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REVIEW PAPER

Anaerobic co-digestion of organic wastes

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Abstract Over the last years anaerobic digestion has been successfully established as technology to treat organic wastes. The perspective of turning, through a low-cost process, organic wastes into biogas, a source of renewable energy and profit, has certainly increased the interest around this technology and has required several studies aimed to develop methods that could improve the performance as well as the efficiency of this process. The present work reviews the most interesting results achieved through such studies, mainly focusing on the following three aspects: (1) the analysis of the organic substrates typically co-digested to exploit their complementary characteristics; (2) the need of pre-treating the substrates before their digestion in order to change their physical and/or chemical characteristics; (3) the usefulness of mathematical models simulating the anaerobic co-digestion process. In particular these studies have demonstrated that combining different organic wastes results in a substrate better balanced and assorted in terms of nutrients, pre-treatments make organic solids more accessible and degradable to microorganisms, whereas mathematical models are extremely useful to predict the co-digestion process performance and therefore can be successfully used to choose the best substrates to mix as well as the most suitable pre-treatments to be applied.

Keywords Anaerobic · Co-digestion · Pre-treatments · Mathematical model

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1 Introduction

Anaerobic Digestion (AD) is a technology that has been around for over a century, mainly and successfully applied for stabilizing the organic sludge produced from Municipal Waste Water Treatment

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Plants (WWTPs) and for cleaning wastewaters from food process. Recently the interest in using this technology for treating other organic solids (e.g. organic wastes and energy crops) has gone rapidly growing thanks to the boost impressed by new and stricter regulations on organic waste disposal as well as by the need of finding new sources of energy alternative to the fossil fuels (Lema and Omil 2001; Lettinga 2001).

The use of AD for treating organics is attractive for several reasons that involve economic as well as environmental aspects (Lettinga 2001; Barton et al. 2008): this technology not only reduces the volume of material to be disposed and avoids soil and groundwater pollution, but also makes available a renewable and inexpensive energy, e.g. biogas, that, unlike the fossil fuels, keeps stable in the atmosphere the balance of greenhouse gases, such as CO_2. Additionally the AD of organic solids represents an affordable low-cost and low-technology system to supply energy for rural areas in developing as well as underdeveloped countries where the main cause of their economic and social backwardness can be reasonably attributed to the lack of suitable energy. Beside biogas that is already successfully used to produce electricity and heat as well as to feed gas networks (Bekkering et al. 2010), a further source of income produced by AD of organic solids is represented by the semi-solid by-product of this process, called digestate, that thanks to its high content of nutrients can be used in agriculture directly as fertilizer or processed into compost to increase its quality (Tambone et al. 2009; Rehl and Müller 2011).

The AD, as biological process, is strongly dependent on the environmental conditions (Mata-Alvarez et al. 2000) such as temperature, pH, nutrients content, carbon/nitrogen (C/N) and carbon/phosphorus (C/P) ratio, presence of inhibitors, substrate typology, microelements availability and particles size, that, in particularly unfavourable situations, can be responsible for undesirable drops in performance and even for detrimental failures. Therefore, an accurate setting of the operating conditions and their continuous monitoring during all the evolving of the AD can prevent unwanted inconveniences and also improve the efficiency of the process. From this point of view interesting results have been obtained treating simultaneously several solid as well as liquid organic wastes. This process is commonly known as co-digestion and has been studied during the last 15–20 years (Cecchi et al. 1988; Rintala and Ahring 1994; Pohland 1996; Hamzawi et al. 1998a; Sosnowski et al. 2003). The above-mentioned investigations take into account different types of organic solids with regard to their nature and origin. The outcomes showed a synergistic effect. In particular mixing organic substrates can result in the production of a mixture with a C/N ratio included in the optimal range 20/1–30/1 as reported in literature (Hawkes 1980). Further benefits of the co-digestion process are: (1) dilution of the potential toxic compounds eventually present in any of the co-substrates involved; (2) adjustment of the moisture content and pH; (3) supply of the necessary buffer capacity to the mixture; (4) increase of the biodegradable material content; (5) widening the range of bacterial strains taking part in the process.

All these benefits concur in an improved stability and performance of the process and in a higher biogas and energy production (Tchobanoglous et al. 1993).

However, the studies reported in literature indicate that the optimal operational conditions in terms of percentages of co-substrates cannot be univocally defined but should be investigated for each specific case (Fujita et al. 1980; Hills 1980; Fischer et al. 1983; Hashimoto 1983; Weiland and Hassan 2001; Callaghan et al. 2002; Kaparaju et al. 2002; Lehtomäki et al. 2007; Labatut et al. 2011; Ponsá et al. 2011; Esposito et al. 2012). Moreover, with regard to the physical and biochemical processes prevailing in a co-digestion system, only some aspects have been investigated and other questions require to be further studied such as the effect of the temperature on the process performance; the need for pre-treatments; the feeding systems of the solid mixture; the effect of the solid mixture moisture content; the definition of the optimal stirring of the solid mixture in the bioreactor. For these studies an useful tool is represented by mathematical models (simulating the co-digestion process according to the specific sets of operational conditions) that can indicate the best settings of them to maximize the performance of the process.

2 Co-digestion substrates

All sorts of biomass containing carbohydrates, proteins, lipids, cellulose and hemicelluloses, as main components, are suitable to be used as substrates for biogas production. Sewage sludge from aerobic wastewater treatment, animal manure, harvest residues, organic wastes from agriculture-related factories, meat and fish industrial wastes, dairy wastes, food waste, collected municipal organic solid waste from markets and households and energy crops are the substrates commonly used for feeding anaerobic digesters.

The theoretical gas yield varies with the content of carbohydrates, proteins, and lipids. Lipids provide the highest biogas yield, but require a long retention time due to their slow biodegradability, whereas carbohydrates and proteins show faster conversion rates but lower gas yields.

Lipids are characterized as fats, liquid (oils) and solid (greases). They are commonly present in food wastes and in some industrial wastewaters, such as those produced by slaughterhouses, dairies or fat refineries (Li et al. 2002). Lipids are attractive for biogas production due to the high number of C and H atoms in their molecule, which implies a high theoretical methane potential. However, they can also present several problems such as inhibition of methanogenic bacteria and adsorption onto biomass that can cause sludge flotation and washout (Neves et al. 2009).

