Moringa oleifera Lam. as an energy crop for biogas production in developing countries

Fulvia Tambone*, Matteo Pradella, Floriana Bedussi and Fabrizio Adani

Ricicla Group Labs - Dipartimento di Scienze Agrarie e Ambientali – Produzione, Territorio, Agroenergia - Università degli Studi di Milano - Via Celoria 2 – 20133 Milano, Italy

(*) Corresponding author: e-mail: fulvia.tambone@unimi.it

Abstract

Moringa oleifera Lam. (moringa) is a typical plant of tropical climates used as food, feed and natural medication. This plant, rich in oil, could be valorised in the bioenergy sector, as infeed to produce biofuel, useful to save fossil sources and to limit GHG emissions. This paper has evaluated its potential methane production by the anaerobic digestion process, in comparison to corn and giant cane, two typical food and non-food energy crops. Biogas production has been correlated to the content of fats, waxes, cellulose, hemicellulose, proteins and lignin in the three plants and to the different carbon-types detected by solid-state Nuclear Magnetic Resonance (NMR) technique. Chemical and spectroscopic analysis showed that organic matter of moringa contains more than 40% of fats having high potential to produce biogas. Although the quantity of biogas produced from corn was higher, among the three samples, the content in methane for corn and moringa was not statistically different. In particular, the methane yields for the giant cane, moringa and corn were

363±11, 442±9 and 452±12 m^3 Mg DM^{-1} respectively: moringa produced less biogas but it was richer in methane. Methane concentration was positively correlated to the sum of fats, waxes, resins, hemicellulose and proteins ($R^2=0.97$; $n=3$; $p<0.05$). All these results seem to indicate that effectively moringa can be used as substrate to produce biogas by anaerobic digestion. When combined with small-scale low-tech digesters, this can represent a good opportunity for bioenergy production in developing countries.

Keywords:

anaerobic digestion; biogas; chemical composition; fats; moringa; renewable energy

1. Introduction

Moringa oleifera Lam. (moringa) (Moringaceae family) is a fast-growing softwood tree indigenous to sub-Himalayan tracts of Northern India. This tree is mainly found in the Middle East and in African and Asian countries, but, due to its adaptability, it is spreading to other areas affected by drought in tropical and subtropical lands [1]. Moringa, until a few years ago, was known and used only by the population of the tropical belt, where moringa grows spontaneously. More recently, due to its easy propagation and its various uses, moringa is receiving growing interest at international level, both from scientists and private companies. The main use of moringa is as food, to combat malnutrition in the poorest areas of the world, and as forage, but it is also used in medicinal applications for different diseases, by virtue of the numerous active principles contained in this plant [2]. Nevertheless, the literature is contradictory regarding the use of moringa as food or forage. Makkar and Becker [3] reported that the particular bitter taste in fresh moringa, due to the presence of alkaloids, saponins and glucosinolates, has as a consequence that dairy cows fed with fresh moringa produce milk with a bad taste or smell. However, in another experiment [4], where moringa was used as forage, the organoleptic analysis of milk showed no evidence of quality problems. These results lead us to suppose, as a precautionary measure, the possibility to use only limited quantities of moringa as a dietary supplement, both for human beings and for other animals. In this context, some authors have proposed the use of moringa as a source of bioenergy, in particular biodiesel or biogas [5-8]. With the aim of improving the methane content in the biogas, some pretreatments of the infeed biomasses are proposed such as ozonation, alkaline or acid hydrolysis, shredding, press extruders, ultrasound, pre-digestion by microorganism, enzymes or use of additives [9]. Biodiesel can derive from different vegetable oils (*i.e.* cottonseed, palm, soybean) and a review of the literature indicates that moringa oil could be one of the prospective sources of biodiesel that has lower impact in terms of emissions of carbon monoxide (CO), carbon dioxide (CO_2), Particulate Matter (PM_{10} and $PM_{2.5}$) compared to fossil fuels [10], as atmospheric pollutants affect the air quality [11].

