BIOCHEMICAL METHANE POTENTIAL TESTS OF DIFFERENT AUTOCLAVED AND MICROWAVED LIGNOCELLULOSIC ORGANIC FRACTIONS OF MUNICIPAL SOLID WASTE.

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ABSTRACT

The aim of this research was to enhance the anaerobic biodegradability and methane production of two synthetic Organic Fractions of Municipal Solid Waste with different lignocellulosic contents by assessing microwave and autoclave pre-treatments. Biochemical Methane Potential assays were performed for 21 days. Changes in the soluble fractions of the organic matter (measured by soluble chemical oxygen demand,

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Abbreviations: A, autoclave; AD, anaerobic digestion; BMP, biochemical methane potential; COD, chemical oxygen demand; EB, energy produced in form of biogas; ED, energy demand; EQ, energy recovered in form of heat; ET, energy profit of the pretreatment; GS, gas production sum; MW, microwave; OFMSW, organic fraction of municipal solid waste; sCarb, soluble carbohydrates; sCOD, soluble chemical oxygen demand; SD, standard deviation; sProt, soluble proteins; TS, total solids; TVS, total volatile solids.

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carbohydrates and proteins), the first order hydrolysis constant k_h and the cumulated methane production at 21 days were used to evaluate the efficiency of microwaving and autoclaving pretreatments on substrates solubilisation and anaerobic digestion. Microwave treatment led to a methane production increase of 8.5% for both the tested organic fractions while autoclave treatment had an increase ranging from 1.0% to 4.4%. Results showed an increase of the soluble fraction after pre-treatments for both the synthetic organic fractions. Soluble chemical oxygen demand observed significant increases for pretreated substrates (up to 219.8%). In this regard, the mediocre results of methane's production led to the conclusion that autoclaving and microwaving resulted in the hydrolysis of a significant fraction of non-biodegradable organic substances recalcitrant to anaerobic digestion.

Keywords: Anaerobic Digestion, Biochemical Methane Potential, Organic Solid Waste, Microwave, Autoclave, Lignocellulosic Matter.

1. Introduction

Anaerobic digestion (AD) is an efficient organic waste treatment that has gained interest during the last years as it recovers energy in the form of biogas for use in combined heat and power plants. Nowadays the scientific and technical community is focused in drawing new borders for the development of the process with particular regard to the study of the dark fermentation and the production of biohydrogen (Alibardi and Cossu, 2015; Cappai et al., 2014; De Gioannis et al., 2013) and the application of pretreatments to enhance methane production from lignocellulosic and non-lignocellulosic substrates (Ariunbaatar et al., 2014a, Cesaro and Belgiorno, 2014).

The Organic Fraction of Municipal Solid Wastes (OFMSW) contains a high content of lignocellulosic fiber that is not readily digestible. Alibardi and Cossu (2015) studied OFMSW composition investigating five fractions (on weight basis, % w/w): meat-fish-cheese (0.3 - 12%); fruit (12.7 - 24.8%); vegetables (18.2 - 42.3%); pasta-bread (1.3 - 12.3%); undersieve (13.0 – 17.5%); rejected materials as paper and cardboard, kernels, etc (17.0 – 22.2%). This latter category and yard waste are typical lignocellulosic fractions which are significant parts in Tuscan OFMSW (Pecorini et al., 2013). For instance, wood fiber of yard waste typically comprises around 25-30% hemicellulose and 45% cellulose, on a dry weight basis (Perez et al., 2002). The encasing of cellulose and hemicelluloses in lignin may considerably restrict anaerobic degradation in which the limiting factor is the hydrolytic phase due to constrained accessibility of particulate substrates by enzymes and/or the complexity of compounds that need to be hydrolyzed (Delgenès et al., 2003). The rupture of the complex structure is essential for enzymatic attack and efficient bioconversion to processes such as hydrolysis, fermentation and biomethanogenesis. Pretreatments of OFMSW to enhance hydrolysis can be used to solubilize organic matter prior to AD in order to improve the overall AD process in terms of faster rates and degree of OFMSW degradation, thus increasing methane production (Cesaro and Belgiorno, 2014; Shahriari et al., 2012). Moreover, substrate pre-treatments have been shown to be a useful step to enhance aerobic biodegradation processes as composting (Ibrahim et al., 2011) and for pathogens destruction (Ariunbaatar et al., 2014a).