Carbohydrates are the main components of organic wastes from agriculture-related factories, food waste and collected organic fraction of municipal solid waste (OFMSW) from households and markets. The anaerobic degradation of such wastes is strongly dependent on the ratio between the acidification process rate and the methanogenic process rate. In particular, if the acidification process is faster than the methanogenic process, volatile fatty acids (VFAs) tend to accumulate in the reactor causing progressive drops in pH that stress and inhibit the activity of methanogenic archaea (Siegert and Banks 2005).

Wastes rich in proteins and consequently with a high nitrogen content are mainly produced by meat processing factories, slaughterhouses and farms (animal slurry and manure). These wastes present a high organic content, high biological oxygen demand (BOD) but low C/N ratio (Callaghan et al. 2002; Edström et al. 2003; Cuetos et al. 2010). High ammonia concentration in animal wastes is considered a factor of inhibition for anaerobic treatments (Nielsen and Angelidaki 2008). This problem is particularly serious when the digesters are fed with wastes rich in proteins because, during their fermentation, a significant increase of the ammonia concentration occurs (Chen et al. 2008).

Cellulose wastes (CWs) are produced by paper and cardboard factories or from textile factories. CWs are also part of the municipal solid wastes (MSWs) that are not source-separated and could be added to the organic solid wastes to be anaerobically treated. CWs have a high C/N ratio ranging from 173/1 to higher than 1,000/1 (Zhang et al. 2008), while the suggested optimum C/N ratio for anaerobic digestion is in the range of 20/1–30/1 (Hawkes 1980).

In a co-digestion process wastes rich in proteins can provide the buffering capacity and a wide range of nutrients, while wastes with a high carbon content can balance the C/N ratio for all substrates characterized by a low C/N ratio, decreasing the risk of ammonia inhibition (Hills and Roberts 1981; Hashimoto 1986). Therefore, the anaerobic co-digestion with the OF-MSW is recommended for the treatment of wastes with a high nitrogen content (Buendía et al. 2009) thanks to a better C/N ratio in the resulting mixture. As well as a better balance of the C and N content is obtained when animal manure is co-digested with crop residues resulting in a higher methane yields (Lehtomäki et al. 2007). The optimal C/N ratio seems to be the most interesting synergistic effect when different substrates are co-digested and therefore this aspect has been widely studied. An attempt to determine the optimal ratio was done by Li et al. (2009), using pre-treated corn stalks to co-digest cattle manure at four mixing ratios (manure VS/corn stalks VS = 1/1, 1/2, 1/3, and 1/4). The authors reported an optimal value of C/N ratio for biogas production developing the co-digestion process when the ratio manure VS/corn stalks VS was equal to 1/3. Wang (2009) observed that wheat straw co-digested with swine manure can increase the methane productivity up to 10 % when 46 % wheat straw is added to the digester. In a recent study Wu et al. (2010) indicated that a significant increase of the volumetric biogas production can be achieved by adding carbon rich agricultural residues, such as corn stalks, oat straw and wheat straw to the co-digestion process with swine manure. The amounts of crop residues added to swine manure were calculated to set the C/N ratio equal to 16/1, 20/1, and 25/1, where nitrogen is calculated as Total Kjeldahl Nitrogen (TKN). The best performance of the anaerobic reactor in terms of biogas productivity was obtained with a C/N ratio equal to 25/1. The synergistic effect of five substrates in a digester was investigated by Zitomer et al. (2008). In particular the authors noticed that the addition of yeast waste produced an increase in biogas production higher than 50 %, probably due to trace of micronutrients (Fe, Ni, Co) present in the yeast waste (Zitomer et al. 2008).

Further promising results have been obtained by co-digesting OFMSW with CWs (Zhang et al. 2008), with agricultural residues (Converti et al. 1997; Kübler et al. 2000), with wastes rich in lipids (Ponsá et al. 2011). Interesting results were also obtained by co-digesting organic solid wastes such as OFMSW with sewage sludge (Fernández et al. 2005; Zhang et al. 2008; Neves et al. 2009), manure with harvest residues (Wu et al. 2010), manure with crops residues (Lehtomäki et al. 2007), manure with cheese whey (Kacprzac et al. 2010), fruit and vegetable waste with fish waste, slaughterhouse waste and wastewater activate sludge, respectively (Bouallagui et al. 2009). Particularly remarkable is the use of energy crops for biogas production. Energy crops, unlike the other substrates listed before, are not wastes, as they are expressly grown and used to produce green power and they can perform high biogas production even when used as pure substrates (Table 1).

The specific biogas yields from the co-digestion of different organic mixtures are reported in Table 2. However, when comparing such values it should be taken into account that they were obtained under different operational conditions in terms of working volume (V), temperature (T), organic load rate (OLR), hydraulic retention time (HRT), reactor hydrodynamic regime (batch, plug flow or completely stirred), moisture content, substrate/inoculum ratio, etc. In Table 2 are indicated only the operational conditions in terms of substrates ratio, V and T because these data are present in all papers accessed and where decimal numbers as well as standard deviation value are not reported, it means that this information is not present in the original paper.

Table 1 Biogas potential of different crops (Weiland 2010)

Crop	Biogas yield (Nm^3 $ton^{-1}VS^*$ added)	Methane content (%)
Sugar beet	730–770	53
Fodder beet	750–800	53
Maize	560–650	52
Corn cob mix	660–680	53
Wheat	650–700	54
Triticale	590–620	54
Sorghum	520–580	55
Grass	530–600	54
Red clover	530–620	56
Sunflower	420–540	55
Wheat grain	700–750	53
Rye grain	560–780	53

VS volatile solids

3 Co-digestion pre-treatments

During recent years, various studies have been conducted on pre-treatments suitable to improve the co-digestion of a mixture of two or more different substrates, such as mechanical particle size reduction, alkaline hydrolysis, thermal and ultrasonic treatment, enzymatic degradation and ensiling (Table 3). The aim of these pre-treatment methods is to increase the solubilization of the complex matrices in order to accelerate the hydrolysis step, which is the slowest and limiting process for complex substrates (Eastman and Ferguson 1981; Noike et al. 1985).