Rashid et al. [5] proposed moringa oil as potential biomass to produce biodiesel with interesting results and yields. Nevertheless, some authors reported high values of cloud and pour points for the biodiesel from moringa oil which presents a problem in cold temperatures [6] and others reported low oxidation stability of biodiesels that could be overcome only by adding antioxidants [7]. Another possibility consists in the use of moringa [6] with particular interest in the leaves and branches to produce biogas [8] leaving seeds for biodiesel production or food purposes.

This last opportunity, for bio-energy production, could be particularly interesting in developing countries where energy needs are growing. In Sub-Saharan Africa, for example, the access to energy systems is difficult, despite the presence of several reserves of petroleum, natural gas and coal. This causes high consumption of virgin wood and charcoal, as the principal sources of energy, with negative implications for the environment (deforestation and greenhouse gases – GHGs emission) and human health (respiratory infections), particularly in rural areas [12]. The main victims of these risks are the women and children who generally do the cooking and harvesting of biomasses [13]. In this context, anaerobic digestion (AD) can play a central role in reducing conflicts between energy combustion and environmental conservation, adopting low-tech technologies [12-16]. In order to optimize the production of biogas and, at the same time, the methane content, it is now common to use the so-called energy crops for anaerobic digestion. Several annual and perennial plants are proposed as suitable to produce energy by AD, both food and no-food crops. The most important perennial species are grass, rye grass and miscanthus, for which potential methane yields (PMY) have been reported [17] to be 298-467 m^3 Mg^{-1} VS; 390-410 m^3 Mg^{-1} VS; 179-218 m^3 Mg^{-1} VS, respectively. Among the annual energy crops, maize is most widely used but also, wheat, sorghum, triticale and sugarbeet [17]. For these crops PMY are 205-450 m^3 Mg^{-1} VS; 384-426 m^3 Mg^{-1} VS; 295-372 m^3 Mg^{-1} VS; 337-555 m^3 Mg^{-1} VS; 236-381 m^3 Mg^{-1} VS respectively [17]. The great variability is due to the energy crop type, chemical composition, area of cultivation and time of harvest. However, the potential biogas production cannot be the only parameter to evaluate the quality of an energy crop and other aspects must be considered such as the climatic context and the agro-ecological impact [18]. Other agronomic aspects, in particular water and nutrients use efficiency, can play key roles in the choice of the most suitable energy crop [19]. For example, Gissén et al. [20], reporting the performance in methane production and relative costs related to six different energy crops (hemp, sugar beet, maize, triticale, grass/clover ley, winter wheat), concluded that, despite the good energy yields in some contexts in European countries, it would be difficult to achieve a real advantage in terms of costs and benefits in the long term. In this context, recently *Arundo donax* L. (giant cane) has been proposed as a non-food energy crop [21]. Giant cane is characterized by its very high biomass production and, despite its yield in methane being lower, compared to corn, the biomethane production per hectare is much higher, in view of the lower energetic and agronomic inputs for giant cane in comparison with corn [21]. Similarly, one of the main characteristics of moringa, in addition to its remarkable hardiness, is its low need for water, a factor that has allowed it to adapt well to adverse weather conditions. The plant generally produces flowers and pods from the second year of age, sometimes two crops are obtained each year. A moringa plant remains productive for as long as 30 years, all the year round, even during the dry seasons. In fact, today moringa is known and used in many arid areas of the world (i.e. tropical Africa, tropical America, Sri Lanka, India). Comparing the requisites that an energy crop must possess [22] in particular rapid growth, high yield per hectare, absence or limited competition with food production, low needs in terms of water, nutrients and pesticides, moringa seems to agree well with these requirements. Because of the characteristics of moringa above discussed, this crop seems to be potentially useful for producing bioenergy, via biogas production, in developing countries. Unfortunately, few data exist regarding the ability of this crop to produce biogas in relation to its chemical characteristics.