Several methods have been assessed for their technical feasibility at pre-treating residues. These include enzymatic (Bru et al., 2012), chemical (Dewil et al., 2007), ultrasonic (Cesaro et al., 2014), thermal (Ariunbaatar et al., 2014b; Li and Jin, 2015; Wang et al., 2010), hydrothermal (Lissens et al., 2004, Tampio et al., 2014) and microwave (Marin et al., 2010, Shahriari et al., 2012) treatments. The present research focuses its attention on these latter methods in order to study the anaerobic biodegradability and methane production of two different OFMSW by assessing Autoclaving (A) and Microwaving (MW). The two methods were tested since the former is able to release the cellulosic materials enmeshed in lignin resulting in an increase of smaller molecules available for further processing (Heerah et al., 2008; Papadimitrou et al., 2010) while the latter is an optimal method to solubilize organic solids and as such is a suitable candidate to treat OFMSW (Shahriari et al., 2013).

Autoclaving is a hydrothermal treatment where water is used as a reagent at increased temperature and pressure to hydrolyze and solubilise sugars, starch, proteins and hemicelluloses (Tampio et al., 2014). Autoclaving involves the high pressure sterilization of waste by steam which cooks the waste and destroys any bacteria in it (Ibrahim et al., 2011). The main factors influencing the process are temperature, pressure and time. Several studies investigated the effect of these process parameters by studying lighter and more aggressive treatment conditions. Time and temperature depend on the volume of waste feed into autoclave usually ranging between 120–160°C within 1 hour (Ibrahim et al., 2011). Marchesi et al. (2013) studied the biochemical methane potential (BMP) of organic waste after autoclaving for 15-30 minutes at 2 bars and 134°C while Heerah et al. (2008) autoclaved lignocellulosic biomass at 95°C and 1 bar for four consecutive cycles each lasting 45 minutes. Papadimitriou (2010) autoclaved commingled household waste for 1 hour at 200°C and 15.5 bars, Tampio et al. (2014) treated source segregated food waste at 160°C and 6.2 bars and Wilson and Novak (2009) studied secondary sludge at 220°C and 28.7 bars for 2 hours. Most of the detected results showed an increase in soluble COD (Heerah et al., 2008; Marchesi et al., 2013; Papadimitriou, 2010) and an increase in methane production (Heerah et al., 2008, Lissens et al., 2004). Bougrier et al. (2008) and Tampio et al. (2014) reported that more aggressive thermal and hydrothermal pre-treatments at higher temperatures (around 180°C) decrease biodegradability and biogas production. This is attributable to the formation of complex and inhibitory Maillard compounds, produced by reactions between amino acids and carbohydrates. Another possible drawback of the treatment is the release of a high total ammonia nitrogen load due to protein solubilisation (Wilson and Novak, 2009) that could induce a methanogenic inhibition.

Microwave irradiation is an electromagnetic radiation with a wavelength between 0.001 and 1 m, corresponding to an oscillation frequency of 300–0.3 GHz (Appels et al., 2013; Eskicioglu et al., 2007). Domestic "kitchen" microwave ovens and industrial microwave generators are generally operating at a frequency of 2.45 GHz with a corresponding wavelength of 0.12 m and energy of 1.02·10-5 eV (Appels et al., 2013; Beszédes et al., 2008). MW is an alternative method to conventional thermal pre-treatments as it is able to break organic molecules. The cell liquor and extracellular organic matter within polymeric network can release into the soluble phase increasing the ratio of accessible and biodegradable component. This effect could be manifested by different ratio of soluble and total COD and the increased rate of biogas production (Beszédes et al., 2008). The main factors influencing the treatment are temperature, power and irradiation time. Literature reports a range of application of the power between 440-500 W (Elagroudy and El-Gohary, 2013; Rani et al., 2013; Sólyom et al., 2011) and 1250 W (Coelho et al., 2011; Eskicioglu et al., 2007; Marin et al., 2010). The applied temperature covers a wide range of values: from 30°C (Kuglarz et al., 2013) to 175°C (Marin et al., 2010). The irradiation time is generally found to be in the order of few minutes (1-10 minutes) even if some works present irradiation time higher than 40 minutes (Marin et al., 2010; Shahriari et al., 2012). As for autoclaving, MW with high temperatures, long irradiation time and thus a significant applied energy (e.g. until 12000 kJ/kg in Bészedes et al., 2008 and until 2333 kJ//kg in Rani et al., 2013) could lead to the formation of refractory compounds inhibiting the digestion (Marin et al., 2010; Shahriari et al., 2012).

The enhancement of methane production due to the application of pre-treatments is generally analyzed through BMP tests (Beszédes et al., 2008; Elagroudy and El-Gohary, 2013; Eskicioglu et al., 2007; Kuglarz et al., 2013; Lissens et al., 2004; Marchesi et al., 2013; Marin et al., 2010; Rani et al., 2013; Shahriari et al., 2012; Sólyom et al., 2011; Zhou et al., 2013) while the solubilisation effect is usually monitored through analysis on the soluble fractions of the organic matter.