These pre-treatments could improve the waste stabilization and the methane production but their application should be proved to be commercially viable in relation to the additional processing costs.

3.1 Physical pre-treatments

Some authors in literature propose to improve the anaerobic co-digestion with mechanical processes in order to reduce the particle size of the influent substrate. The performance of a digester operating on solid wastes is dependent on this parameter, as the available specific surface and the release of intracellular components represent an option for increasing the degradation rate and accelerating the digestion process.

The effect of organic materials comminution on their anaerobic biodegradability was investigated by Palmowski and Müller (1999). In particular the authors studied the effect of comminution on different substrates such as a mixture of potatoes, apple and carrot, meat, sunflower seeds, hay and leaves co-digested with sewage sludge and found a biogas production improvement of 24, 22, 17, 15, and 10 %,

Table 2 Bio-methane potential of different organic mixtures

Co-substrates	Ratio	Operational conditions	CH_4 yield (Nm^3 ton^{-1} VS added)	References
Pig manure: corn stover	75:25 (on VS content)	V = 30 L; T = 39 °C	210	Fujita et al. (1980)
Pig manure: potato waste	85:15 (on VS content)	V = 3.5 L;	210–240	Kaparaju et al. (2002)
	80:20 (on VS content)	T = 35 ± 1 °C	300–330	Kaparaju et al. (2002)
Pig manure: wheat straw	75:25 (on VS content)	V = 20 L;	240	Fischer et al. (1983)
	50:50 (on VS content)	T = 35 °C	220	Fischer et al. (1983)
Cow manure: wheat straw	50:50 (on VS content)	V = 0.3 L;	70	Hashimoto (1983)
	25:75 (on VS content)	T = 35 °C	30	Hashimoto (1983)
	10:90 (on VS content)		100	Hashimoto (1983)
Cow manure: forage beet silage	83:17 (on VS content)	V = 20 L; T = 35 °C	400	Weiland and Hassan (2001)
Cow manure: fruit and vegetable waste	80:20 (on weight)	V = 18 L;	380	Callaghan et al. (2002)
	70:30 (on weight)	T = 35 ± 0.5 °C	340	Callaghan et al. (2002)
	60:40 (on weight)		380	Callaghan et al. (2002)
	50:50 (on weight)		450	Callaghan et al. (2002)
Cow manure: barley straw	80:20 (on volume)	V = 100 L; T = 35 °C	160	Hills (1980)
Cow manure: sugar beet tops	90:10 (on VS content)	V = 1.5 L;	149 ± 12	Lehtomäki et al. (2007)
	80:20 (on VS content)	T = 35 ± 1 °C	200 ± 16	Lehtomäki et al. (2007)
	70:30 (on VS content)		229 ± 54	Lehtomäki et al. (2007)
	60:40 (on VS content)		220 ± 30	Lehtomäki et al. (2007)
Cow manure: grass silage	90:10 (on VS content)	V = 1.5 L;	143 ± 16	Lehtomäki et al. (2007)
	80:20 (on VS content)	T = 35 ± 1 °C	178 ± 9	Lehtomäki et al. (2007)
	70:30 (on VS content)		268 ± 29	Lehtomäki et al. (2007)
	60:40 (on VS content)		250 ± 16	Lehtomäki et al. (2007)
Cow manure: straw	90:10 (on VS content)	V = 1.5 L;	145 ± 9	Lehtomäki et al. (2007)
	80:20 (on VS content)	T = 35 ± 1 °C	159 ± 19	Lehtomäki et al. (2007)
	70:30 (on VS content)		213 ± 17	Lehtomäki et al. (2007)
	60:40 (on VS content)		188 ± 19	Lehtomäki et al. (2007)
Buffalo manure: maize silage	70:30 (on VS content)	V = 0.5 L; T = 35 ± 1 °C	358.23 ± 44.15	Esposito et al. (2012)
Dairy manure: cheese whey	75:25 (on VS content)	V = 0.25 L;	252.4	Labatut et al. (2011)
	90:10 (on VS content)	T = 35 ± 1 °C	237.6	Labatut et al. (2011)
Dairy manure: potatoes	75:25 (on VS content)	V = 0.25 L; T = 35 ± 1 °C	227.7	Labatut et al. (2011)
Dairy manure: plain pasta	75:25 (on VS content)	V = 0.25 L;	353.5	Labatut et al. (2011)
	90:10 (on VS content)	T = 35 ± 1 °C	224	Labatut et al. (2011)
Dairy manure: meat pasta	75:25 (on VS content)	V = 0.25 L;	285.6	Labatut et al. (2011)
	90:10 (on VS content)	T = 35 ± 1 °C	232.1	Labatut et al. (2011)
Dairy manure: switchgrass	75:25 (on VS content)	V = 0.25 L; T = 35 ± 1 °C	207.8	Labatut et al. (2011)
Dairy manure: used oil	75:25 (on VS content)	V = 0.25 L; T = 35 ± 1 °C	360.6	Labatut et al. (2011)

Table 2 continued

Co-substrates	Ratio	Operational conditions	CH_4 yield (Nm^3 ton^{-1} VS added)	References
OFMSW: vegetable oil	83:17 (on dry weight)	V = 1 L; T = 37 °C	699 ± 6	Ponsá et al. (2011)
OFMSW: animal fat	83:17 (on dry weight)	V = 1 L; T = 37 °C	508 ± 16	Ponsá et al. (2011)
OFMSW: cellulose	83:17 (on dry weight)	V = 1 L; T = 37 °C	254 ± 10	Ponsá et al. (2011)
OFMSW: protein	83:17 (on dry weight)	V = 1 L; T = 37 °C	288 ± 7	Ponsá et al. (2011)

V working volume; *T* temperature

respectively. Furthermore the authors investigated for each of these substrates the effect of comminution on the technical digestion time, that is defined as the time needed to produce 80 % of the maximal digester biogas yield. They found that this pre-treatment leads to a significant decrease of the digestion time for all the substrates analysed. The most effective decrease of the digestion time occurred for the substrates which needed a longer time to be digested, such as the leaves and hay stems. Thus, the authors demonstrated that the comminution could guarantee a harmonization of the digestion time in case of a heterogeneous feed consisting of materials with different biodegradability.

