatment techniques on the chemical composition and on the methane yields of the Opuntia ficus-indica (prickly pear) biomass

P. S. Calabrò[a,*], E. Catalán[b], A. Folino[a], A. Sánchez[b], D. Komilis[b,c]

[a] *Università degli Studi Mediterranea di Reggio Calabria – Dipartimento di Ingegneria Civile, dell'Energia, dell'Ambiente e dei Materiali; via Graziella, loc. Feo di Vito, 89122 Reggio Calabria, Italy.*
[b] *Universitat Autònoma de Barcelona, Engineering College, Dept. of Chemical, Biological and Environmental Engineering, Bellaterra, Spain*
[c] *Democritus University of Thrace, Dept. Of Environmental Engineering, Xanthi, Greece.*
**Corresponding author: tel +39 0965 1692 222, email: paolo.calabro@unirc.it*

ABSTRACT

The Opuntia ficus-indica (OFI) is an emerging biomass that has the potential to be used as substrate in anaerobic digestion. The goal of this work was to investigate the effect of three pretreatment techniques (thermal, alkaline, acidic) on the chemical composition and the methane yield of OFI biomass. A composite experimental design with 3 factors and 2 to 3 levels was implemented and regression modelling was employed using a total of 10 biochemical methane potential (BMP) tests. The measured methane yields ranged from 289 to 604 NmL/gVS_{added}, according to the results, only the acidic pretreatment (HCl) was found to significantly increase methane generation. However, the experimental values being quite high with regards to the theoretical methane yield of the substrate, this effect still needs to be confirmed via further research. The alkaline pretreatment (NaOH) did not noticeably affect methane yields (an average reduction of 8% was recorded), despite the fact that it did significantly reduce the lignin content. Thermal pretreatment had no effect on the methane yields or the chemical composition. Scanning electron microscopy images revealed changes in the chemical structure after the addition of NaOH and HCl. Modelling of the cumulated methane production by the Gompertz modified equation was successful and aided in understanding kinetic advantages linked to some of the pretreatments. For example, the alkaline treatment (at the 20% dosage) at room temperature resulted to a μ_{max} (maximum specific methane production rate [$NmLCH_4/(gVS_{added} \cdot d)$]) equal to 36.3 against 18.6 for the control.

KEYWORDS

Anaerobic digestion; biogas; cactus biomass; cellulose; hemicellulose; lignin; Opuntia ficus-indica; pretreatment.

Introduction

The large-scale feedstock cultivation for biofuel production has changed dramatically agricultural land use, decreased food availability and boosted food price dynamics, especially in developing countries (Ruane et al., 2010; Valentine et al., 2012). Due to these negative impacts, production is shifting to second generation biofuels and research for new feedstocks is intense. The choice of the most adapt substrate has to be performed analysing several issues, such as availability of agro-industrial residues, adaptation of second-generation energy crops to local soil and climate, transportation costs and environmental impacts (Ruane et al., 2010; Chandra et al., 2012; Moraes et al., 2014; Pierie et al., 2015).

The organic substrate tested in this paper is a cactus named Opuntia ficus-indica L. (Mill.), commonly known as "prickly pear". Opuntia ficus-indica (OFI) has a crassulacean acid metabolism (CAM) system, that allows to adapt in areas with very limited rainfall (Consoli et al., 2013). The plant's native area is Mexico (Cushman et al. 2015), but it is also completely adapted to the Mediterranean area and to semiarid zones of North and South America, Africa and Australia. In some areas it is cultivated to obtain edible fruit, or to be used as a vegetable, cattle fodder and forage production (García de Cortázar and Nobel, 1992). The cladodes (the plant's green branches) of OFI are a potential excellent source of lignocellulosic biomass with a yield of 10 to 50 Mg dry mass/(year·ha) (García de Cortázar and Nobel, 1992; Consoli et al., 2013; Gabriel et al., 2014; Liguori et al., 2014). Higher values (Liguori et al., 2014) of biomass production refer to high density plantings (24 plants/m^2), while lower values are set for orchards optimised for fruit production (0.24 plants/m^2).

The average chemical composition of OFI biomass is shown in Table 1. According to the table, the material can be classified as a lignocellulosic feedstock.

Lately, there is an increasing interest on the use of the OFI biomass as a substrate for anaerobic digestion and bioethanol production (Obach and Lemus, 2006; Haladová et al., 2011; Jigar et al., 2011; Ortiz-Laurel et al. 2014; Ramos-Suárez et al. 2014; Yang et al. 2015; de Souza Filho et al. 2016; Santos et al., 2016). The main sources of such biomass are due to the pruning of prickly pear orchards. For optimal fruit production, the "scozzolatura" (a Sicilian term without an equivalent in English), is normally

performed in June and consists in the removal of the first flush of cladodes and flowers to promote a return bloom and a retarded and improved fruit production (Barbera et al., 1991; Liguori and Inglese, 2015). According to Rodiguez-Felix and Cantwell (1988), the crude fiber and protein content of young cladodes decrease with growth. This fact must be taken into account when optimizing the management of a plantation of OFI to be used as substrate for anaerobic digestion.

Table 1. Average chemical composition of OFI

Constituent	*g/100g (dry weight basis)*
Moisture (wet basis)	88 – 95
Carbohydrates (total polysaccharides)	64-71
Ash	17-24
Lignin	8-16
Cellulose	7 – 22
Hemicellulose	9 – 19
Protein	4 – 10
Lipid	1 – 4

Adapted from Gabriel and Victor, 2014; do N. Santos et al., 2016; Yang et al., 2015; Malainine et al., 2015.

As already mentioned, OFI (prickly pear) is native of Mexico but it is present in other areas of the World; in some of them (e.g. in Southern Italy) its presence is part of the natural landscape and ecosystem while in others (e.g. U.S., Australia, South Africa) it is considered a weed and active measures for its control are taken (Austalian Invasive Cacti Network, 2017; Brutsch and Zimmermann, 1993; United States Department of Agriculture, 2014).

Obach and Lemus (Obach and Lemus, 2006) have evaluated, during an experiment in a semi-continuous 1 m^3 mesophilic digester, the biogas potential of OFI with a reported methane yield equal to around 500 $NmLCH_4/gVS$. Ortiz-Laurel et al. (2014) reported a production of 244 $NmLCH_4/gVS$ while Ramos-Suarez et al. (2014), using a similar species (Opuntia maxima), measured a production of 142 $NmLCH_4/gVS$ during a batch experiment, the low production was attibuted to a possible destabilization of the process due to the high amount of soluble carbohydrates available. The same authors carried out also a semi-continuous experiment, where the same feedstock was used in codigestion with 25% microalgae (Scenedesmus sp., whose biomethane potential measured during a batch experiment was 140 $NmLCH_4/gVS$) reporting a much higher production equal to 308 $NmLCH_4/gVS$. The high difference was attibuted to a synergistic effect between the two substrates. Table 2 summarizes the main findings from recent anaerobic experiments performed with OFI without any pretreatment.

Table 2. Experimental data and operating parameters from recent anaerobic digestion experiments on OFI without any pretreatment

Reference	Ortiz-Laurel et al., 2014	Obach and Lemus, 2006	Ramos-Suárez et al., 2014	Ramos-Suárez et al., 2014	Santos et al., 2016
Substrate	OFI	OFI	Opuntia maxima	Opuntia maxima (75%)+Scenedesmus sp. (25%)	OFI
Operating mode	N.A.	Semi-continuous	Batch	Semi-continuous	Semi-continuous
Methane yeld [NmL/gVS_{added}]	203.6	501	142.4	308	517
Temperature of the experiment [ºC]	N.A.	Mesophilic	37	37	-
Digester volume [m^3]	N.A.	1	0.001	0.003	N.A.
Duration of experiment [days]	N.A.	83	40	15 (hydraulic retention time)	N.A.
Notes			$VS_{inoculum}$ / $VS_{substrate}$ = 2.0, C/N=51, yield from *Scenedesmus sp* = 140.3 NmL/gVS	Organic loading rate = 5.33 gVS/(L·d)	

OFI: *Opuntia ficus indica* (prickly pear biomass); N.A: Not available

To allow the conversion of lignocellulosic substrates to the highest possible degree through biological processes, it is necessary to increase the accessibility of cellulose by bacteria by "breaking the lignin seal" (Antonopoulou et al., 2015). To make cellulose more accessible to biodegradation, several pretreatment techniques have been used (Taherzadeh and Karimi, 2008; Kumar et al., 2009). In addition to the above, a pretreatment method can enhance hydrolysis, but it should also avoid the degradation of carbohydrates or of other readily biodegradable compounds, avoid the formation of byproducts that are inhibitory to subsequent processes (e.g. hydrolysis) and guarantee technical feasibility and economical sustainability (Taherzadeh and Karimi, 2008).