As previously mentioned, many works have already investigated the effect of pretreatments on the anaerobic digestion of several substrates. Nevertheless, it is still not clear whether these treatments are effective on lignocellulosic materials such as it might be the OFMSW. Under this perspective the present work aims at evaluating microwave and autoclave pretreatments on the anaerobic digestion of lignocellulosic OFMSW giving a first indication on which of the two methods is more suitable for a richer or for a meager lignocellulosic OFMSW. Focusing the attention on the lignocellulosic fraction of biowaste, the study was conducted by varying the lignocellulosic content of OFMSW while the pretreatment conditions were not changed. As such it has been selected a single condition for A and MW characterized by low treatment energy with the intention of limiting the energy expenses and prevent the formation of refractory compounds. The objective of this work is therefore to study the enhancement of the anaerobic biodegradability and methane production of two synthetic OFMSW with different lignocellulosic content (M1 and M2) by assessing microwave (M1_MW and M2_MW) and autoclave (M1_A and M2_A) pre-treatments. BMP assays were performed for 21 days (Cossu and Raga, 2008). Changes in the soluble fractions of the organic matter (measured by soluble COD, carbohydrates and proteins), the first order hydrolysis constant k_h and the cumulated methane production (BMP_{21}) were used to evaluate the efficiency of microwaving and autoclaving on substrates solubilisation and AD process.

2. Materials and methods

2.1 Organic waste and inoculum

Two different samples of OFMSW with different lignocellulosic contents were assessed. The two samples were achieved taking into account the main fractions of Italian OFMSW (Alibardi and Cossu, 2015) and varying the different amounts in order to control proteins (meat), carbohydrates (pasta) and fibers content (fir sawdust and vegetables). Similarly to Shahriari et al. (2013), M1 sample was characterized by (% w/w): fir sawdust (10%), grass (30%), carrot (10%), cabbage (10%), spinach (10%), cooked meat (7.5%), raw meat (7.5%) and cooked pasta (15%). M2 sample was composed by (% w/w): fir sawdust (25%), grass (20%), carrot (10%), cabbage (10%), spinach (10%), cooked meat (5%), raw meat (5%) and cooked pasta (15%). Pasta and meat were cooked for 10 minutes and then strained. For each fraction proteins, carbohydrates, lipids and fibers (sum of hemicellulose, cellulose and lignin) contents were measured in accordance with the methodologies presented in paragraph 2.3. The average values of proteins, carbohydrates, lipids, fibers and ashes content expressed in total solids percentage (%TS) are presented in Table 1 for M1 and M2. The table reports a higher composition of proteins, carbohydrates and lipids for M1 and a higher fiber composition for M2. The compositions were in line with previous studies. In particular, M1 and M2 were found richer in terms of organic groups contents than what reported by Nielfa et al. (2015) for a typical OFMSW (lipids 0.47 %TS, carbohydrates 6.95 %TS, proteins 6.44 %TS, fibers 35.13 %TS), while superior contents were reported by Alibardi and Cossu (2016) for organic waste mixtures (lipids 15-48 %TS, carbohydrates 34-64 %TS, proteins 12-45%, fibers as sum of hemicellulose and cellulose 4-6 %TS). In order to reduce the particle size to 3 mm diameter each fraction was treated in a food processor and sift with a strainer. Supplemental tap water was then added to the samples leading to two mashes to guarantee a TS content suitable for an anaerobic plant with wet fermentation technology (9.1 %TS for M1 and 10.0 %TS for M2). As such, dilution ratios were determined 1.7 l/kg for M1 and 2.5 l/kg for M2. The mashes were then stored at 4°C until use. Samples characterization is presented in Table 2. Digested sludge from an anaerobic reactor treating OFMSW was used as the mesophilic inoculum. It had a pH of 7.9 while TS and Total Volatile Solids (TVS) contents were about 2.6±0.1% (w/w) and 61.2±4.6% on TS basis respectively.

Here Table 1.

2.2 Microwave and Autoclave pre-treatments

A commercial domestic microwave oven (2450 MHz frequency, 850 W) was used to irradiate the mashes. The microwave heating was performed in batch at 96°C using 500 g of mash placed in a closed vessel to avoid losses caused by hot spot formation during the treatment (Appels et al., 2013; Rani et al., 2013) for a period of 4 minutes (in the range tested by Kuglarz et al., 2013, Rani et al., 2013 and Appels et al., 2013). According to Heerah et al. (2008) autoclaving was carried out using a batch system composed by a conventional pressure cooker operating at a maximum of 134°C and 2 bars (Marchesi et al., 2013) heated by a hot plate operating at 400 W. The retention time consisted of 15 minutes to lead the mixtures from atmospheric conditions to 134°C and 2 bars followed by 30 minutes of heating at constant conditions. The pretreatment was performed on 1700 g of mash. Both pre-treatments configurations were assessed to avoid high temperature and pressure conditions which result in expensive treatments (Cesaro and Belgiorno, 2014) and could lead to the formation of Maillard compounds.