Møller et al. (2004) considered manure solid–liquid separation as physical pre-treatment. This produces a concentrated solid fraction which increases significantly the biogas potential per digested volume unit. An example of this application at industrial scale is in Denmark, where several co-digestion plants are operated successfully thanks to a substitution of some of non pre-treated manure with the solid manure fraction, that has a higher volatile solid concentration (Møller et al. 2004). Separation technologies such as decanting centrifuge, chemical treatment with subsequent dewatering and faeces/urine separation are used. For instance substituting 25 % of the pig manure fed to a co-digestion plant with the solid fraction separated from the same pig manure resulted in a biogas production increase from 20 to 25 Nm^3CH_4 m^{-3}substrate (Møller et al. 2004).

In literature there are already encouraging results to improve the performance of digesters with ultrasonic pre-treatment that is commonly used to break down complex polymers in the treatment of sewage sludge (Ward et al. 2008). Simonetti et al. (2010) investigated the influence of the ultrasonic pre-treatment on the solubilization and anaerobic biodegradability of co-digested waste activated sludge (WAS) with OFMSW. The results indicated that ultrasonic treatments at low frequencies and high ultrasonic power density present significant benefits for enhancing the availability of organic matters in WAS, but do not lead to a marked enhancement of the OFMSW solubility. However, in terms of biogas production the authors observed a positive effect of the ultrasonic pre-treatment for all tested matrices. In particular this pre-treatment improved the biogas production by a factor of 4, 2.25 and 3.5 after a 15-min long ultrasonic treatment compared with the untreated samples of OFMSW and two WAS coming from two different WWTPs, respectively.

3.2 Biological and physical–chemical pre-treatments

Biological and physical–chemical pre-treatments promote the substrates hydrolysis, breaking down the polymer chains into soluble components (Vavilin et al. 2007).

Del Borghi et al. (1999) tested the aptitude of hydrolytic micro-organisms to solubilize a mixture of 50 % sewage sludge and 50 % OFMSW (v/v). This mixture was treated with a high temperature alkaline pre-treatment followed by a bacterial hydrolysis in order to obtain an optimal solubilization of the organic substances. The results indicated a specific biogas production of 870 Nm^3 ton^{-1}VS for the hydrolysed mixture, higher than the biogas production of 360 Nm^3 ton^{-1}VS obtained from the untreated mixture.

Table 3 Effect of different pre-treatments methods on biogas production

Pre-treatment methods	Substrate	Biogas yield variation[a] (%)	References
Mechanical comminution[b]	Mix of apples, carrots and potatoes	(+24)	Palmowski and Müller (1999)
	Meat	(+22)	Palmowski and Müller (1999)
	Sunflower seeds	(+17)	Palmowski and Müller (1999)
	Hay	(+15)	Palmowski and Müller (1999)
	Leaves	(+10)	Palmowski and Müller (1999)
Solid–liquid separation	Solid fraction of pig manure-liquid fraction of pig manure	(+145)	Møller et al. (2004)
Bacterial hydrolysis and alkaline addition at high temperature	Sewage sludge and OFMSW	(+140)	Del Borghi et al. (1999)
Ensilage	Willow	(+22)[c]	Wang (2009)
	Miscanthus	(+1.13)[c]	Wang (2009)
	Mix of timothy, red clover and meadow fescue grass.	(+17)	Pakarinen et al. (2008)
Alkaline pre-treatment	Mix of sugar beet tops, grass, hay straw	(+17)[d]	Lehtomäki et al. (2004)
	Summer and winter switchgrass	(+32)	Frigon et al. (2008)
	10 % SFW and 90 % WAS	(+63)	Heo et al. (2003)
	30 % SFW and 70 % WAS	(+59)	Heo et al. (2003)
	50 % SFW and 50 % WAS	(+16)	Heo et al. (2003)
	70 % SFW and 30 % WAS	(+1.9)	Heo et al. (2003)
	Sewage sludge and OFMSW	(+31)[e]	Hamzawi et al. (1998b)
Thermal pre-treatment	Sewage sludge and OFMSW	(+6)	Hamzawi et al. (1998b)
	Slaughterhouse waste	(+268)	Edström et al. (2003)
	Dairy manure and bio-wastes	(+14)–(+18)	Paavola et al. (2006)
Thermal-chemical pre-treatment	Sewage sludge and OFMSW	(−5)	Hamzawi et al. (1998b)
Hydrothermal pre-treatment	Manure	(+14)	Qiao et al. (2011)
	Fruit and vegetable waste	(+16)	Qiao et al. (2011)
	Municipal sewage sludge	(+65)	Qiao et al. (2011)
Wet explosion	Wheat straw and swine manure	(−12)	Wang (2009)
Utrasonic pre-treatment	WAS and OFMSW	(+124)–(+296)	Simonetti et al. (2010)
Wet oxidation	Mix of source-sorted food waste, yard waste and digested bio-wastes	(+35)–(+70)	Lissens et al. (2004a)
	Miscanthus[f]	(−20)–(−39)	Wang (2009)
	Corn stalker	(−18)–(−36)	Wang (2009)
	Wheat straw	(−6)–(−11)	Wang 2009
	Willow	(+80)	Wang 2009

[a] [(biogas from pre-treated substrate–biogas from raw substrate)/biogas from raw substrate] × 100

[b] The comminution takes place together with sewage sludge

[c] After 5 months in BMP tests

[d] 2 % NaOH addition after 72 h

[e] Particle size = 8 mm and TS concentration = 23 % and alkaline dosage = 150 meq L^{-1}

[f] The values are related to different ratios of crops with swine manure

Another type of biological pre-treatment is the ensilage process, that is particularly useful to keep unchanged the natural moisture content of crops used to produce biogas (Egg et al. 1993). In the ensilage process, the soluble carbohydrates contained in biomass undergo lactic acid fermentation. This reaction produces a drop in pH and a loss of organic material originally present in the substrate (Wang 2009).

An increase in biogas production obtained using this pre-treatment is probably due to the degradation of the plants structural polysaccharides into more easily degradable intermediates (Egg et al. 1993). In literature different attempts are described to implement this technique for pre-treating various energy crops, such as silage ray, maize, barley, milky, willow and miscanthus (Egg et al. 1993). With regard to willow and miscanthus, Wang (2009) investigated the effect of ensilage pre-treatment on methane production and biomass losses. The author observed that the loss of organic matter due to ensilage when willow and miscanthus were pre-treated was not higher than 2 and 3 % respectively, therefore negligible.