Acid pretreatment with sulphuric or hydrochloric acids, for example, allows the transformation of hemicellulose and can increase the accessibility of cellulose for the subsequent enzymatic hydrolysis (Mosier et al., 2005; Kumar et al., 2009). Drawbacks of acid pretreatment are typically the degradation of hemicellulosic sugars and the formation of undesired compounds such as furfurals (Taherzadeh and Karimi, 2008). Alkaline pretreatment (with NaOH, KOH, lime, ammonia or urea) has been implemented on several organic substrates and often provides good results with regard to neutralization of pH, alteration of the lignin structure, solubilisation of hemicellulose and the increase of the accessibility of cellulose (Galbe and Zacchi, 2007; Kumar et al., 2009; Sambusiti et al., 2013a; Sambusiti et al., 2013b; Montgomery and Bochmann, 2014).

Thermal pretreatment can be used alone or in combination with chemical ones to reach similar objectives (Mosier et al., 2005; Kumar et al., 2009; Antonopoulou et al., 2015).

The objective of this paper is the evaluation of the effect of the thermal, alkaline and acidic pretreatments on the composition and on the biochemical methane potential (BMP) of the OFI cladodes (herein referred to as OFI). The pretreatment procedure has been kept as simple as possible to simulate the actual process that can be applied in a full-scale facility.

Even though OFI has been extensively studied for agronomic purposes (e.g. fruit and forage production), scientific literature is very scarce on issues related to its exploitation as an anaerobic digestion feedstock (Obach and Lemus, 2006; Haladová et al., 2011; Jigar et al., 2011; Ortiz-Laurel et al., 2014; Ramos-Suárez et al., 2014; Santos et al. 2016). In addition, a detailed characterization of the OFI biomass is completely lacking from the literature.

The novelty of this work lies on the following:

- the methane potential of OFI after various pretreatment techniques is evaluated for the first time;
- a thorough characterization of the OFI biomass into its lignocellulosic and elemental content is presented;
- a statistical approach, based on best reduced regression modelling, is presented to accurately evaluate the effects of the pretreatments techniques on both the chemical composition and the methane potential of the OFI biomass. We believe that the statistical modelling aspect in our work is a novelty and strengthens the discussion of our experimental findings.

Materials and methods

Sampling and basic characterisation

A total amount of 5 kg of OFI cladodes (approximately one year old) was collected from two different randomly selected spontaneous trees, growing near the campus of Università Mediterranea di Reggio Calabria on non–irrigated and non–cultivated land. To ensure the homogeneity of the feedstock for characterisation and experiments, the cladodes were immediately manually chopped (average volume 2 cm^3) with a knife, milled using a commercial blender at maximum power (Silvercrest Blender, 550 W) and then placed in closed containers at 4 °C until further use. Due to the high-water content, cladodes were easily converted to a slightly dense fluid.

Total solids (TS), volatile solids (VS) and pH were measured according to conventional standard methods (Eaton and Franson, 2005) in duplicates.

Elemental (ultimate) analysis (C, H, N, S) of all substrates (raw and after pretreatment) was performed with an elemental analyzer (Thermo-Electron, USA, model: EA-1110, CHNS-O) (Komilis et al. 2012). One to two milligrams of the dried and pulverized samples was placed in tin capsules. The samples were combusted in a column that contained electrolytic copper and copper oxide catalysts placed in an oven kept at 1000ºC. The combustion resulted in the generation of CO_2, N_2, H_2O and SO_2 that were measured via gas chromatography and a thermal conductivity detector (TCD) to respectively quantify solid C, N, H and S. Helium flow was maintained at 100 ml/min and oxygen injection lasted 60 s. The chromatographic column was a 2 m Teflon PQSW packed column. The GC oven temperature was kept steadily at 60°C. Oxygen (O) was calculated indirectly as the difference of the sum of C, H, N and S from the organic matter (volatile solids) content. Using the results of the elemental composition, an empirical formula was

developed for all substrates.

Sugars and lignin contents were measured in triplicates according to (Pettersen et al., 1984) and (Sluiter et al., 2012) with modifications presented in (Komilis and Ham, 2000). In brief: approximately 150 mL of hot water (at 55 to 60ºC) passed through 1 g (per triplicate) of dried and ground material. The amount removed was labeled as hot water-soluble sugars (HWSS) and was determined with weight difference (in g DM). Then, a second extraction was performed on the same material with a mixture of toluene:ethanol (2:1) to remove fats/waxes/lipids. That group of compounds was also quantified via dry mass difference. From the remaining amount, 0.3 mg were removed (per replicate) and were acid digested with 72% sulfuric acid. After digestion, 63 mL of water were added and the solution was autoclaved for 1 hour at 122ºC and 1.5 bar. Then, the material was filtered through a glass filter (0.45 μm). The solid residue captured on filter was dried and lignin was quantified via the loss on ignition at 550ºC (Pettersen et al., 1984; Komilis and Ham, 2000). In the resulting filtrate, solid powdered $CaCO_3$ was gradually added to achieve a final pH close to 6. Then, the filtrate was centrifuged at 3500 rpm for 10 min to separate the $CaCO_3$ and the supernatant (now with a pH between 6 to 7) was filtered via a syringe type 0.22 μm filter prior to injection into the HPLC. Cellulose was quantified as D-glucose (SIGMA®, purity >99%) and hemicellulose was quantified as the sum of L-arabinose, D-mannose, D-galactose and D-xylose (SIGMA®, purity >99%). A REZEX® RPM monosaccharide column was used (300x7.8 mm), with lead as the ionic form, and with distilled water as the mobile phase. The mobile phase flowrate was kept at 0.6 mL/min while a guard column was also used. The HPLC system was a DIONEX® Model Ultimate 3000. No cellobiose peaks were detected in any of the chromatographs, since that would indicate the partial efficiency of the acid digestion step. A four-point linear calibration was developed for each of the five aforementioned sugars and the achieved R^2 of the calibration line was always higher than 0.99 for all. All measurements were done in triplicates (starting with 1 g per replicate). A recovery test was performed with pure cellulose and a 96% recovery was calculated. The same procedure was followed without any samples (blanks) and no peaks of any type of sugars (e.g. cellobiose) were detected that would indicate incomplete digestion of cellulose. A verification of the calibration was always performed prior to the initiation of a series of measurements. Results of all five groups (HWSS, fats/waxes, cellulose, hemicelluloses and lignin) are presented on a TS basis of the initial dry sample prior to any extractions. All sugars and lignin analyses were performed in triplicates.

The theoretical methane yield was calculated according to both the Buswell chemical equation 1 (Buswell and Mueller 1952, Raposo et al., 2011) by knowledge of the empirical formula of the material (without including the N and S), and, by knowledge of the organic fraction composition (lipids, proteins, and carbohydrates), by equation 2 (Raposo et al., 2011):

$$C_nH_aO_b + (n - a/4 - b/2)\ H_2O \rightarrow (n/2 + a/8 - b/4)\ CH_4 + (n/2 - a/8 + b/4)\ CO_2 \quad (1)$$

$$B_{o-ThOFC} = 415 \cdot \%Carbohydrates + 496 \cdot \%Proteins + 1014 \cdot \%Lipids \quad (2)$$

With $B_{o-ThOFC}$ methane yield calculated by knowledge of the organic fraction composition. The percentage of all fractions in %TS.

SEM observation of the dried OFI biomass was performed with a PHENOM SEM equipped with microanalysis at about 1000X and 9600X.

Experimental design and regression modeling

A composite factorial experimental design was employed to assess the effect of the alkali, acidic and thermal pretreatments on the chemical composition and the methane yields. The experimental design is included in Table 3.

The pretreatment conditions (i.e. chemical dosages of up to 20% and temperature up to 80ºC) were chosen on the basis of existing literature (Kumar et al., 2009; Sambusiti et al., 2013a; Antonopoulou et al., 2015; Calabrò et al., 2015; Zhang et al., 2015). In particular, the highest dosage was chosen to verify an "extreme" condition which is probably non-easily applicable in field operations for economic-technical reasons but still investigated by researchers.