The applied energy resulted in 408 kJ/kg for MW and 424 kJ/kg for A. Each sample was then stored at 4°C until use. The characterization of microwaved and autoclaved samples (M1_MW, M2_MW, M1_A and M2_A) is presented in Table 2.

Here Table 2.

2.3 Analytical parameters

TS, TVS and pH were determined in order to characterize the inoculum and each substrate according to standard methods (APHA, 2006). Ashes and moisture contents were then obtained in accordance with TS and TVS measurements. According to Angelidaki et al. (2006), due to the acidic condition of each substrate, TS determination was performed at 90°C instead of 105°C until constant weight in order to avoid the volatilization of VFA.

In order to investigate the solubilisation effect of the pre-treatments, soluble COD (sCOD), soluble Carbohydrates (sCarb) and soluble Protein (sProt) were analyzed before and after pre-treatments. sCOD was analysed to investigate the solubilisation of organic materials in the entire samples while sProt and sCarb were analysed to investigate the behaviour of two macromolecular organic components. The soluble part of each substrate was determined after centrifugation at 12000g for 30 min and subsequent filtration 0.45 μm microfiber filter paper (Marin et al., 2010; Rani et al., 2013).

Proteins, lipids and fibers contents were measured in accordance with the European Commission Regulation 2009/152/EC of 27 January 2009. Carbohydrates were then calculated by subtracting to the total amount, the contents of humidity, ashes, proteins, lipids and fibers. COD on the filtrate was measured according to APAT (2003).

The increase in the soluble fraction was calculated as given in the following equation Eq. (1) (Rani et al., 2013) where *X* represents sCOD, sProt and sCarb alternately.

$$\Delta X(\%) = \frac{(X_{after\,pretreatment} - X_{before\,pretreatment})}{X_{before\,pretreatment}} \times 100 \quad (1)$$

2.4 Specific energy

The specific energy demand (E_D) was calculated according to Kuglarz et al. (2013) taking into account the power of the microwave/autoclave heating system, the exposure time applied for each treatment and the mass of treated mash in kgTVS. E_D (kJ/kgTVS) was calculated according to Eq. (2):

$$E_D = \frac{P_D \cdot t_D}{M_{TVS}} \quad (2)$$

where:

P_D: power of microwave generator or hot plate (kW);

t_D: exposure time (s);

M_{TVS}: mass of treated mash (kgTVS);

2.5 Energy balance of the pre-treatment

According to Kuglarz et al. (2013) specific energy profit of the pre-treatment E_T (kJ/kgTVS) was calculated taking into account the E_D of the pre-treatment, the energy produced in the form of biogas and the theoretical amount of energy produced in the form of heat (Eq. (3)):

$$E_T = E_B + E_Q - E_D \tag{3}$$

where:

E_B: amount of energy produced in the form of biogas after subtracting the energy generated by raw substrates (kJ/kgTVS);

E_Q: amount of energy produced in the form of heat (kJ/kgTVS);

E_D: amount of energy used for samples pre-treatment performed in certain conditions (kJ/kgTVS).

E_B was based on an average CH_4 energetic value of 37 kJ/dm^3 and BMP_{21}. E_Q (kJ) was calculated as follows (Eq. (4)):

$$E_Q = M_S \cdot C_p \cdot \Delta T \quad (4)$$

where:

M_S: the mass of substrate equivalent to unit of volatile solids;

C_p: the specific heat capacity of substrates (kJ/kg·°C);

ΔT: the temperature difference between the mash after pretreatment and the temperature (37°C) of the mesophilic digestion.

C_p was based on ratio of water and solids. The values of C_p used for calculations amounted to 4.18 and 1.95 kJ/kg°C for water and solids respectively (Kim and Parker, 2008; Kuglarz et al., 2013).