Furthermore the author noticed that the ensilage of willow can produce during 1, 3 and 5 months an increase of the total methane production equal to 12, 22 and 22 %, respectively, compared with the raw willow. The ensilage of miscanthus produced during 1, 3 and 5 months no significant increase of methane production compared with the raw substrate. Woodard et al. (1991) estimated an increase of 15 % in methane production by ensiling elephantgrass and energycane compared with the fresh crops. Additionally Pakarinen et al. (2008) studied the effect of the ensilage on a mixture of timothy, red clover and meadow fescue, during a period of time variably long from 2 to 11 months, with and without the addition of biological material containing both enzymes and lactic acid bacteria. In this last case (i.e. with no addition of biological material) the authors obtained an increase of 19 % in methane production compared with the raw substrate, considering as pre-treatment only a 6 months long ensilage. A further increase of the methane production was noticed in grass ensiled for 6 months with formic acid addition (35 % increase compared with fresh crops), whereas with an inoculation of lactic acid the increase was equal to 4 %.

A more common chemical pre-treatment proposed in literature is the alkaline pre-treatment that produces the breakage of the soluble organic matter such as carbohydrates, proteins and lipids into lower molecular compounds through the alkali hydrolysis. Application of alkaline solutions such as NaOH, $Ca(OH)_2$ or ammonia efficiently increases the accessibility of enzymes to cellulose and the saccharification (Mouneimne et al. 2003; Fox et al. 2003). During alkaline pre-treatment the first reactions are solvation and saponification. This makes the substrate more accessible to bacteria.

The effect of saponification is the breakage of cross-linking, that produces an increase of the swelling capacity and the porosity. This produces an increased diffusivity for the hydrolytic enzymes but also facilitates the enzyme-substrate interactions (Mouneimne et al. 2003; Fox et al. 2003).

Lehtomäki et al. (2004) reported the effect of this pre-treatment on a mixture of sugar, beet, tops grass, hay straw under three different conditions: 2 % NaOH addition for 24 and 72 h, 3 % $Ca(OH)_2$ + 4 % Na_2CO_3 addition for 72 h. The biogas production increased by 9 and 17 % when a 2 % NaOH solution was added for 24 and 72 h, respectively. In the case of 3 % $Ca(OH)_2$ + 4 % Na_2CO_3 addition for 72 h an increase in biogas production of 7 % was noticed compared with the untreated substrate.

Frigon et al. (2008) reported that alkaline pre-treatment of summer and winter switchgrass with 7 g L^{-1} NaOH for 3 h can result in a 32 % increase of the maximal biogas production if compared with the untreated substrate. Heo et al. (2003) considered a mixture of simulated food waste (SFW) and WAS pre-treated with 45 meq L^{-1} NaOH at 35 °C and carried out biochemical methane potential (BMP) tests to define the anaerobic digestibility of the pre-treated waste activated sludge (PWAS). In particular the authors investigated the cumulative methane yield varying the ratios of the feed mixture (SFW/WAS and SFW/PWAS) used as substrate on the basis of the total VS content of the two solid wastes as follows: 10/90, 30/70, 50/50, 70/30 and 90/10. The results indicated that methane production in anaerobic co-digestion is considerably affected by the pre-treatment of WAS fractions. The anaerobic digestion performance in terms of methane production at the different ratios of the components in the mixture (SFW/PWAS equal to 10/90, 30/70, 50/50, 70/30 and 90/10) improved by 63, 59, 16, 1.9 and 1.7 %, respectively, compared with the corresponding feed mixture with untreated WAS.

Also lab-scale experiments of Hamzawi et al. (1998b) demonstrated that the alkaline pre-treatment can increase the biodegradability and the methane production of sewage sludge/OFMSW mixture when compared with the untreated mixture. The authors tested the effects of alkaline pre-treatment on digestion process performance varying the particles size and the total solids (TS) concentration of the feeding substrate and found as *optimum* the operating condition with a high TS concentration (23 %), a high alkaline dosage (150 meq L^{-1}) and a large particles size (8.0 mm). This particle size is larger than the optimal particles size found for untreated mixture, resulting in a 31 % increase of the biogas production and a 32 % increase of the TS removal. This is probably due to the methanogens inability to handle the increased hydrolytic activity induced by the feed pre-treatment. Under the same values of particle size and TS content the authors conducted further tests using a thermal pre-treatment where temperature, pressure and heating time were 130 °C, 1 atm and 1 h, respectively and a thermo-chemical pre-treatment by adding 185 meq L^{-1} sodium hydroxide, finding a biogas production variation equal to 6 and −5 %, respectively, when compared with the untreated substrate. An innovative physical–chemical pre-treatment for organic wastes is the wet oxidation (Wang 2009). This technique aims at enhancing the contact between molecular oxygen and the organic matter to be degraded. The organic substrate is oxidized by oxygen dissolved in water or an oxidizing agent such as hydrogen peroxide (H_2O_2) at high temperature (above 120 °C) and pressure (in the range of 0–12 bars) to increase the oxidation rate (Wang 2009).

Lissens et al. (2004a) used wet oxidation to improve the anaerobic biodegradability and increase the biogas yield from source-sorted food waste, yard waste and digested bio-wastes treated in a full-scale biogas plant. The authors noticed an increase in the biogas yield by approximately 35–70 % from raw and digested lignocellulosic bio-wastes.

In further experiments Lissens et al. (2004b) used organic municipal solid waste enriched with wheat straw treated by wet-oxidation as pre-treatment for subsequent enzymatic conversion and fermentation into bio-ethanol. The authors assessed the effect of high oxygen pressure under alkaline conditions for an extensive delignification (up to 67 %) of household waste, resulting in 62–65 % ethanol yield from the cellulose fraction of the waste.

Wang (2009) investigated the co-digestion of swine manure with both raw and wet oxidation pre-treated agriculture residue or energy crops, such as wheat straw, corn stalker, willow and miscanthus. For all crops, the soluble sugar content increased after pre-treatment, but higher methane production was only obtained from willow.