Table 3. Experimental design

# Experiment	*Reagent used*	*Dosage [g/100gTS]*	*Pretreatment duration [h]*	*Temperature [°C]*
Baseline*	-	-	-	Room Temp.
1	-	-	24	80
2	NaOH	2	24	Room Temp.
3	NaOH	20	24	Room Temp.
4	NaOH	2	24	80
5	NaOH	20	24	80
6	HCl	2	1	Room Temp.
7	HCl	20	1	Room Temp.
8	HCl	2	1	80
9	HCl	20	1	80

Two (2) replicates per BMP experiment were performed.
*untreated sample (raw OFI)

According to Table 3, the three factors statistically examined in this work were: i) the chemical reagent, ii) the chemical dosage and iii) the temperature. Results were analysed with a regression equation using the aforementioned three factors as continuous variables with the levels being:

- Chemical reagent type (3 levels): None, NaOH, HCl;
- Chemical dosage (3 levels): 0%, 2%, 20%;
- Temperature (2 levels): Room temperature (i.e. 24ºC), 80 ºC.

The following generic regression model was fitted to the data whilst all variables were treated as continuous.

$$\text{Parameter} = a \bullet \text{Temp} + b \bullet \text{HCl} + c \bullet \text{NaOH} \quad (3)$$

where: Parameter is either the HWSS, cellulose, hemicellulose or lignin contents (or the methane yield) in % TS (or mL/gVS); Temp: Temperature in ºC; HCl: dosage in %; NaOH: dosage in %; a, b, c: regression coefficients.

During regression modelling, the best reduced models were calculated according to the procedure described by (Brown and Berthouex, 2002), which is based on the idea that the non-significant terms are gradually removed from the initial complete regression model, until a simpler (reduced) model (best regression model) is reached that contains only the statistically significant terms.

Two (2) replicates per BMP experiment were performed. The solids analysis, on the other hand, was performed in triplicates (3 replicates analysed per untreated / pretreated solid sample).

Pretreatment procedure

Powdered NaOH (reagent grade, Sigma-Aldrich®) and HCl solution 36% (Alfa Aesar) were added to the milled OFI without dilution; the mixture was then manually stirred for about 2 minutes. Pretreatment was carried out on closed beakers (glass, 250 mL, non-hermetically sealed) that, in the case of 24h duration treatments, were manually shaken every 8 hours.

Thermal and thermo-chemical pretreatment was carried out by placing the beakers in an oven at 80 ±0.5 °C. All the pretreatment operations ended at the same time and biochemical methane potential (BMP)

tests and characterisation of pretreated material started immediately after the end of pretreatment to avoid any potential change from storage.

BMP experiments

Biochemical methane potential (BMP) tests were performed in duplicates under mesophilic conditions (35±0.5°C). Tests were performed using a custom-made method based on Schievano et al. (2008) and Calabrò et al. (2015). The method employs 1.1 L bottles with three necks (two side necks, equipped with septa and the central main neck) that were placed in a thermostatic cabinet at 35±0.5 °C. The contents (substrate and inoculum) of each bottle were mixed by a magnetic stirrer throughout the 30 d period. About three times per week, biogas was slowly transferred into a second bottle (alkaline trap) containing 0.5 L of a 3M NaOH solution using a 100 mL syringe (Schievano et al., 2008). Through a side opening of the second bottle, a tube allowed to transfer biogas by the syringe. The carbon dioxide present in the biogas was absorbed into the alkaline solution. The pressure increase in the alkaline trap provoked the displacement of an amount of the alkaline solution that was transferred by a tube connected to another side opening in the bottle to a graduated volumetric cylinder. The total volume of the alkaline solution displaced by the gas was considered equal to the volume of methane present in the biogas. The volume of carbon dioxide was calculated by the difference of the methane volume from the total biogas volume.

All samples were inoculated with an anaerobic inoculum that was taken from the second stage of a digester operated under mesophilic conditions and fed on agro-residuals (mainly cattle manure, agriculture residuals and dairy residues). Immediately after sampling, inoculum was sieved (<1mm) to remove large fibrous materials (e.g. straw) and was then kept under endogenous anaerobic conditions at 35 °C for about 15 days to reduce non-specific biogas generation. The inoculum had a solid content of 52.6g TS/L and 36.2g VS/L and a pH of 8.4. Each batch was prepared by mixing 150 mL of inoculum and 2 g of $NaHCO_3$ (food grade, Solvay, Italy), added to ensure an adequate buffering capacity), then, immediately at the end of the pretreatment period, substrate was added by keeping an inoculum to substrate ratio (ISR, on a VS basis) at around 3. Since the measurement of VS from each substrate tested needs about 48h, the ISR was based on preliminary evaluations derived from the experience gained on other substrates. The actual ISR values varied, eventually, between 2.1 and 4.9 (mean 3.18±0.67). The total solids (TS) content of the material in the bottles was in the range 6.2 – 7.5% (mean 6.8%±0.31%).

The net specific biochemical methane production (BMP_{30}) was calculated by equation 4:

$$BMP_{30} = \frac{(V_{CH4,s} - V_{CH4,blank})}{VS_s \cdot V_s} \quad (4)$$

where: BMP_{30} in $NmLCH_4/gVS_{added}$; $V_{CH4,s}$ is the 30 d gross methane production from all treatments (substrate + inoculum) in $NmLCH_4$; $VCH_{4,blank}$ is the 30 d methane production of the inoculum itself in $NmLCH_4$; VS_s is the concentration of volatile solids from the feedstock present in the bottle at the beginning of the test (g VS/L) (i.e. VS added) and V_S is the liquid volume (L) in the BMP bottle. Note that the BMP yields in this work are expressed per g of VS added substrate (contribution of inoculum corrected).

Modeling of methane profiles and empirical equation

The specific cumulative methane production of BMP experiments was modelled using the modified Gompertz equation (Lo et al., 2010):

$$y = A\ exp\left\{-exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\} \quad (5)$$

where: y is the specific methane accumulation [$NmLCH_4/gVS_{added}$] at time t; t is the time [d] over the test period; A is the specific methane production potential at infinite time [$NmLCH_4/gVS_{added}$]; μ_m is the maximum specific methane production rate [$NmLCH_4/gVS_{added} \cdot d$]; λ is the lag phase duration [d] and e is the Euler's constant.

The results of BMP tests were fitted to the modified Gompertz equation using the least square methods by applying the routine "Solver" of Microsoft Excel.

Results and discussion

Feedstock composition

Table 4 includes the basic characterization of the substrates from all experiments in terms of pH, TS and VS (initial TS and VS correspond to those of untreated material).

Table 4. Basic characterization of all feedstocks

#	*Pretreatment*	*Initial pH**	*Final pH***	*TS after pret. [%]*	*VS after pret. [%TS]*
0	Raw substrate	4.8	4.8	6.6	78.2
1	80ºC	4.8	4.7	7.8	78.5
2	NaOH, 2%, Amb.	9.2	6.2	6.1	80.5
3	NaOH, 20%, Amb.	12.1	10.8	7.6	54.2
4	NaOH. 2%, 80ºC	9.2	6.2	8.3	77.7
5	NaOH, 20%, 80ºC	12.1	11.2	11.4	62.4
6	HCl, 2%, Amb.	3.2	3.9	6.4	80.8
7	HCl, 20%, Amb.	0.7	0.9	5.7	81.3
8	HCl. 2%, 80ºC	3.2	3.7	5.7	80.2
9	HCl, 20%, 80ºC	0.7	1.2	5.3	80.5

*: initial pH recorded right upon addition of the chemicals; **: final pH recorded at the end of the pretreatment period

According to Table 4, the raw OFI biomass was acidic and it is evident how the nature of the reagent used (acidic or alkaline) greatly influenced the final pH of the treated material. The use of hydrochloric acid led to a very low final pH which could have a negative effect on the anaerobic digestion process as long as there was not enough buffering capacity present. The experiments with the 20% HCl dosage, in particular, reduced pH to around 1. The pH with the alkaline pretreatments at the 2% dosage was kept close to 6, indicating the buffering capacity of the material. The thermal pretreatment apparently did not alter the pH (4.8) of the initial material.

The same table reveals that the TS content after pretreatment varied. The NaOH pretreatment resulted in an increase of the solid content, most probably due to a build up of salts (Montgomery and Bochmann, 2014); this effect was amplified when alkali treatment was combined with the thermal one. The use of hydrochloric acid led to a reduction of the total solids (from 3% to about 20%), the effect was clearly dependent upon the dosage of the reactant and on the temperature and could be probably linked to hydrolisation / solubilisation processes.

Table 5 presents the four dominant fractions of the OFI biomass. Although fats/lipids were also quantified in this work, they were practically less than 0.1% TS in all cases, and are therefore not included in the table. Closures were close to 100% in almost all cases, except for the NaOH treatment at the 20% dosage. This might be explained by the fact that the addition of NaOH eventually increased the TS content. This was confirmed by the relatively low VS contents recorded for those two samples (see Table 4).