2.6 Anaerobic biodegradability assays

Anaerobic biodegradability assays were performed for 21 days in order to determine the biogas (GS, gas sum, Cossu and Raga, 2008) and the methane (BMP) production of the evaluated substrates. The analysis were conducted using a modified method of Ponsà et al. (2008) following the basic guidelines and advices included in Angelidaki et al. (2009). The test was performed in quadruplicate for each sample using stainless steel batch reactors (1 L, 2 bar proof pressure) manufactured at DIEF (Department of Industrial Engineering of Florence, Pecorini et al., 2012). The vessels were incubated in a water bath at 37°C and tightly closed by a special cap provided with a ball valve to enable the gas sampling. After set-up the bottles were flushed with inert gas to ensure anaerobic conditions in the headspace of the batches. The bottles were daily shaken to guarantee homogeneous conditions in the assay vessels (Angelidaki et al. 2009). Each reactor was loaded with different amounts of substrate, depending on the characteristics of the materials, to achieve a concentration of substrate of about 1 gTVS/100 ml solution in each batch. This concentration is a compromise of, one hand, the need to use a large sample to have a good representativeness and to get a high easy-to-measure gas production, and, on the other hand, to avoid too large and impractical volumes of reactors and gas production and keep the solution dilute to avoid inhibition from accumulation of volatile fatty acid (VFA) and ammonia (Hansen et al., 2004). Previous tests were assessed with different substrate concentrations (2 and 4 gTVS/100 ml) resulting in the same methane potential (data not shown). This finding guarantees that the methane potential of substrates is not underestimated due to overload or potential inhibition (Angelidaki et al., 2009) and that the use of different amounts of substrates does not affect BMP results. The inoculum to sample ratio was about 1.5:1 TVS basis and kept under 10:1 weight basis according to Ponsà et al. (2008) for fresh feed-in substrate (the amount of inoculum should be enough to prevent the accumulation of VFA and acid conditions). To determine the background methane production a blank assay with only the inoculum was done in triplicate. The inoculum was degassed for 5 days in order to deplete the residual biodegradable organic matter (Angelidaki et al., 2009) until the achievement of an endogenous metabolism phase. Prior to the tests, the inoculum response toward a “standard” substrate was checked in duplicate with cellulose with a concentration of 2 gTVS/100 mL solution (Angelidaki et al., 2009) in order to assure the suitability of the sludge for the experiment. The inoculum activity was then found to be 0.13±0.04 gCH_4-COD/(gTVS d). The value obtained agreed with those recommended by Angelidaki et al., 2009 who reported that sludge inoculum must have a minimum specific activity of 0.1 gCH_4-COD/(gTVS d). Therefore, the inoculum used in this study was suitable for performing the anaerobic tests.

Biogas production was daily estimated by measuring the pressure in the head space of each reactor and then converting to volume by the application of the ideal gas law. Pressure was measured using a membrane pressure gauge (Model HD2304.0, Delta Ohm S.r.L., Italy). The measured values of pressure were converted into biogas volume as following, Eq. (5):

$$V_{biogas} = \frac{P_{measured} \cdot T_{NTP}}{P_{NTP} \cdot T_r} \cdot V_r \tag{5}$$

where:

V_{biogas}: volume of daily biogas production, expressed in Normal litre (Nl);

$P_{measured}$: headspace pressure before the gas sampling (atm);

T_r and V_r: temperature (K) and volume (l) of the reactor's headspace;

T_{NTP} and P_{NTP}: normal temperature and pressure, (273.15 K and 1 atm respectively).

The GS was determined as the cumulated biogas production (sum of the daily biogas productions) divided by the TS and the TVS content contained in each batch.

In order to determine the methane production, the methane content of the gas was then measured by using an IR gas analyser (ECOPROBE 5 – RS Dynamics). As such, the BMP was calculated as the cumulated methane production (sum of the daily methane productions), divided by the TS and the TVS content contained in each batch.

Results were lastly reported at normal conditions after 21 days and presented as GS_{21} and BMP_{21}.The increase in GS_{21} and BMP_{21} was calculated on TVS basis as given in the following equation, where X is GS_{21} and BMP_{21} alternately. Eq. (6):

$$\Delta X_{21}(\%) = \frac{(X_{21_{after\ pretreatment}} - X_{21_{before\ pretreatment}})}{X_{21_{before\ pretreatment}}} \times 10 \qquad (6)$$

According to Angelidaki et al. (2009) results from BMP tests were used to obtain further information on the studied substrates. The first order hydrolysis constant k_h (d^{-1}) was calculated thanks to the following equation, Eq. (7):

$$ln\frac{B_\infty - B}{B_\infty} = -k_h t \qquad (7)$$

where:

B_∞: value of the ultimate methane production;
B: methane produced at a given time t.

k_h and B_∞ were obtained from experimental data using a fitting procedure that minimized the sampling variance.

2.7 Statistical analysis

The differences between pre-treatment results (sCOD, sCarb, sProt, GS_{21} and BMP_{21}) for the two different mashes (M1 and M2) were compared by one-way ANOVA followed by Tukey-test, with the level of significance set at < 0.05 (Kuglarz et al., 2013). Data significantly equivalent were indicated by the same letters. GS_{21} and

BMP_{21} values subjected to statistical analysis were means of four independent replicates (n = 4) ± SD (standard deviation) while sCOD, sCarb, sProt were means of three independent replicates (n = 3) ± SD.