The author conducted BMP tests on different crops at different co-digestion ratios with swine manure, obtaining lower performance of the reactor in terms of cumulative methane yield in comparison with the digestion of raw crops.

In particular a decrease of fractional methane yield in the range 20–39 % for miscanthus, 18–36 % for corn stalker and 6–11 % for straw was observed. The reason why biogas production decreased when wheat straw, corn stalker and miscanthus were used as substrates could be related to their structure, that is softer and easier to be digested if compared with woody crop willow. However, only for willow a significant increase in the methane production equal to 18–20 % was noticed. This is probably due to the fact that willow is more woody compared with wheat straw or corn stalker. After pre-treatment a particle size reduction was noticed in the case of willow that implies an increase of the available surface area and release of sugar (Qiao et al. 2011).

Another innovative physical–chemical pre-treatment is the wet explosion, developed by Ahring and Munck (2005) as a combination of steam explosion and wet oxidation. It enables to operate with high substrate concentration. The aim of this treatment is to heat the substrate with high temperature water and provide an oxidation reaction by adding an oxidizing agent such as H_2O_2 under high pressure. This process is similar to the wet oxidation process, whereas in the next step, the substrate undergoes a sudden pressure decrease that is a characteristic of the steam explosion process.

Wang (2009) applied this type of pre-treatment for anaerobic co-digestion of wheat straw and swine manure in a continuous operated system. Despite the high release of soluble sugars, the methane obtained from the wet-exploded wheat straw was lower than the methane obtained from the unpre-treated mixture, with a negative efficiency (−12 %) in terms of methane production. According to the author this was probably due to the formation of inhibitory compounds, that could occur inside the reactor when the high temperature pre-treatment is applied before the fermentation process.

3.3 Thermal pre-treatments

The thermal technique is commonly used as conditioning process for raw or digested sludge and improves the dewaterability, the sludge solubilization, the viscosity and pathogen microorganism reduction of such wastes because the heat treatment alters the structure of the insoluble fraction to make it more amenable to biodegredability (Bougrier et al. 2007).

Co-digestion of animal by-products and other solid substrates such as food waste from restaurants and food distributors as well as sludge from a slaughterhouse wastewater treatment plant have been studied at laboratory and pilot scale with heating pre-treatment (at 70 °C for 1 h) by Edström et al. (2003). The authors in this case obtained an increase of potential biogas yield (1.14 L biogas g^{-1} VS) compared with non-pasteurized animal by-products (0.31 L biogas g^{-1} VS).

Lately Paavola et al. (2006) also applied the same pre-treatment to mixtures of dairy manure and bio-wastes with particles size <12 mm and obtained a methane production enhancement of 14–18 % compared with the raw wastes.

Recently Cuetos et al. (2010) tried to apply heat and pressure pre-treatments (for 20 min at 133 °C and pressure >3 bar) to solid slaughterhouse waste (SHW) co-digested with OFMSW. In this case the authors found a decrease of methane production (53 % from treated SHW + OFMSW and 61.9 % from the untreated mixture) because foaming problems and accumulation of fats occurred in the reactor. This was probably due to the formation of conjugated and refractory compounds, which could have been resulted in toxic effects for the AD process, thus explaining the long chain fat acids (LCFAs) and VFAs accumulation observed in reactors fed with pre-treated SHW.

Another type of thermal treatment is the hydrothermal process, that has been used in municipal sewage sludge digestion at industrial scale (Qiao et al. 2011). Using the hydrothermal pre-treatment, municipal sewage sludge is dissolved into a liquid phase that skips the slow biological enzyme hydrolysis. Qiao et al. (2011) applied this pre-treatment at high temperature as well as pressure to remove water from sludge and also to oxidize the following organic substrates: fruit/vegetable waste, cow manure, pig manure and sludge that were diluted by adding tap water before heating whereas the collected food waste was firstly screened to remove bones, plastic and metals and then crushed before heating. In particular temperatures equal to 120, 170, and 190 °C and 10, 15, 20, 30, 45, and 60 min heating times were used to set the operational conditions of the test. The BMP tests were carried out in order to investigate the effect of this pre-treatment on methane production, resulting in an increase of the methane production from treated pig manure, fruit/vegetable waste, and municipal sewage sludge of 14.6, 16.1 and 65.5 %, respectively.

The effects of the previously described pre-treatments on the biogas production from the co-digestion process are summarized in Table 3.

4 Co-digestion mathematical modelling

During the last decades many researchers have studied the anaerobic co-digestion technology with the aim to assess the effect of mixing two or more substrates in an anaerobic digester (Tables 4, 5). To know the correct combination of different substrates many experiments are needed (i.e. BMP tests).

An accurate modelling can help to define a correct ratio between different organic substrates to be digested. However, for this purpose typical mono-substrates models are not suitable as they are not capable to simulate the biodegradation behaviours of different substrates in terms of kinetics, particle size, nutrient balance, etc.

4.1 Mathematical models not based on the ADM1 approach

The first co-digestion model was proposed by Bozinis et al. (1996). It is a steady state mathematical model which simulates the degradation of the main component groups (i.e. lipids, carbohydrates and proteins).

A mathematical model for co-digesting piggery, olive-mill and dairy wastewaters based on batch kinetic experiments was developed by Gavala et al. (1996). This model considers a four steps process (hydrolysis, acidogenesis, acetogenesis, and methanogenesis). The model cannot predict the methane concentration, and does not consider the inhibitory effect by high ammonia concentration, volatile fat acids (VFAs), long chain fat acids (LCFAs) and hydrogen.

Successively Kiely et al. (1997) developed and validated a two-stage mathematical model of acidogenesis and methanogenesis, including ammonia inhibition and pH prediction.