Table 5 reveals that the dominant component of the OFI was the HWSS which was approximately 50% of

the total dry weight. Cellulose was the next most abundant component with contents between 8% to 11%, which is in agreement with literature findings (see Table 1).

Hemicellulose and lignin were approximately similar with around 6% to 8%TS each. From the four sugars used to quantify hemicelluloses, the dominant ones were arabinose and mannose (both detected as one peak in the chromatogram) and comprised between 62% to 92% of the total hemicellulose polymer. The next fraction was galactose, whilst xylose was negligible (almost 0%) in all treatments.

Table 5. Composition of the OFI biomass

Treatment	*HWSS* (%TS)*	*Diff.***	*Cellulose (%TS)*	*Diff**.*	*Hemicellulose (%TS)*	*Diff.***	*Lignin (%TS)*	*Diff.***
Raw substrate	48.2%^{BC}±1.4%	-	7.7%^{C}±0.41%	-	8.6%^{A}±0.43%	-	8.3%^{A}±0.32%	-
80ºC	51.8%^{A}±0.42%	7%	8.1%^{C}±0.55%	5%	6.7%^{BCD}±0.51%	-22%	7.4%^{AB}±1.3%	-11%
NaOH, 2%, Amb	51.5%^{A}±1.3%	7%	8.0%^{C}±0.07%	4%	6.1%^{DE}±0.35%	-29%	7.2%^{ABC}±0.23%	-13%
NaOH, 20%, Amb	48.3%^{BC}±0.77%	0%	11.1%^{A}±0.57%	44%	6.6%^{CD}±0.31%	-23%	5.7%^{CD}±0.36%	-31%
NaOH. 2%, 80ºC	49.9%^{ABC}±0.63%	4%	8.5%^{BC}±0.39%	10%	7.8%^{AB}±0.28%	-9%	6.2%^{BCD}±0.02%	-25%
NaOH, 20%, 80ºC	47.8%^{C}±0.65%	-1%	11.7%^{A}±0.15%	52%	7.4%^{BC}±0.30%	-14%	5.2%^{D}±0.19%	-37%
HCl, 2%, Amb	51.7%^{A}±0.93%	7%	7.5%^{C}±0.44%	-3%	6.6%^{CD}±0.30%	-23%	7.5%^{AB}±0.20%	-10%
HCl, 20%, Amb	50.1%^{ABC}±1.3%	4%	9.3%^{B}±0.18%	21%	6.1%^{DE}±0.08%	-29%	6.6%^{BCD}±0.51%	-20%
HCl. 2%, 80ºC	49.5%^{ABC}±0.89%	3%	8.3%^{BC}±0.14%	8%	7.5%^{ABC}±0.39%	-13%	6.5%^{BCD}±0.15%	-22%
HCl, 20%, 80ºC	50.7%^{AB}±0.99%	5%	8.3%^{BC}±0.78%	8%	5.2%^{E}±0.70%	-40%	6.1%^{BCD}±0.51%	-27%

Means ± standard deviation based on n=3 (solids analyses were performed in triplicates)

*Hot Water-Soluble Sugars; **Diff. = Difference from the corresponding content of the untreated OFI

Different letters on the same column indicate statistically different means at $p<0.05$; Amb: Ambient (room) temperature.

Since this is the first study dealing with pretreatment of OFI biomass, only comparisons with studies dealing with other biomasses are possible. According to Table 5, the changes in cellulose, hemicellulose and lignin are higher than those reported in (Zhu et al., 2010), while they are sufficiently in agreement, especially for hemicellulose and lignin, with those reported elsewhere (Monlau et al., 2012; Sambusiti et al., 2013b; Antonopoulou et al., 2015; Zhang et al., 2015). The apparent marginal increase in cellulose content could be explained by the fact that part of the hemicellulose is transformed to D-glucose (Mosier et al., 2005; Taherzadeh and Karimi, 2008; Kumar et al., 2009) during chemical pretreatment. The raw substrate contains amounts of hemicellulose and lignin that are statistically higher than almost all other treatments. This indicates that pretreatment, with both chemicals (NaOH, HCl), effectively reduced the hemicellulose and lignin contents. Hemicellulose reduction lied in the range of 9% – 40%, while lignin reduction was in the range of 10 – 37%. In agreement with (Sambusiti et al., 2013a), an increase in NaOH dosage (from 2% to 20%) led to a higher lignin removal.

The HWSS slightly increased (3-7%) when biomass was subject to almost all treatments (excepted 20% NaOH). Cellulose changes were higher and ranged from -3% to +52%. According to the statistics of Table 5, it appears that there is a group of pretreatments (indicated with letter C) that do not show statistical differences with respect to raw OFI; in this group, cellulose change ranged from -3 to +10%; the only treatments that significantly affected the cellulose content were the ones with HCl treatment at 20% dosage at ambient temperature (+21%), as well as the NaOH treatments at 20% dosage at ambient temperature and at 80 °C (+44% and +52%, respectively). However, it is not easy to draw solid conclusions with the pairwise comparisons only. Regression modelling can provide clearer information on the effect of pretreatment on chemical composition as explained in the next section.

Table 6 reports the elemental composition of the samples and the theoretical methane production calculated according to equations 1 and 2. During the application of equation 2, carbohydrates were calculated as the sum of HWSS, cellulose and hemicellulose. Lignin was not considered part of the carbohydrates, due to its well known negligible biodegradation in anaerobic environments. Lipids were neglected due to the very low amount present. Proteins were calculated using according to the N amount and using a conversion factor of 5.36 (Salo-väänänen & Koivistoinen, 1996).

Table 6. Elemental composition of the raw and pretreated substrates and theoretical methane yields

Treatment	*C (%TS)*	*N (%TS)*	*H (%TS)*	*C/N*	*Empirical formula **	*Theoretical CH_4 yield according to eq. 1 ($NmLCH_4/gVS$)*	*Theoretical CH_4 yield according to eq.2 ($NmLCH_4/gVS$)*
0	32.8%±3.5%	1.23%±0.18%	5.2%±0.43%	26.7	$C_{31}H_{59}O_{28}N$	403	385
1	33.6%±1.6%	1.13±0.09%	6.2%±0.40%	29.7	$C_{35}H_{77}O_{29}N$	453	390
2	32.2%±1.0%	1.26%±0.08%	5.2%±0.91%	25.6	$C_{30}H_{69}O_{28}N$	412	380
3	25.4%±0.46%	0.74%±0.10%	5.2%±0.07%	34.3	$C_{40}H_{98}O_{27}N$	558	542
4	33.2%±2.1%	1.11%±0.08%	5.9%±0.18%	29.9	$C_{35}H_{74}O_{30}N$	443	392
5	27.9%±0.85%	0.73%±0.08%	5.9%±0.18%	38.2	$C_{45}H_{113}O_{33}N$	526	476
6	34.6%±1.4%	1.23%±0.04%	6.5%±0.35%	28.1	$C_{33}H_{74}O_{27}N$	459	378
7	26.0%±3.3%	1.1%±0.14%	6.4%±0.30%	23.6	$C_{28}H_{81}O_{38}N$	313	370
8	30.3%±3.3%	1.0%±0.14%	6.3%±0.74%	30.3	$C_{35}H_{88}O_{37}N$	387	371
9	29.4%±2.3%	0.97%±0.11%	7.0%±0.54%	30.3	$C_{35}H_{101}O_{39}N$	397	363

Mean ± stdev (n=3); *: the O in the chemical formula was calculated indirectly according to O = VS - C-H-N (% TS)

In most of the cases, the theoretical yields calculated by the 2 equations were in fairly good agreement (difference less than 15%). Only in two cases, treatment 6 (HCl, 2% dosage at room temperature) and 7 (HCl, 20% dosage at room temperature), the differences observed were around 18%, which is considered

still a relatively low difference.

The values calculated on the basis of the organic matter composition (equation 2) are more uniform than those calculated according to Buswell formula except the two sample treated with NaOH with 20% dosage. However for these two specific cases, and only for these, the closures (carbohydrates+proteins+lipids) are significantly higher that 100% (140 and 122% respectively) and therefore calculations cannot be considered fully reliable.

Regression modelling to describe chemical composition

The best reduced models to describe the content of all four groups contained in the OFI as a function of the three factors are included in Table 7. Note that the models contain only the statistically significant terms. The duration time was also included in the first phase of the modelling, but proved not to be a statistically significant factor worth of including in the statistical modeling. Eventually, the model had a data set of n=30 (i.e. 10 experiments with 3 replicated solid measurements per experiment) and a maximum number of parameters fitted (p) equal to 3 (i.e. the modelling had n-p-1=26 degrees of freedom).