The aim of this paper is to assess the potential methane production of moringa in comparison with corn and giant cane. In addition, corn, giant cane (this work) and *Miscanthus x giganteus* (literature data as suggested by referees) have been chosen for comparison because they represent, respectively, the typical energy crop characterized by high methanogenic power, but not feasible in developing countries with food safety problems, and two emerging non-food energy crops with a good methanogen potential. Moreover, biogas produced by energy crops has been correlated with chemical characteristics, *i.e.* fats, waxes, resins, cellulose, hemicellulose, proteins and lignin contents detected by the different carbon-types assessed by the Cross Polarization Magic Angle

Spinning ^{13}Carbon Nuclear Magnetic Resonance (CPMAS ^{13}C NMR) technique.

2. Materials and methods

2.1 Experimental approach

To verify the aptitude of *Moringa oleifera* to be used as an energy crop for biogas production its chemical characterization, as well as organic carbon detection by CPMAS ^{13}C NMR, were carried out. All these data have then been correlated with the Anaerobic Biogas Potential (ABP) test. In addition, two other energy crops used to produce biogas, i.e. corn (annual crop) and giant cane (perennial crop) have been considered for comparison.

2.1 Chemical characterization of biomasses under investigation: moringa, corn and giant cane

Samples of plants of *Moringa oleifera* Lam. (moringa - M) (leaves and branches of adult plants of 4-5 years), *Zea mays* L. (corn - C) and *Arundo donax* L. (giant cane - GC) (for these last two, whole plants at the end of the cycle without roots) were used for the experiment. Corn and giant cane, sampled from an AD full-scale plant (Lombardy Region – North Italy), were previously subjected to an ensiling process. The samples of moringa came from a cultivated crop located in Haiti (Port-au-Prince). The samples were dried for 24 h at 80 °C and then shredded in a blender to pass through a 2-mm mesh. All the samples were analysed for their pH (fresh samples for C and GC), volatile solids (VS), Total Organic Carbon (TOC) by the dichromate method and total N-Kjeldahl (TKN) content [23]. Since all the biomasses studied were of vegetable origin and to better understand if there was a relationship, as expected, between the production of biogas with some specific class of organic compounds, we performed a detailed quantification of the different classes of compounds characterized by different biodegradability rates and so different potential biogas production. For this purpose, macromolecular composition analyses were performed by using different solvents, as reported in a previous work [24]. In details the following components were determined: fraction I, soluble in organic solvents (hexane-ethanol 50:50 v/v, and ethanol 16.87 mol L^{-1}) for lipids, resins, tannins, part of proteins; fraction II, soluble in H_20 and hot H_2SO_4 0.94 mol L^{-1} under reflux for 2 h for hemicellulose, part of proteins, sugar; fraction III, soluble in H_2SO_4 13.50 mol L^{-1} at 4°C for 24 h for cellulose; fraction IV, insoluble in H_2SO_4 13.50 mol L^{-1} for lignin. All analyses were performed in triplicate.

2.2 Carbon-type determination of biomasses under study detected by CPMAS ^{13}C NMR technique

CPMAS ^{13}C NMR is a technique useful to provide qualitative and quantitative information on the composition of a biomass by the identification of the main carbon-types of which the organic matter consists [25]. In this paper, this technique has been combined with the chemical characterization of the samples in order to find possible correlations between biogas production and moringa composition. The solid-state CPMAS ^{13}C NMR spectra of the samples, dried and ground (diameter ! 0.2 mm), were acquired at 10 kHz on a Bruker AMX 600 spectrometer (Bruker BioSpin GmbH, Rheinstetten) using a 4-mm CP-MAS probe. The pulse repetition rate was set at 0.5 s, the contact time at 1 ms, and the number of scans was 3200. The chemical shift scale of CP MAS ^{13}C

NMR spectra were referred to tetramethylsilane (δ = 0 ppm). Spectra were elaborated using TOPSPIN 1.3 software (Bruker BioSpin GmbH, Rheinstetten, Germany).