In 1997 Jeyaseelan proposed a simplified mathematical model that simulates the anaerobic co-

Table 4 Mathematical models not based on the ADM1 approach

Author	Steady state model	Dynamic model	Design model	Calibration	Validation	Substrates used in calibration/validation
Bozinis et al. (1996)	x	–	–	–	–	–
Gavala et al. (1996)	–	x	–	x		Olive mill wastewater, pig manure, dairy wastewater
Kiely et al. (1997)	–	x	–	x	x	OFMSW and primary sludge
Jeyaseelan (1997)	–	x	–	–	–	–
Angelidaki et al. (1999)	–	x	–	x	x	Manure and glycerol triolate, manure with gelatine, manure with protein
Dinsdale et al. (2000)	–	–	x	x	–	WAS, fruit and vegetable mixture
Buendía et al. (2009)		x		x	x	Waste sludge, cow manure, ruminal waste, and pig and cow waste slurries (PCS)
Sosnowski et al. (2008)		x		x	x	OFMSW and sewage sludge
Ponsá et al. (2011)	x			x	x	Sewage sludge and pure substrates

Table 5 Mathematical models based on the ADM1 approach

Author	Steady state model	Dynamic model	Design model	Calibration	Validation	Substrate used in calibration/validation
Lübken et al. (2007)		x		x	x	Cattle manure
Esposito et al. (2008)		x				
Fezzani and Cheikh (2009)		x		x	x	Olive mill waste and olive mill solid waste
Derbal et al. (2009)		x		x	x	OFMSW and sewage sludge
Esposito et al. (2011a)		x				
Esposito et al. (2011b)		x		x	x	Synthetic OFMSW and sewage sludge

digestion of different wastes, defining the waste by its general composition. The model takes into account only two conversion processes, i.e. hydrolysis/acidogenesis and methanogenesis.

A more complete dynamic model was developed by Angelidaki et al. (1999). The model, based on a model previously proposed (Angelidaki and Ahring 1993), describes the anaerobic degradation of complex material and the co-digestion of different types of wastes. The model includes 2 enzymatic hydrolytic steps, 8 bacterial steps and involves 19 chemical compounds (carbohydrates, lipids, and proteins, intermediates such as VFAs and LCFAs, and important inorganic components, i.e. ammonia, phosphate, cations, and anions). The model also includes pH prediction and free ammonia, acetate, VFAs and LCFAs inhibition.

Dinsdale et al. (2000) used a model of the acidogenic stage based on the work of Eastman and Ferguson (1981) in order to show that the hydrolysis in a two-stage anaerobic digester could be achieved during a retention time of 3.1 days, as reported by Ghosh (1991).

Buendía et al. (2009) carried out anaerobic batch experiments combined with a mathematical model to study the anaerobic biodegradability of meat industry wastes. The authors proposed a simple mathematical model based on the assumption that the biodegradable fractions of the organic waste were divided into readily and slowly biodegradable fractions in agreement with Spanjers and Vanrolleghem (1995) and De Lucas et al. (2007). Organic matter degradation is described using Monod kinetics without mutual interactions by a modification of the "Methane Production model" proposed by Rodríguez et al. (2007). The model was calibrated with experimental data (cumulative methane production and VS degradation) from mesophilic anaerobic batch experiments in order to estimate the different biodegradable fractions (readily, slowly and inert) of the co-digested waste.

Sosnowski et al. (2008) carried out batch experiments with sewage sludge and OFMSW in large scale. The aim of such experiments was the determination of the process carbon balance and the proposal of a simple kinetic model of the anaerobic digestion. This is a two-stage acidogenesis and methanogenesis mathematical model which does not distinguish particular groups of microorganisms and includes carbon dioxide formation both in hydrolytic and methanogenic steps. VFAs inhibition is also taken into account.

Ponsá et al. (2011) proposed a first-order model based on a previous work of Tosun et al. (2008). To address the limitations of the models described by Tosun et al. (2008), the new model was developed to obtain the three different fractions in which organic matter can be classified in terms of biodegradability: readily biodegradable, slowly biodegradable and inert fraction. Several co-digestion experiments were carried out to calibrate the model. In such experiments vegetable oil, animal fats, cellulose and proteins were used to improve the anaerobic digestion of OFMSW.

4.2 Mathematical models based on the ADM1 approach

In 2002 the International Water Association (IWA) Task Group for Mathematical Modelling of Anaerobic Digestion Processes developed a comprehensive mathematical model known as ADM1-Anaerobic Digestion Model no. 1 (Batstone et al. 2002), which was based on the knowledge of modelling and simulation of anaerobic digestion systems emerged over the previous years.

However, this model neglects some processes involved in the anaerobic digestion such as sulphate reduction, acetate oxidation, homoacetogenesis, solids precipitation and inhibition due to sulphide, nitrate, LCFAs, weak acid and base (Fuentes et al. 2008). Some of the previous aspects have been studied and modelled afterwards; for instance two ADM1 extensions were published in 2003, concerning respectively the sulphate reduction (Fedorovich et al. 2003), and the $CaCO_3$ precipitation (Batstone and Keller 2003). A further extension aimed to remove the ADM1 discrepancies in both carbon and nitrogen balances was published in 2005 (Blumensaat and Keller 2005).

As reported in Mata-Alvarez et al. (2011), when the ADM1 was applied under co-digestion conditions all authors considered the following two assumptions: (1) the ADM1 model components for composites cannot be used as an inflow fraction, and substrate characterisation should be done in terms of carbohydrates, proteins and lipids (Lübken et al. 2007; Fezzani and Cheikh 2009; Galí et al. 2009; Zaher et al. 2009), and (2) the disintegration/hydrolysis step is generally considered the rate-limiting step during the anaerobic digestion process (Lübken et al. 2007; Esposito et al. 2008, 2011a, b; Galí et al. 2009; Zaher et al. 2009). The first assumption was not used for the modified version of the ADM1 proposed by Esposito et al. (2008) that considers two different input substrates (i.e. sewage sludge and OFMSW), with the possibility to apply different kinetics for each of them (Fig. 1).

Lübken et al. (2007) proposed a modified version of the ADM1 where energy production by co-digesting cattle manure and energy crops is evaluated. Fezzani and Cheikh (2009) published an extension of the ADM1 aimed at including phenol compounds degradation in the anaerobic digestion process.

Applications of the ADM1 in the co-digestion of OFMSW and sewage sludge were proposed by Esposito et al. (2008) and Derbal et al. (2009). Derbal et al. (2009), using data obtained from a full scale anaerobic digester, showed the ADM1 potential as a tool for assisting in system operation and process control.