According to equation A, the HWSS (the dominant component of the organic substrate) was affected only by the NaOH treatment. That is, as NaOH dosage increased, the HWSS content decreased; this might indicate that the soluble sugars are degraded by the alkaline added. Fats and lipids, which were anyway negligible for all runs, were not affected by any type of pretreatment.

Table 7. Regression modeling to study the effect of pretreatment on chemical composition

Equation	*Dependent variable*	*Best reduced model*
A	HWSS	0.51 – 0.12 NaOH
B	Fats / Lipids	No terms significant (i.e. not affected by pretreatment)
C	Cellulose	0.079 + 0.176 NaOH + 0.049 HCl
D	Hemicellulose	0.073 – 0.082 HCl
E	Lignin	0.079 – 0.094 NaOH – 0.048 HCl

All dependent variables in % TS; HCl: chemical dosage in %; NaOH: chemical dosage in %.

According to equation C, it appears that both types of chemical pretreatment apparently affected cellulose content. Equation C also indicates that as the NaOH and HCl pretreatment dosages increased, cellulose content increased too. Actually, the magnitudes of the coefficients in equation C indicate that NaOH

affected cellulose 2 times more than HCl. Equation D reveals that hemicellulose was affected only by the acidic pretreatment. It appears that as the acid increased, the hemicellulose content slightly decreased, probably due to breakdown to monomeric sugars. This agrees with the findings of Antonopoulou et al., 2015, who had worked with sunflower straw biomass and had found that hemicelluloses is affected more by acid than by NaOH.

According to equation E, both the alkaline and the acidic additions resulted in the reduction of the lignin content. This is reasonable, since this is usually the objective of those pretreatment techniques, as has been confirmed by several other researchers for several types of organic materials (Taherzadeh and Karimi, 2008). Equation E reveals that the alkaline addition resulted in a higher (almost double) lignin reduction compared to the acidic pretreatment, as clearly indicated by the magnitude of the coefficients in equation E. This is in accordance to the findings of equation C, since cellulose was more affected (reduced) by the NaOH treatment than the HCl treatment. Although, the coefficient of the temperature term was initially found to be statistically significant in equation E, this coefficient was close to 0 (0.00013) indicating that the temperature had practically no effect on the lignin content, despite its statistical significance. Therefore, the temperature term was removed from equation E and the best reduced regression model is the one finally shown in Table 6. The temperature term was not found to be statistically significant in any of the other equations as well indicating that temperature (at least up to 80ºC as was used here) did not affect the chemical composition of OFI.

SEM observations

SEM observations confirm that the pretreatment altered the OFI biomass structure (see Figure 1). The Figure exemplifies the alterations of the chemical structure of the material under different pretreatment processes. According to the images, pretreatment causes a collapse of the structure of the OFI by forming cracks and pores (samples 1, 3, 8 and 9). In many cases (e.g. sample 8), the presence of grains of deposited materials was evident. This is most likely calcium oxalate (weddellite form) (Malainine et al., 2003).

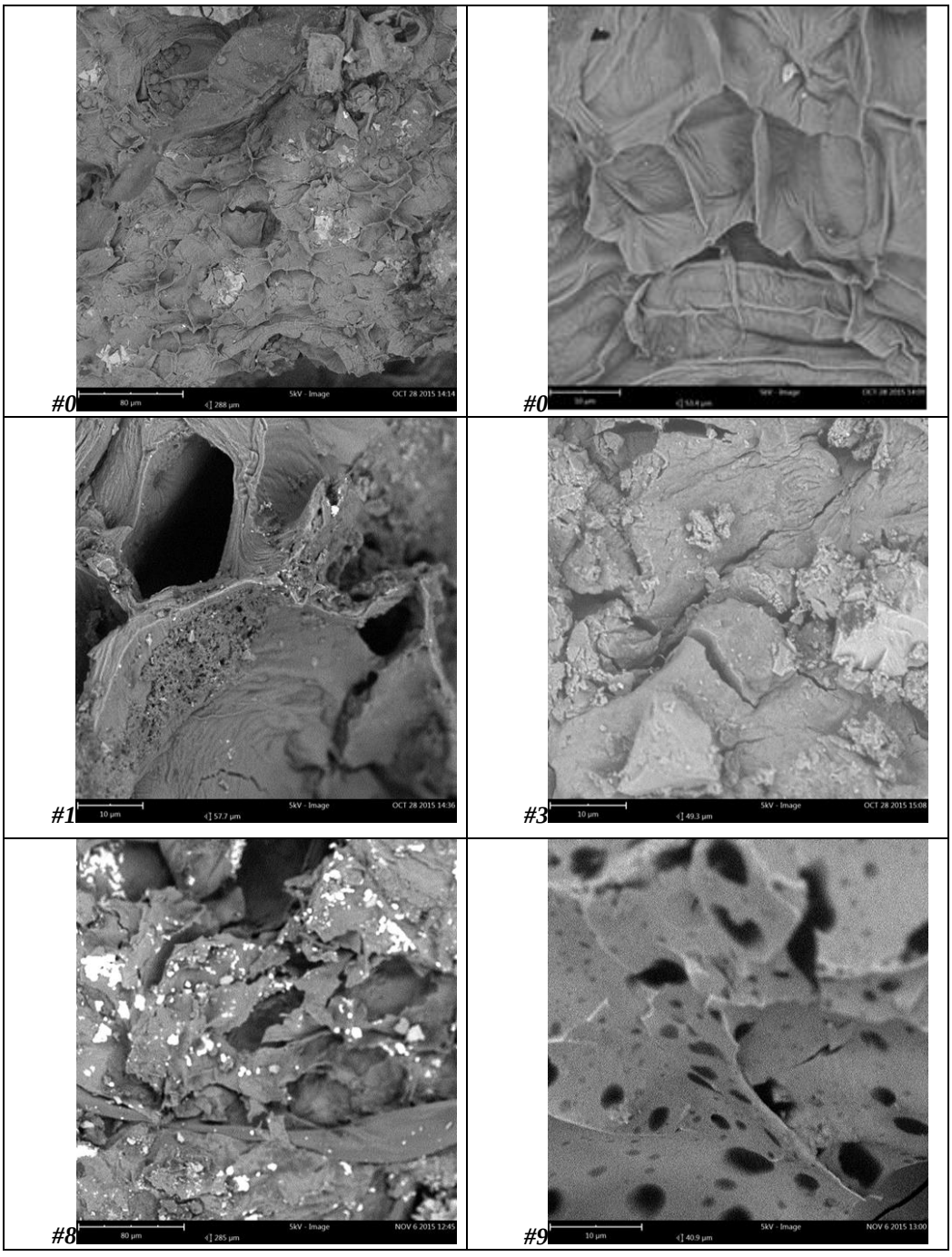

Figure 1. SEM images of the raw and pretreated OFI biomass. (0#) raw OFI biomass, (1#) thermally treated sample, (3#) alkaline treatment at room temperature at 20% dosage, (#8) acidic treatment at 80 °C at 2% dosage and (#9) acidic treatment at 80 °C at 20% dosage.

BMP tests results and regression modeling

Table 8 and Figure 2 summarize the results of the BMP tests. Table 8 also includes T_{50} (time needed to reach half of the total production over the 30 days period) and the $BMP_{30,wOFI}$, which is the specific net methane production expressed in terms of raw OFI entering the pretreatment. This latter parameter allows

a holistic consideration of the process of pretreatment and of anaerobic digestion, since it takes into account the weight reduction of the raw material during the pretreatment step.

Table 8. Methane yields and other operating parameters calculated during the BMP tests

#	*pH initial*[1]	*pH final*[2]	*BMP_{30}*[3] *[$NmLCH_4/gVS_{added}$]*	*Difference from raw OFI [%]*	*T_{50}*[4] *[d]*	*Average $BMP_{30,wOFI}$ [$NmLCH_4$/g wet OFI]*	*Difference from untreated wet OFI [%]*
0	8.4	7.7	424±14	-	15±2	21.9	-
1	8.6	7.8	350±63	-17%	22±0	23.6	8%
2	8.3	7.7	414±7	-2%	18±2	20.2	-8%
3	8.5	7.9	545±14	29%	7±1	22.2	1%
4	8.3	7.8	289±8	-32%	18±1	20.8	-5%
5	8.6	7.9	324±14	-24%	24±1	17.8	-19%
6	8.3	7.7	447±25	5%	12±1	22.2	1%
7	7.4	7.5	539±16	27%	9±0	24.5	12%
8	8.2	7.7	495±0	17%	15±0	21.7	-1%
9	7.6	7.6	604±20	42%	15±0	25.4	16%

1: pH measured in the batch at the start of the BMP test; 2: pH measured in the batch at the end of the BMP test; 3: mean ± std (n=2); 4: time needed to reach 50% of BMP_{30} yield.