2.3 Biogas determination by anaerobic potential biogas production (ABP) test performed on moringa, corn and giant cane

The ABP test was performed in a 100-ml serum bottle using 0.62 g of dried sample added to 37.5 ml of inoculums in stable methanogenic activity and 22 ml of de-ionized water. Volatile fatty acids losses during samples' drying (corn and giant cane) have not been taken in consideration because their content is very limited (1-2% DM) [26]. All batches were sealed with Teflon hermetic caps, flushed with an N_2 atmosphere, and incubated at 37 ± 1°C, until no further biogas production was detected (around 100 days) [27]. The same analysis was performed on the inoculums alone as controls. Test bottles were periodically analysed for both quantitative and qualitative determination of biogas production. Quantitative biogas production was estimated by withdrawing extra-pressure gas with a syringe. Biogas composition (CH_4-CO_2 ratio) was assessed by gas chromatograph (Agilent 3000A Micro GC). All tests were run in duplicate.

2.4. Statistical analyses of the data

Chemical analyses were performed in triplicate. Average and standard deviation values were calculated according to standard procedures. The results were analysed by ANOVA, and the Tukey test was used to compare mean values. All statistical analyses were carried out using SPSS statistical software (SPSS, Chigago, IL).

3. Results and discussion

3.1 Chemical characterization of biomasses under investigation vs. potential biogas production for moringa, corn and giant cane.

Chemical characteristics can affect biomass degradability and so biogas production.–The moringa chemical characteristics, i.e. C, TKN and C to N ratio, are comparable to those reported in the literature [28], but differ from those determined for corn and giant cane (Table 1). In particular corn and giant cane are characterized by lower pH values than moringa biomass as a consequence of the presence of volatile fatty acids [29] produced during the ensilage process. On the other hand, the organic carbon (OC) content was very similar in all biomasses, as indicated by the low standard deviation obtained (average value 550±9 g kg^{-1} DM). This parameter can indicate the suitability for a biomass to produce biogas, because it represents the substrate potentially degradable by the microbial community responsible for the anaerobic digestion process [30]. Nitrogen content (TKN) in moringa was four and five times higher than corn and giant cane, respectively, because of the high content of organic nitrogen (*i.e.* protein) [2]. The availability of N *vs.* C content for microorganisms, *i.e.* the C/N ratio, was much lower for moringa (12.1) than for corn (49.7) and giant cane (69.7). This parameter is important to define the rapidity of degradation of a biomass: lowest values promote degradation [31].

More interesting are the data reported in Figure 1 concerning the macromolecular composition of the organic matter (OM) contained in the three plants, as the potential biogas production of a biomass depends on both quantitative aspects, OC content as previously reported, and qualitative factors such as the content of fats, carbohydrates, proteins, cellulose, hemicellulose, and lignin [32]. Moringa samples were characterized by a significantly higher fraction of fatty compounds, i.e. 43.8±5.2 % OM for moringa, 20.7±0.0% OM for corn, and 14.6±0.9% OM for giant cane, than fibrous material (hemicellulose, cellulose and lignin). Substrates rich in fats [32], which are organic molecules characterized by a lower oxidation state, are able to produce more biogas in comparison to other biomasses with similar organic matter content-

3.2 Carbon-type determination of biomasses under study detected by CPMAS ^{13}C NMR technique vs. potential biogas production

The results of solid-state CPMAS ^{13}C NMR analyses, reported in Table 2, provide qualitative information on the composition of the three biomasses studied, by identifying the main carbon-type that composed organic matter. C type distribution agrees with the wet analysis before discussed. In particular, moringa is characterized by the presence of fat (aliphatic C) (spectra region 0-47 ppm) that is of 29% of total C, and so much higher than those identified for corn and giant cane (about 8%). This means that moringa is rich in both short and long chain linear structures like suberin, cutin and waxes (peaks at 25–33 ppm) [33, 34] that confirm the high fraction of fatty compounds determined by solvent extraction (Figure 1). This resulted in a lower presence of C types in the spectra region 47-110 ppm (polysaccharides) (Table 2).