3. Results and discussion

3.1 Substrate solubilisation by Microwave and Autoclave treatments

MW and A treatments led to the solubilisation of the organic material of both the OFMSW samples. Table 3 presents the mean parameters and the SD of MW, A and un-treated substrates. Significant difference ($p < 0.05$), underlined by different letters, was found for pretreatment results compared to un-treated samples.

Here Table 3.

sCOD, sCarb and sProt were found higher for M1 substrates than M2 substrates. This feature is concurring with the OFMSWs initial composition (Table 1) which shows a higher content of proteins, carbohydrates and lipids for M1 mash.

An increase of sCOD was found for both treatments and both OFMSW tested samples. This trend was found particularly relevant for the MW treatment with an increase of about 219.8% for M1_MW and 142.4% for M2_MW. The increase of sCOD for MW treatment agrees with Coelho et al. (2011), Elagroudy and El-Gohary (2013), Kuglarz et al. (2013), Marin et al. (2010) and Toreci et al. (2008). The solubilisation effect on carbohydrates and proteins was registered for both treatments (in agreement with Marin et al., 2010; Rani et al., 2013) but it was mainly relevant for the A treatment. Compared to the abovementioned studies, results showed a lower solubilisation effect of carbohydrates and proteins. This is due to the application of treatments characterized by low temperatures and short duration times that translates into the application of little energy per treatment. The higher increase found for sProt and sCarb for A is attributable to the higher temperature reached in A compared to MW. Indeed, as reported by previous studies (Appels et al., 2010; Wilson and Novak, 2009), the increase in temperature is associated to a major release of soluble proteins and carbohydrates. In particular, the thermal effect acts on both decomposition of extracellular polymer substances and cell lysis (Appels et al., 2010; Eskigcioglu et al., 2007). Furthermore, as reported in Table 2, the lower pH found after all treatments could be associated to a release of organic acids during the process (Heerah et al., 2008; Papadimitriou, 2010).

3.2 Anaerobic biodegradability assays results

Figure 1 represents the methane production profiles of each substrate on TVS basis. Table 4 reports the results of the BMP test with GS_{21}, BMP_{21} on TS and TVS basis, mean methane content and k_h.

Results showed a higher biogas and methane production for all M1 substrates compared to M2 substrates (M2 was characterized by a higher fibre and lignocellulosic content) which is attributable to the sample composition which is more suitable for anaerobic bacteria. Also the mean methane content found during the monitored period was registered higher for M1 (ranging between 59.9% and 61.6%) than M2 substrates (between 56.2% and 58.1%).

MW led to a BMP_{21} increase of 8.5% for both the tested OFMSW while A had an increase of about 1.0% for M1 and 4.4% for M2. The increase in GS_{21} was found more significant with values of 14.7%, 10.0% and 6.7, 8.0% for MW and A treatments respectively. As such, MW was found to be an efficient treatment for both OFMSW while A was found to be more suitable for a lignocellulosic substrate. This statement is concurring with Lissens et al. (2004) who determined a higher increase in BMP for a yard waste rather than a food waste substrate after a wet oxidation treatment.

The increase in biogas and methane production was found directly proportional to sCOD release with the highest increase of biogas production found together with the highest release of sCOD (Figure 2). The coefficient of determination (R^2) was calculated for both mashes and found above 0.98 guarantying a good approximation of the linear correlation. This finding concurs with Beszédes et al., (2008) and Elagroudy and El-Gohary, (2013) which reported an increase of methane production together with an increase in sCOD. Although this, the significant increase in sCOD does not correspond with a similar increase in methane production (e.g. for MW1: ΔsCOD = 219.8% while ΔBMP_{21} = 8.5%). This outcome suggests that most of the released sCOD was not biodegradable and it was not converted into methane. Indeed, 1 gram of biodegradable COD produces around 400 mL of CH_4 (Field et al., 1988) and according to the increase of sCOD, the increase in methane production does not reflect this relation supporting the case that the sCOD produced from MW or A was mainly composed of non-biodegradable substances. In this regard, the non-biodegradable fraction can be associated to recalcitrant compounds such as lignin or lipids hydrolysis products (Alibardi and Cossu, 2016; Chen et al., 2008).