According to Table 8, the BMP_{30} of the untreated OFI agrees with the values reported by (Obach and Lemus 2006). Table 8 also reveals that the acidic pretreatment led to a higher methane generation compared to the alkaline and thermal treatments. However, the BMP_{30} of three acid pretreated samples (#7, #8, #9), with values close to or higher than 500 NmL/gVS_{added} , were higher than those of the other samples and than the typical values reported (Angelidaki and al., 2004).

In some cases, the experimental BMP_{30} value was significantly (more than 10%) higher than the theoretical value. This is true (both theoretical values are significantly exceeded) for samples treated with hydrochloric acid at 80 ºC (both dosages) and at 20% dosage at room temperature. BMP_{30} of untreated OFI is on average 2% higher than theoretical yields indicating that OFI is highly biodegradable.

A plausible explanation for that is that the extremely acidic pretreated OFI added in the bottles, without any prior washing or neutralization, affected the inoculum sludge as well in the same way that it affected the main substrate, thus increasing the endogenous methane production from the sludge itself. This increased production did not occur in the blanks, which can explain the overestimation of biomethane production in the acidically pretreated experiments.

Regarding the alkaline pretreatment, only the sample treated with 20% NaOH (run 3) at room temperature showed a rather large increase of methane production. According to Table 7, a clear increase (> 10%) of methane production in comparison to raw OFI was observed with the NaOH treatment at ambient temperatures and at the 20% dosage. In addition, almost all acidic treatments resulted in a net methane

increase compared to the control. All these treatments presented high levels of hemicellulose and lignin removal. Thermal and thermo-alkaline treatments resulted in a reduction of methane production.

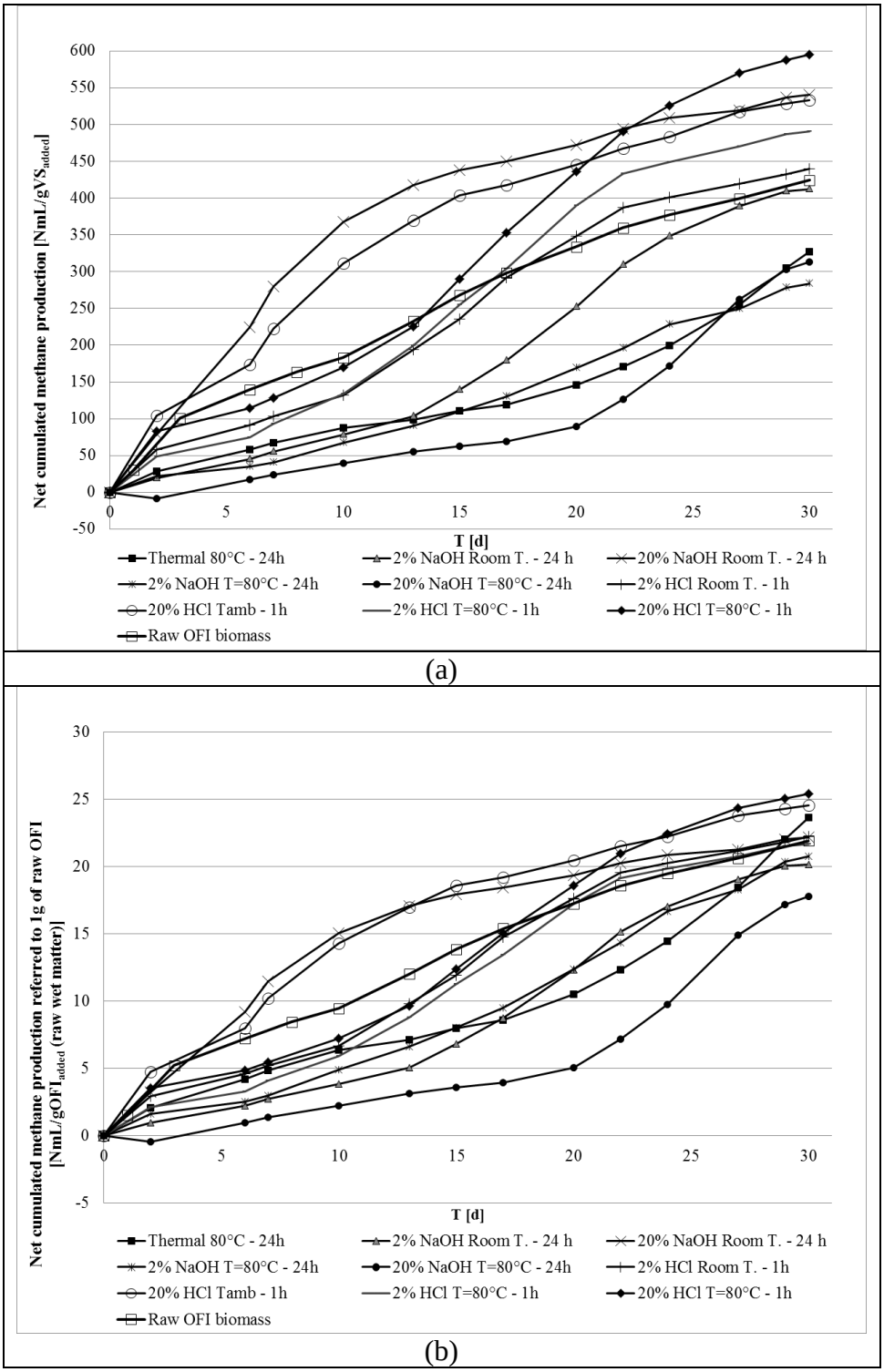

Figure 2. Methane generation profiles for all experiments (a: methane production expressed per g of VS added of the pretreated material; b: methane production expressed per g of initial wet OFI).

If the whole process (pretreatment and anaerobic digestion) is considered by the BMP_{30} referred to wet OFI in input to pretreatment ($BMP_{30,wOFI}$) it is clear that only acidic treatments at 20% dosage gave a clear advantage respect to the direct anaerobic digestion of raw OFI. Moreover, it seems that NaOH and HCl treatments at ambient temperature and 20% dosage provided a kinetic advantage as witnessed by the lower T_{50}.

To further aid in investigating the effect of the treatment techniques, regression modelling was employed for the chemical composition. In this case, since duplicate BMP measurements were performed per experiment, n=20 and the degrees of freedom during modelling become n-p-1=20-3-1=16. The best reduced regression model that was finally developed is shown in equation (6):

$$\text{Methane yield } (NmL/gVS_{added}) = 405.5 + 854\ HCl \quad (6)$$

Where HCl is in % dosage units. According to the above equation, only acidic pretreatment results in a statistically significant increase of the methane production. That is, as the dosage of HCl increases, the methane yield increases too. The validity of this statement obviously depends on the range of dosages used in the experiments, since a further increase in HCl concentration could cause an increase of the release of inhibitory by-products (e.g. furfurals). The fact that acidic addition resulted in a net methane increase agrees with the results of several researchers that had impemented acidic addition to other agricultural wastes (Antonopoulou et al., 2015). Equation 6 reveals that if no acid is added to the OFI, the expected BMP production of the raw substrate should be around 406 mL/gVS_{added}, which agrees with the experimental findings in this work since the raw substrate produced 424 mL/gVS_{added} (Table 8).

Modeling of the methane profile

BMP results have been modelled using the Gompertz modified equation in order to provide kinetic parameters. Table 9 includes the kinetics parameter calculated through the modelling process and Figure 3 illustrates the fitting procedure for 4 typical runs.

The selected model always fitted data adequately (the R^2 values were always higher than 0.995 in most cases). Only the significance of parameters calculated for experiments #5 and 9 is questionable.