3.3 Biogas determination by anaerobic potential biogas production (ABP test) performed on moringa, corn and giant cane

To assess the potential of moringa in producing biogas, Table 3 reports a comparison with the average of ten samples of different ingestates [35] composed by mixes of the organic fraction of municipal solid waste, pig and cow slurry, milk serum, and corn silage. Data reported in Table 2 show that, in terms of composition especially for aliphatic C, moringa is very similar to typical infeed mixtures, which leads us to propose that it has a good potential for the production of biogas. In this regard, Figure 2 reports the trends relative to biogas production during the 100 days of ABP test. Figure 2 clearly shows similar cumulative trend (no inhibition) and production (Table 3) of biogas for moringa (670±6 m^3 Mg DM^{-1}) and giant cane (686±2 m^3 Mg DM^{-1}) but higher values for corn (781±13 m^3 Mg DM^{-1}). Nevertheless, data should also be analysed to compare the specific methane yield potential (SMY) for the samples. The results, expressed on the base of DM and VS, (442±9 m^3 Mg DM^{-1} i.e. 412 m^3 Mg VS^{-1}; 452±12 m^3 Mg DM^{-1} i.e. 430 m^3 Mg VS^{-1}; 363±11 m^3 Mg DM^{-1} i.e. 349 m^3 Mg VS^{-1} for moringa, corn and giant cane respectively) are significantly different, because the percentage of methane in biogas is taken into consideration. Biogas produced during the anaerobic digestion process principally consists of methane (50–80% v/v) and carbon dioxide [35]. As a result, the greater the percentage of methane, the higher the energy power of the produced biogas will be. The concentration of methane in the biogas, measured during the ABP test, is reported in Table 3 and the values are 66.8±1.5 %, 58.9±1.3 % and 53.2±1.4% for M, C and GC respectively. In particular, CH_4 (% Biogas) concentration was moringa>corn>arundo. This means that, in comparison to giant cane, moringa is able to produce less biogas which, however is richer in methane. As a consequence, the total CH_4 produced, in terms of m^3 Mg D.M.$^{-1}$, for corn and moringa was not statistically different (Table 3). Potential biomethane production from moringa appeared very interesting also when it was compared with *Miscanthus x giganteus*, i.e. a typical perennial crop proposed for biogas production (literature data). In fact, bibliographic data relative to the quantity of methane that can be produced by *Miscanthus x giganteus* vary from 150 up to 325 m^3 Mg D.M.$^{-1}$ [36-38] depending mainly on the harvest time during the year. For our samples, methane concentration was positively correlated to the sum of fats, waxes, resins, hemicellulose and proteins (R^2=0.97; n=3; $p<0.05$) and, as expected, negatively correlated to lignin content (R^2=0.70; n=3; $p<0.05$). In moringa, despite the lower content of carbohydrate (i.e. hemicellulose and cellulose) in comparison to corn (about +40% for corn), the higher content of fats (about +53% for moringa) was able to contribute in a significant manner to the production of bioenergy during anaerobic digestion.

4. Conclusions

Moringa's chemical and spectroscopic characteristics, in comparison with those of corn and giant cane, highlighted a content of compounds with high methanogenic power (i.e. fats) and the potential biogas production test has confirmed that the plant can effectively be valorised as a substrate suitable for bioenergy production by anaerobic digestion. The use of moringa, mixed with other available organic waste, could therefore be an excellent opportunity for the production of biogas by small-scale low-tech digesters. Moringa, also by virtue of its characteristics of hardiness, rapid growth, low water and fertilizer requirements could play a multifunctional role in socioeconomic and environmental terms in developing contexts.