The higher increase in biogas production and sCOD recorded for MW compared to A is attributable to the different action mechanism of the pre-treatments, and, in particular, to the athermal effect of MW. Indeed while A increases the ionized products of water which are able to hydrolyze the macromolecules at elevated temperature and pressure (Yin et al., 2014), MW can improve the rupturing of the cell wall in two different ways: thermal and athermal effect (Cesaro and Belgiorno, 2014; Houtmeyers et al., 2014; Solyom et al., 2011). The former corresponds to degradation caused by temperature increase. The latter occurs when the alternating electric field of microwaves is able to force the polarized side chains of the cell wall macromolecules to break their hydrogen bonds, and thus alter their structure. As reported by previous studies (Eskicioglu et al., 2007; Pino-Jelcic et al., 2006), the athermal effect of MW is manifested through a difference in sCOD and/or increased rates of biogas production compared to other treatments.

Here Table 4.

Here Figure 1.

Here Figure 2.

An inverse correlation was found between the increase of methane production and the increase of protein solubilisation. Indeed, the more is the protein solubilisation, the less is the increase in methane production (M1_A and M2_A results). Analysing the ratio sCarb/sProt calculated for both treatments and presented in Table 3, this parameter was directly proportional to methane and biogas production. Even in the presence of a relevant increase in sCarb, the increase in sProt reduces the ratio and simultaneously the methane production.

The increase in methane production noticed for MW and A is in agreement with what reported in previous batch studies (Eskicioglu et al., 2007; Kuglarz et al., 2013; Sólyom et al., 2011 for MW and Marchesi et al., 2013 for A). The different pretreatments applied did not affect significantly biogas and methane potential ($p > 0.05$) which is agreement with previous studies (Kuglarz et al., 2013).

Results on the first order hydrolysis underlines what previously reported. k_h was found higher for M1 samples than M2 ones; furthermore for M1 k_h was registered superior for MW while for M2 k_h was determined slightly superior for A underlining the efficiency on the hydrolysis phase of MW on a meager lignocellulosic substrate and A on a rich lignocellulosic substrate.

3.3 Energy balance of the pre-treatment

Energy efficiency is a crucial factor influencing the economic feasibility and justifying the mash pre-treatment (Kuglarz et al., 2013). E_D, E_B, E_Q and E_T for the different treatments and substrates are presented in Table 5.

Here Table 5.

Analysing the specific energy balance, no energy profits were registered for all treatments. This was mainly due to the low increase in biogas production compared to raw substrate digestion and to laboratory scale conditions (Cesaro and Belgiorno, 2014). In particular, the amount of E_B and E_Q did not balance E_D. A negative energy balance was also reported by previous studies performing low-energy treatments: Houtmeyers et al., 2014 and Appels et al., 2013 carried out microwaving by applying 96 kJ/kg and 336 kJ/kg respectively. Under this perspective, energy balances proved that under these conditions pre-treatments were not energetically feasible. Comparing the two methods applied in the present study, even if with negative results, MW showed better energetic response than A. Other studies showed relevant increase in total energy. Kuglarz et al. (2013) studied the energy balance of the application of various microwave and thermal pre-treatments on secondary sludge finding the best energy balance for the most severe treatment conditions (E_D 8094 kJ/kgTVS) which result in high E_B and E_Q values, not found in the present work. Further investigations with different pre-treatment conditions are necessary to examine the feasibility of such pre-treatments on lignocellulosic OFMSW.

4. Conclusions

The application of A and MW to lignocellulosic substrates resulted in an increase of methane production and solubilisation. Microwaving was proved effective for both the tested OFMSW with an increase of BMP_{21} and sCOD. Autoclaving showed lower increases in biogas production compared to MW with the best responses found for the most lignocellulosic OFMSW. Although this, the significant increase in sCOD did not correspond with a similar increase in methane production. This finding suggests that most of the sCOD produced from MW or A was composed of non-biodegradable substances that were not converted into methane.

Also in the matter of k_h, analysis underlined the better impact on the hydrolytic phase of MW on the meager lignocellulosic substrate and A on the richer lignocellulosic substrate. No energy profit was registered for any tested pretreatment due to the low increase in biogas production. Despite this, even if with negative results, MW showed better energy balance than A.

Further investigations with different treatment conditions and lignocellulosic contents are required to better probe the pre-treatment efficiency on the AD of OFMSW.

Acknowledgements

Prof. Lidia Lombardi is fully acknowledged for her precious suggestions. Many thanks to the researchers of the Department of Industrial Engineering of Florence (DIEF) for support and collaboration, in particular thanks to Donata Bacchi.

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Table 1

Proteins, lipids, carbohydrates, fibers and ashes content expressed in % of dry matter (%TS) for M1 and M2 synthetic OFMSW.

	Proteins [%TS]	Lipids [%TS]	Carb. [%TS]	Fibers [%TS]	Ashes [%TS]
M1	17.8	11.7	35.1	32.0	3.5
M2	10.5	6.4	26.2	54.8	2.2

Table 2

Substrates characterization. pH, TS and TVS/TS with mean and standard deviation values.