Esposito et al. (2008) modified the ADM1 in order to include the possibility to model the disintegration of two different input substrates. In particular, their model considers first order kinetics for sewage sludge disintegration and surface-based kinetics to model the OFMSW disintegration. When organic solid wastes are present in the reactor influent, the disintegration process is the rate limiting step of the overall co-digestion process and the main advantage of the proposed modelling approach is that the kinetic constant of such a process does not depend on the waste particles size distribution (PSD), but only on its nature and composition. This model has been upgraded in Esposito et al. (2011a) in order to simulate the effect of LCFAs production in pH prediction, also including the possibility to separate each product of the disintegration process (i.e. carbohydrates, proteins and lipids) into two fractions, i.e. a readily biodegradable fraction and a slowly biodegradable fraction. BMP tests conducted on synthetic organic waste have

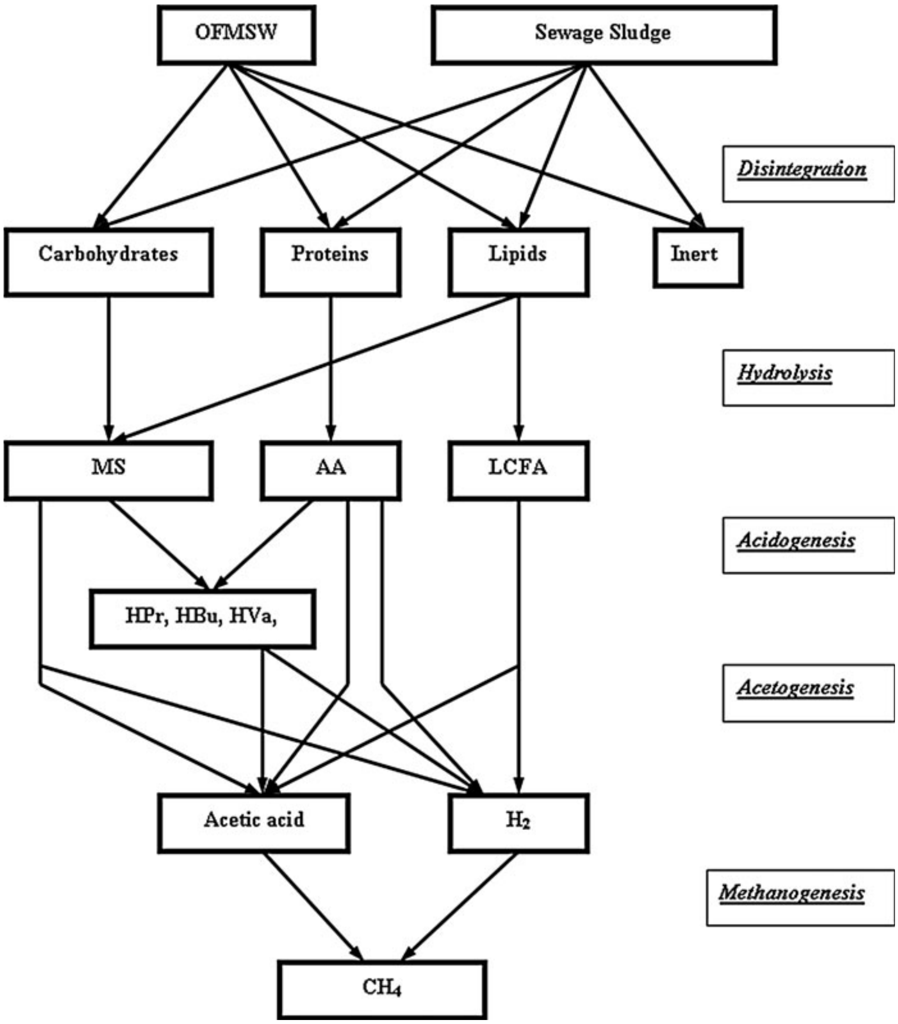

Fig. 1 Flow chart of the modified version of the ADM1 proposed by Esposito et al. (2008). *MS* monosaccharides, *AA* amino acids, *LCFA* long chain fatty acid, *HPr* propionic acid, *HBu* butyric acid, *HVa* valeric acid

been used to calibrate and validate this model (Esposito et al. 2011b).

All above cited models consider completely stirred tank reactor (CSTR) hydrodynamic conditions and thus hydrodynamic calibration/validation is not required.

5 Conclusions

Co-digestion can result in an important increase of the bio-methane potential when the substrates mixture is prepared with proper percentages of the different organic substrates to be digested. The beneficial effect of the co-digestion is mainly due to the optimization of the nutrient balance in the substrates mixture when co-digesting nitrogen rich substrates with carbon rich substrates. The higher specific methane yield of 686 Nm^3 $ton^{-1}VS$ is achieved by co-digesting OFMSW and vegetable oil (83/17 on dry weight), but also OFMSW with animal fat (83/17 on dry weight) and cow manure with fruit and vegetable waste (60/40 on weight) give very high methane yield of 490 and 450 Nm^3 $ton^{-1}VS$, respectively.

Several pre-treatment methods can be applied to increase further the biogas production of a co-digestion process, such as mechanical comminution, solid–liquid separation, bacterial hydrolysis and alkaline addition at high temperature, ensilage, alkaline, ultrasonic and thermal pre-treatments. However, other pre-treatment methods, such as wet oxidation and wet explosion, can result in a decrease of the methane production efficiency.

Bio-methane potential data when experimentally determined are only valid for the co-substrates and the operating conditions (temperature, OLR, HRT, moisture content, substrate/inoculum ratio, etc.) applied during the experiments. However, they can be used to calibrate mathematical models capable to simulate the co-digestion process and then such models can be used to predict the bio-methane potential achievable with different co-substrates and under different operating conditions. For such purpose only mathematical models specifically aimed at simulating the co-digestion of different co-substrates are suitable, as mono-substrate models are not capable to take into account the peculiarities of different substrates in terms of physical and biochemical characteristics and their synergistic effects (e.g. in terms of pH, alkaline and nutrient balance).

Recent co-digestion models in literature are mainly based on the ADM1, with various modifications to upgrade it from the mono-substrate to the multi-substrate system. Such models, if compared to models not based on the ADM1, offer the advantage to simulate the main steps of the anaerobic digestion process with a complete and advanced approach as that included in the ADM1.

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