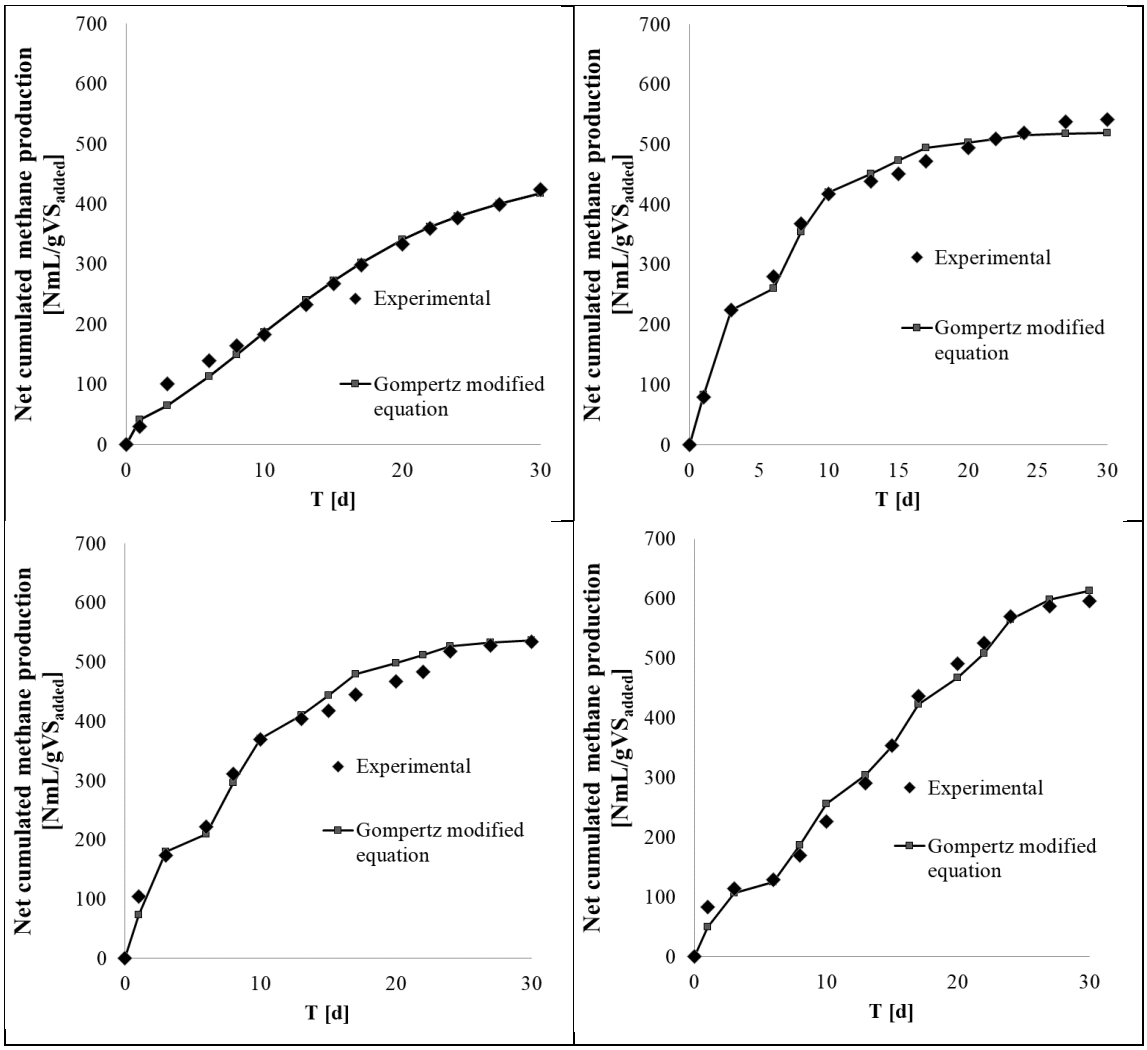

Figure 3. Fitting of data to the Gompertz model for four runs (first row from right to left: raw OFI, 20% NaOH at ambient temp., second row from right to left: 20% HCl at ambient temp., 20% HCl at 80°C)

Table 9. Parameters of the Gompertz modified equation

Experiment	*A* *[NmLCH$_4$/gVS$_{added}$]*	*μ_{max}* *[NmLCH$_4$/ (gVS$_{added}$*d)]*	*λ* *[d]*
0	462	18.6	0.0
1	536	9.4	0.8
2	641	19.7	7.2
3	523	37.3	0.0
4	531	12.1	5.9
5	1718	29.4	20.0
6	528	19.5	2.2
7	554	30.0	0.0
8	584	24.0	3.9
9	837	24.3	2.5

A: maximum theoretical methane yield at infinite time; μ_{max}: maximum specific increase rate of methane generation; λ: theoretically calculated lag time (d).

The Gompertz modified equation parameters shows that pretreatment resulted in most cases to higher methane yields at infinite time (A) than the raw substrate. A clear increase in the μ_{max} was also indicated for some of the treatments, namely the alkaline (3) and the acidic ones (7, 8, 9). On the other hand, experiments 8 and 9 (2 and 20% HCl at 80 °C), despite their high maximum specific increase rate of methane generation, had rather large lag times (3.9 and 2.5 d) indicating that the acid addition coupled with thermal treatment, retarded the initiation of the biodegradation process.

Conclusions

The conclusions of this work are:

- Both the acidic and the alkaline pretreatments resulted in a statistically significant decrease of the lignin. Alkaline pretreatment, specifically, affected the cellulose and lignin contents almost two times more than the acidic pretreatment. Hemicellulose decreased significantly upon acid addition only.
- OFI is a suitable substrate for anerobic digestion. Methane yields ranged from approximately 420 NmL/gVS_{added} for the raw substrate to up to approximately 600 NmL/gVS_{added} for OFI after acidic pretreatment (at a 20% HCl dosage) at 80ºC.
- The experimental values for the specific methane yields were, in some cases, higher than the calculated theoretical maximal values, according to substrate composition. Further research will be needed to confirm the high energy density of OFI cladodes.
- Only the acidic pretreatment led to a statistical significant increase of the methane yield of the OFI biomass. The positive effect of the acidic pre-treatment on methane production rates and yields was also confirmed by the modelling of the methane generation data using the Gompertz equation.
- If the high specific methane yields of OFI cladodes can be confirmed in further works, then, this substrate can be classified as highly degradable material, and in that case, no pretreatment would be strictly required prior to AD, except particle size reduction, unless it is demonstrated the economic advantage of using a pretreatment for further increasing reaction rate thus allowing the reduction of the volume of the digester.

Aknowledgements

The authors are grateful to Prof. Patrizia Frontera for carrying out the SEM observations and to Ms. Spyridoula Gerassimidou for her support during experimental activities.

References

Antonopoulou G, Dimitrellos G, Vayenas D & Lyberatos G (2015) Different Physicochemical Pretreatment Methods on Lignocellulosic Biomass: the Efeect on Biochemical Methane Potential. In: Proceedings of the 14th International Conference on Environmental Science and Technology, Rhodes (Greece), 3-5 September 2015.

Austalian Invasive Cacti Network Control of cacti. http://www.aicn.org.au/cactuswebsite-eradication.html (Accessed 01/05/2017)

Barbera G, Carimi F & Inglese P (1991) The reflowering of prickly pear Opuntia ficusindica (L) Miller: Influence of removal time and cladode load on yield and fruit ripening. *Advances in Horticultural Scence*, vol 5, 77–80.

Brown LC & Berthouex P (2002) Statistics for Environmental Engineers, Second Edition CRC Press: Boca Raton, FL.

Brutsch MO & Zimmermann H G (1993) The Prickly Pear (Opuntia ficus-indica [Cactaceae]) in South Africa: Utilization of the Naturalized Weed, and of the Cultivated Plants. *Economic Botany*, vol 47, 154–162.

Calabrò PS, Greco R, Evangelou A, & Komilis D. (2015) Anaerobic digestion of tomato processing waste: Effect of alkaline pretreatment. *Journal of Environmental Management*, vol 163, 49–52.

Calabrò PS, Pontoni L, Porqueddu I, Greco R, Pirozzi F & Malpei F (2016) Effect of the concentration of essential oil on orange peel waste biomethanization: Preliminary batch results. *Waste Management*, vol 48, 440–447.

Chandra R, Vijay VK, Subbarao PMV & Khura TK (2012) Production of methane from anaerobic digestion of jatropha and pongamia oil cakes. *Applied Energy*, vol 93, 148–159.

Consoli S, Inglese G & Inglese P (2013) Determination of evapotranspiration and annual biomass productivity of a cactus pear (Opuntia ficus-indica L(Mill) orchard in a semi-arid environment. *Journal of Irrigation and Drainage Engineering*, vol 139, 680–690.