	M1	M1_MW	M1_A	M2	M2_MW	M2_A
TS [%]	9.2 ± 0.1	9.1 ± 0.1	11.1 ± 0.0	10.0 ± 0.1	8.6 ± 0.4	11.9 ±0.1
TVS/TS [%]	96.5 ± 0.1	96.6 ± 0.1	96.9 ± 0.1	97.8 ± 0.1	97.6 ± 0.0	97.8 ± 0.1
pH	3.84 ± 0.01	3.51 ± 0.02	3.41 ± 0.02	4.22 ± 0.02	3.69 ± 0.02	3.46 ± 0.01

Table 3

Substrates solubilisation in terms of soluble COD, carbohydrates, proteins and sCarb/sProt ratio. Soluble carbohydrates and proteins data are expressed in % of dry matter (%TS). ΔsCOD, ΔsCarb and ΔsProt represent the relative error referred to untreated substrates M1 and M2 (data analyzed statistically for each mash separately, the same letters showing that the values are not significantly different $p > 0.05$).

	M1	M1_MW	M1_A	M2	M2_MW	M2_A
sCOD [mg/l O_2]	19700 ± 4400^{a}	63000 ± 14000^{b}	25500 ± 5600^{a}	17200 ± 3800^{c}	41700 ± 9200^{d}	32200 ± 7100^{d}
ΔsCOD [%]	-	219.8	29.4	-	142.4	87.2
sCarb [%TS]	49.9 ± 4.2^{a}	$54.0 \pm 5.1^{a,b}$	64.1 ± 6.3^{b}	26.2 ± 5.1^{c}	30.8 ± 5.9^{c}	35.4 ± 6.8^{c}
ΔsCarb [%]	-	9.4	29.9	-	17.7	35.5
sProt [%TS]	19.8 ± 5.5^{a}	19.4 ± 5.8^{a}	25.7 ± 6.3^{a}	14.8 ± 3.4^{b}	15.2 ± 3.7^{b}	18.1 ± 4.3^{b}
ΔsProt [%]	-	-2.1	29.8	-	2.9	22.9
sCarb/sProt	-	2.78	2.49	-	2.03	1.95

Table 4

Results of the anaerobic biodegradability assays expressed in terms of methane content, k_h, GS_{21}, BMP_{21}, ΔGS_{21} and ΔBMP_{21}. The percentages represent the relative error referred to untreated substrates M1 and M2 (data analyzed statistically for each mash separately, the same letters showing that the values are not significantly different $p > 0.05$).

	M1	M1_MW	M1_A	M2	M2_MW	M2_A
CH_4 [%]	61.6 ± 0.2	59.9 ± 0.8	60.0 ± 0.9	58.1 ± 0.7	57.2 ± 0.1	56.2 ± 0.5
k_h [d^{-1}]	0.221	0.218	0.202	0.210	0.196	0.200
GS_{21} [Nl/gTS]	193.9 ± 16.6^{a}	216.1 ± 7.7^{a}	208.1 ± 14.3^{a}	147.7 ± 8.1^{b}	159.2 ± 0.7^{b}	158.2 ± 7.8^{b}
GS_{21} [Nl/gTVS]	267.1 ± 20.4^{a}	306.4 ± 13.6^{b}	285.1 ± 28.2^{a,b}	196.9 ± 10.8^{b}	216.6 ± 0.9^{d}	212.7 ± 7.7^{c,d}
ΔGS_{21} [%]	-	14.7	6.7	-	10.0	8.0
BMP_{21} [NlCH_4/gTS]	125.0 ± 8.2^{a}	136.9 ± 8.4^{a}	126.9 ± 8.4^{a}	90.0 ± 3.5^{b}	96.1 ± 0.7^{c}	93.2 ± 4.4^{b,c}
BMP_{21} [NlCH_4/gTVS]	172.1 ± 9.8^{a}	186.7 ± 6.5^{a}	173.8 ± 16.6^{a}	119.9 ± 4.7^{b}	130.2 ± 0.7^{c}	125.2 ± 4.2^{b,c}
ΔBMP_{21} [%]	-	8.5	1.0	-	8.5	4.4

Table 5

Energy demand (E_D), energy produced in the form of biogas (E_B) and heat (E_Q) and profit (E_T) of the pre-treatments.

	E_B [kJ/kgTVS]	E_D [kJ/kgTVS]	E_Q [kJ/kgTVS]	E_T [kJ/kgTVS]
M1_MW	540.2	4445.1	2580.6	- 1324.3
M1_A	62.9	6921.4	4200.0	- 2658.5
M2_MW	410.7	4178.5	2415.5	- 1352.3
M2_A	225.7	6506.3	3931.3	- 2349.3