References

[1] Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S (2016). Moringa oleifera seeds and oil: characteristics and uses for human health. Int J Mol Sci, 17(12): 2141

[2] Gopalakrishnanb L, Lakshmipriya KD, Devarai SK (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. Food Sci Hum Well 5: 49-56

[3] Makkar H, Becker K (1997). Nutrients and antiquality factors in different morphological parts of the Moringa oleifera tree. J Agric Sci 128: 311–332

[4] Mendieta-Araica B, Spörndly R, Reyes-Sánchez N, Spörndly E (2011). Moringa (Moringa oleifera) leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. Livestock Sci, 137: 10-17

[5] Rashid U, Anwar F, Moser BR, Knothe G (2008). Moringa oleifera oil: a possible source of biodiesel. Biores Technol, 99: 8175-8179

[6] Kafuku G, Mbarawa M (2010). Alakline catalyzed biodiesel production from moringa oleifera oil with optimized production parameters. Appl Energy, 87: 2561-2565

[7] Fernandes DM, Sousa RMF, de Oliveira A, Morais SAl, Ritcher EM, Muñoz RAA (2015). Moringa oleifera: a potential source of biodiesel and antioxidant additives. Fuel, 146: 75-80

[8] Gandji K, Chadare FJ, Idohou R, Salako VK, Assogbadjo AE, Glele Kakai RL (2018). Status and utilization of Moringa oleifera Lam: a review. Afric Crop Sci J, 26: 137-156

[9] Panepinto D, Genon G, (2016). Analysis of the extrusion as a pretreatment for the anaerobic digestion process. Ind. Crops Prod., 83, 206–212

[10] Azad AK, Rasul1 MG, Khan MMK, Sharma SK, Islam R (2015). Prospect of Moringa seed oil as a sustainable biodiesel fuel in Australia: A review Proc Engin, 105: 601-606

[11] Panepinto D, Brizio E, Genon G (2014). Atmospheric pollutants and air quality effects: limitation costs and environmental advantages (a cost–benefit approach). Clean. Techn. Environ. Policy, 16:1805-1813

[12] Gwavuya SG, Abele S, Barfuss I, Zeller M, Müller J (2012). Household energy economics in rural Ethiopia: A cost-benefit analysis of biogas energy. Renew Energy, 48: 202-209

[13] Rahman MdM, Hasan MM, Paatero JV, Lahdelma R (2014). Hybrid application of biogas and solar resources to fulfill household energy needs: A potentially viable option in rural areas of developing countries. Renew. Energy, 68: 35-45

[14] Bond T, Templeton MR (2011). History and future of domestic biogas plants in the developing world. Energy Sust Develop 15: 347-354

[15] Gallia A, Veronesi D, Spencer Embalò U, Pongiglione F, Adani F, Schievano A (2015). Domestic low-tech anaerobic digesters in Guine´-Bissau: a bench-scale preliminary study on locally available waste and wastewater. Environ Dev Sustain, 17, 1227-1241

[16] Mungwe JN, Colombo E, Adani F, Schievano A (2016). The fixed dome digester: An appropriate design for the context of Sub-Sahara Africa? Biomass and Bioenergy, 95, 35-44

[17] Murphy J, Braun R, Weiland P, Wellinger A (2011). Biogas from crop digestion. Task 37 - Energy from Biogas. IEA Bioenergy

[18] López-Bellido L, Wery J, López-Bellido RJ (2014). Energy crops: Prospects in the context of sustainable agriculture. Europ. J. Agronomy, 60: 1-12

[19] Jaradat AA (2010). Genetic resources of energy crops: biological systems to combat climate change. Aust. J. Crop Sci. 4: 309-323

[20] Gissén C, Prade T, Kreuger E, Achu Nges I, Rosenqvist H, Svensson SE, Lantz M, Mattsson JE, Borjesson P, Bjornsson L (2014). Comparing energy crops for biogas production e Yields, energy input and costs in cultivation using digestate and mineral fertilization. Biom Bioen, 64: 199-210