Cushman JC, Davis SC, Yang X & Borland AM (2015) Development and use of feedstocks for semi-arid and arid lands. *Journal of Experimental Botany*, vol 66, 4177–4193.

de Souza Filho PF, Ribeiro VT, Santos ES dos, Macedo GR de (2016) Simultaneous saccharification and fermentation of cactus pear biomass evaluation of using different pretreatments. *Industrial Crops*

Production, 89, 425–433. doi: 10.1016/j.indcrop.2016.05.028

Department of Agriculture and Fisheries Biosecurity Queensland The Prickly pear story. https://www.daf.qld.gov.au/__data/assets/pdf_file/0014/55301/IPA-Prickly-Pear-Story-PP62.pdf. (Accessed 01/05/2017)

Eaton AD & Franson MAH (2005) Standard Methods for the Examination of Water & Wastewater. American Public Health Association

Gabriel A & Victor N, (2014) Review Article Cactus pear biomass, a potential lignocellulose raw material for Single Cell Protein production (SCP): A Review. *International Journal of Current Microbiology and Applied Sciences* vol 3, 171–197.

Galbe M & Zacchi G (2007) Pretreatment of lignocellulosic materials for efficient bioethanol production. *Advances in Biochemical Engineering/Biotechnology*, vol 108, 41–65.

García de Cortázar V & Nobel PS (1992) Biomass and Fruit Production for the Prickly Pear Cactus, Opuntia ficus-indica. *Journal of the American Society for Horticultural Science*, vol 117, 558–562.

Haladová D, Cundr O & Pecen J (2011) Selection of optimal anaerobic digestion technology for family sized farm use – Case study of southwest Madagascar. *Agricultura Tropica et Subtropica*, vol 44, 127–133.

Jigar E, Sulaiman H, Asfaw A & Bairu A (2011) Study on renewable biogas energy production from cladodes of Opuntia ficus indica. *Science*, vol 80, n 1, 44–48.

Komilis D & Ham R (2000) A Laboratory Method to Investigate Gaseous Emissions and Solids Decomposition During Composting Of Municipal Solid Wastes. *Compost Science & Utilization*, vol 8, 254–265

Komilis D, Evangelou A, Giannakis G & Lymperis C (2012) Revisiting the elemental composition and the calorific value of the organic fraction of municipal solid wastes. *Waste Management*, vol 32, 372–381.

Kumar P, Barrett DM, Delwiche MJ & Stroeve P (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & Engineering Chemistry Research*, vol 48, 3713–3729.

Liguori G & Inglese P (2015) Cactus pear fruit production: orchard planting and management of Opuntia ficus-indica. In: Proceedings of International Cactus Pear Workshop held at the University of the Free

State (UFS), Bloemfontein, South Africa 27-28 January 2015, 13–17.

Liguori G, Inglese P, Sortino G & Inglese G (2014) Dry matter accumulation and seasonal partitioning in mature Opuntia ficus-indica (L) Mill fruiting trees. *Italian Journal of Agronomy*, vol 9, 44–47

Lo HM, Kurniawan TA, Sillanpää MET, Pai TY, Chiang CF, Chao KP, Liu MH, Chuang SH, Banks CJ, Wang SC, Lin KC, Lin CY, Liu WF, Cheng PH, Chen CK, Chiu HY & Wu HY (2010) Modeling biogas production from organic fraction of MSW co-digested with MSWI ashes in anaerobic bioreactors. *Bioresource Technology*, vol 101, 6329–6335

Malainine ME, Dufresne A, Dupeyre D, Vignon M R & Mahrouz M (2003) First Evidence for the Presence of Weddellite Crystallites in Opuntia ficus indica Parenchyma. *Zeitschrift fur Naturforsch - Section C Biosciences*, vol 58, 812–816.

Monlau F, Barakat A, Steyer JP & Carrere H (2012) Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. Bioresource Technology, vol 120, 241–247.

Montgomery LFR & Bochmann G (2014) Pretreatment of feedstock for enhanced biogas production. Technical Brochure, IEA Bioenergy.

Moraes BS, Junqueira TL, Pavanello LG, Cavalett O, Mantelatto PE, Bonomi A & Zaiat M (2014) Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental, and economic perspectives: Profit or expense? *Applied Energy*, vol 113, 825–835.

Mosier N, Wyman C, Dale B, Elander R, Lee Y Y, Holtzapple M & Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, vol 96, 673–686.

Obach JE & Lemus MP (2006) Bio energy generation using opuntia ficus indica in arid and semi-arid zones of developing countries. In: Proceedings of Venice 2006, Biomass and Waste to Energy Simposium Venice (Italy).

Ortiz-Laurel H, Rössel-Kipping D & Kanswhol N (2014) Energy production balance for biogas generation from cactus prickly in a staged biorefinery. Proceedings of International Conference of Agricultural Engineering, Zurich, 06-10.07.2014.

Pettersen RC, Schwandt VH & Effland MJ (1984) An Analysis of the Wood Sugar Assay Using HPLC: A Comparison with Paper Chromatography. *Journal of Chromatographic Science*, vol 22, 478–484

Pierie F, van Someren CEJ, Benders RMJ, Bekkering J, van Gemert W JT, & Moll HC (2015) Environmental and energy system analysis of bio-methane production pathways: A comparison between feedstocks and process optimizations. *Applied Energy*, vol 160, 456–466

Ramos-Suárez JL, Martínez A & Carreras N (2014) Optimization of the digestion process of Scenedesmus sp. and Opuntia maxima for biogas production. *Energy Conversion and Management*, vol 88, 1263–1270. doi: 10.1016/j.enconman.2014.02.064

Raposo F, Fernández-Cegrí V, De la Rubia MA, Borja R, Béline F, Cavinato C, Demirer G, Fernández B, Fernández-Polanco M, Frigon JC, Ganesh R, Kaparaju P, Koubova J, Méndez R, Menin G, Peene A, Scherer P, Torrijos M, Uellendahl H, Wierinck I & de Wilde V (2011) Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology and Biotechnology*, vol 86, 1088–1098. doi:10.1002/jctb.2622

Ruane J, Sonnino A & Agostini A (2010) Bioenergy and the potential contribution of agricultural biotechnologies in developing countries. *Biomass and Bioenergy*, vol 34, 1427–1439

Salo-väänänen PP & Koivistoinen PE (1996) Determination of protein in foods: comparison of net protein and crude protein (N × 6.25) values. *Food Chemistry*, vol 57, 27–31. doi:10.1016/0308-8146(96)00157-4

Sambusiti C, Ficara E, Malpei F, Steyer JP & Carrère H (2013a) Effect of sodium hydroxide pretreatment on physical, chemical characteristics and methane production of five varieties of sorghum. *Energy*, vol 55, 449–456.

Sambusiti C, Monlau F, Ficara E, Carrère H & Malpei F (2013b) A comparison of different pre-treatments to increase methane production from two agricultural substrates. *Applied Energy*, vol 104, 62–70.

Santos T do N, Dutra ED, Gomes do Prado A, Bezerra de Souza FC, Santos de Fátima Rodrigues R, Moraes de Abreu DC, Simões CA, Ardaillon de Morais Jr D, Menezes MA & Cezar RS (2016) Potential for biofuels from the biomass of prickly pear cladodes: Challenges for bioethanol and biogas production in dry areas. *Biomass and Bioenergy*, vol 85, pp 215–222.

Schievano A, Pognani M, D'Imporzano G & Adani F (2008) Predicting anaerobic biogasification potential of ingestates and digestates of a full-scale biogas plant using chemical and biological

parameters. *Bioresource Technology*, vol 99, 8112–8117.

Sluiter A, Hames B & Ruiz R (2012) Determination of Structural Carbohydrates and Lignin in Biomass Technical Report NREL/TP-510-42618.

Taherzadeh MJ & Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *International Journal of Molecular Sciences*, vol 9, 1621-1651.

United States Department of Agriculture (2014) Field Guide for Managing Prickly Pear in the Southwest.

Valentine J, Clifton-Brown J, Hastings A, Robson P, Allison G & Smith P (2012) Food vs fuel: the use of land for lignocellulosic "next generation" energy crops that minimize competition with primary food production. *GCB Bioenergy*, vol 4, 1–19.

Yang L, Lu M, Carl S, Mayer JA, Cushman JC, Tian E & Lin H (2015) Biomass characterization of Agave and Opuntia as potential biofuel feedstocks. *Biomass and Bioenergy*, vol 76, 43–53. doi: 10.1016/j.biombioe.2015.03.004

Zhang Y, Chen X, Gu Y & Zhou X (2015) A physicochemical method for increasing methane production from rice straw: Extrusion combined with alkali pretreatment. *Applied Energy*, vol 160, 39–48.

Zhu J, Wan C & Li Y (2010) Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. *Bioresource Technology*, vol 101, 7523–7528.