[21] Corno L, Pilu R, Tambone F, Scaglia B, Adani F (2015). New energy crop giant cane (*Arundo donax* L.) can substitute traditional energy crops increasing biogas yield and reducing costs. Biores Technol, 191: 197-204

[22] Dubois J (2011). Requirements for the development of a bioeconomy for chemicals. Curr. Opin. Environ. Sustain. 3: 11-14

[23] APHA (1998). Standard Methods for Examination of Water and Wastewater, 20th ed; American Public Health Association: Washington, DC

[24] Adani F, Genevini P, Tambone F (1995). A new index for compost stability. Compost Sci Util, 3: 25-37

[25] Conte P, Piccolo A, Van Lagen B, Buurman P, de Jager PA (1997). Quantitative aspects of solid-state ^{13}C NMR spectra of humic substances from soils of volcanic systems. Geoderma 80: 327-338

[26] Corno L, Pilu R, Cantaluppi E, Adani F (2016). Giant cane (*Arundo donax* L.) for biogas production: the effect of two ensilage methods on biomass characteristics and biogas potential. Biomass. Bioen., 93: 131-136

[27] 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. Biores Technol, 99: 8112-8117

[28] Mulugeta G, Fekadu A (2014). Industrial and agricultural potentials of Moringa. J Nat Sci Res, 14: 57-63

[29] Zheng Y, Yu C, Cheng Y, Zhang R, Jenkins B, Vander Gheynst JS (2011). Effects of ensilage on storage and enzymatic degradability of sugar beet pulp. Biores Technol, 102: 1489-1495

[30] Fernández J, Pérez M, Romero LI (2008). Effect of substrate concentration on dry mesophilic anaerobic digestion of organic fraction of municipal solid waste (OFMSW). Biores Technol, 99: 6075-6080

[31] Cabrera ML, Kissel DE, Vigil MF (2005). Nitrogen mineralization from organic residues: research opportunities. J Environ Qual 34: 75–79

[32] Schievano A, Scaglia B, D'Imporzano G, Malagutti L, Gozzi A, Adani F (2009). Prediction of biogas potentials using quick laboratory analyses: Upgrading previous models for application to heterogeneous organic matrices. Biores Technol, 100: 5777-5782

[33] Dignac MF, Derenne S, Ginestet P, Bruchet A, Kniker H, Largeau C (2000). Determination of structure and origin of refractory organic matter in biodepurated wastewater via spectroscopic methods. Comparison of conventional and ozonation treatment. Environ Sci Technol 34: 3389–3394

[34] Pichler M, Knicker H, Kögel-Knabner I (2001). Solid-state ^{13}C NMR spectroscopic, chemolytic and biological assessment of pretreated municipal solid waste. J Ind Microbiol Biotechnol, 26: 83–89

[35] Tambone F, Genevini P, D'Imporzano G, Adani F (2009). Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Biores Technol, 100: 3140-3142

[36] Mayer F, Gerin PA, Noo A, Lemaigre S, Stilmant D, Schmit T, Delfosse P (2014). Assessment of energy crops alternative to maize for biogas production in the Greater Region. Biores. Technol., 166: 358-367

[37] Baute K, Van Eerd LL, Robinson DE, Sikkema PH, Mushtaq M, Gilroyed BH (2018). Comparing the biomass yield and biogas potential of *Phragmites australis* with *Miscanthus x giganteus* and *Panicum virgatum* grown in Canada. Energies, 11: 2198, 2-14

[38] Mangold A, Lewandowski I, Hartung J, Kiesel A (2018). Miscanthus for biogas production: influence of harvest date and ensiling on digestibility and methane hectare yield. Glob. Change Biol. Bioen., 11:50–62

[39] Tambone F, Scaglia B, D'Imporzano G, Schievano A, Orzi V, Salati S, Adani F (2010). Assessing amendment and fertilizing properties of

digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere, 81: 577–583