Anaerobic Biodegradation of Solid Substrates from Agroindustrial Activities — Slaughterhouse Wastes and Agrowastes

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Chapter 3

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Abstract

Solid wastes from the meat industry are produced in large amounts resulting in a negative impact on the environment if not properly treated. Due to their high content of proteins and fats, these residues are excellent substrates for anaerobic digestion which holds high potential for methane yield. However, possible toxic compounds may be formed during its biodegradation with a consequent failure of the process under long-term operation. The anaerobic co-digestion of such residues with other co-substrates as those generated in agricultural activities has been proposed as a good alternative to overcome these problems. Nevertheless, today there is very little knowledge to assess on mixture interactions connected to wastes composition, biodegradability, and the kinetics of the anaerobic process when complex materials are utilized in ternary and quaternary mixture, specifically when co-digesting solid cattle slaughterhouse waste with agrowaste. It is therefore important to select the right combination of substrates and ratios to obtain synergy instead of antagonism in those mixtures. This chapter aims to provide an overview of the anaerobic digestion of solid slaughterhouse waste and agrowaste, as well as the influence of mixture interactions on its biodegradation.

Keywords: Agrowaste, anaerobic digestion, co-digestion, synergy, slaughterhouse waste

1. Introduction

The agriculture sector belongs to one of the most important human activities, but at the same time, it is considered as one of the most residue-producing sector in the world. Farmer activities have a huge impact on the environment, and moreover, industries related to agriculture, such as the meat processing industry, generate a large amount of high-strength residues. Due to the growing demand of meat in the world, the amount of organic solid wastes from meat producing industries is increasing every day. There are several attempts to improve the biodegradation of such residues, such as the anaerobic process, the preferred technology to diminish the organic load with an adequate efficiency [1-6]. It is well known that anaerobic digestion (AD) provides both environmental solutions and renewable energy production in rural areas, in most cases, with the corresponding autonomy.

Because of the high content of proteins and fats, slaughterhouse residues are holding high biogas potential and hence are interesting for the anaerobic digestion process. However, potential inhibitory compounds can be formed during the degradation of proteins and lipids, which make this process sensitive and prone to fail [7-9]. A possible way to overcome these problems is the co-digestion with carbon-rich co-substrates, i.e., a mixture of agrowastes with low protein/lipid content. This will lead to a better nutritional balance together with an improvement in the methane yield due to positive mixture interactions. Today, there is very little knowledge to assess mixture interactions connected to wastes’ composition, biodegradability, and the kinetics of the anaerobic process when complex materials are utilized. The aim of this chapter is to describe the behavior of the anaerobic process when slaughterhouse residues are interacting with agro wastes, to provide data on its optimal mixture ratios, methane yield improvement, and the kinetics of the biodegradation process.

2. Characteristics of slaughterhouse wastes and agrowastes

Organic wastes are produced as an integral part of human life. Many anthropologic activities are responsible for the generation of organic wastes, such as the agriculture, the food processing, and the drinks manufacturing industry as well as domestic waste [10]. Agricultural wastes is a wide definition for residues resulting from numerous agricultural activities, such as the production of animals for slaughter (slaughterhouse residues), dairy products, the operation of feedlots, and planting and harvesting of crops [11]. This chapter will focus on both slaughterhouse residues and agrowaste residues.

Specifically, slaughterhouse residues are the result of abattoir operation in which solid and liquid wastes as well as wastewater are generated in larger amounts. In such activities, both the liquid and solid fractions are lumped together [12]. Depending of the slaughterhouse operation, there is a wide range of sources of residues that exist during meat processing. They are determined by the degree of further processing of the slaughtered animals, particularly by the degree of processing of the rumen, stomachs, and intestines in the tripery. Besides, the composition of these fractions also depends on the quality of actions to retain the solid and liquid slaughter residues. The organic matter contained in abattoir effluents is the result of water-cleaning operation from all areas (the slaughtering wastewater, the tripery wastewater, and the washing-down and cleaning water) of the plant, where water comes in contact with manure, carcasses, offal, blood, and waste meat. The principal components of the organic matter presented in abattoir effluents are feces, gut contents, fat, and blood. Other components as coarse separable materials as well as suspended, colloidal, and dissolved organic materials are also presented, including the degradation products of fat and proteins, such as volatile organic acids, amines, and other organic nitrogen compounds. Carbohydrates occur in the wastewater in dissolved or colloidal forms.

Agrowastes are derived from biomass, which is usually comprised of lignocellulosic materials, and they have therefore high contents of cellulose, hemicellulose, and lignin. Table 1 shows a summary on the characterization of diverse animal wastes and agrowaste residues. Agrowastes are considered as the main renewable natural resources utilized widely in the world. The general composition of agrowastes is wood residues (leftover from forestry operations), municipal solid wastes (MSWs), and agricultural and food wastes. Today, 64% of the biomass energy is produced from wood and wood wastes, followed by 24% from MSW, 5% from agricultural waste, and additional 5% from landfill gases [13].

In the last 20 years, the energy crops and their subproducts, mainly in Europe, became and still are a very common feedstock for biofuel production. Governmental regulations, specifically in Germany, provided a scenario, which is quite attractive for energy crops exploitation [14, 15]. Nevertheless, plant wastes and manures have also a high potential to produce biogas cost-effectively [16] without compromising soil utilization for food production.

Substrates	**pH**	**TS (%)***	**VS (%)**	**Total nitrogen (%)***	**Lipids (%)***	**Proteins (%)***	**Carbo-hydrates (%)***	**C/N**	**References**
Animal waste									
Cow rumen	6.1	14.9	89.4	0.3	n.a	n.a	n.a	n.a	[1]
Swine punch waste	5.9	31.7	82.7	0.3	n.a	n.a	n.a	n.a	[1]
Cow blood	7.4	19.8	75.0	2.9	n.a	n.a	n.a	n.a	[1]
Poultry offal, feed, and head	n.a	22.4	68.6	n.d	54	32	n.d	n.d	[17]
Iberia pig slaughterhouse waste	6.24	n.a	n.a	n.a	n.a	n.a	n.a	4.7	[18]
Solid cattle slaughterhouse waste	5.8–6.8	13–26	92–95	2.1–4	17.5–43	13–24	0.1	14.4	[19, 20]

Substrates	pH	TS (%)*	VS (%)	Total nitrogen (%)*	Lipids (%)*	Proteins (%)*	Carbo-hydrates (%)*	C/N	References
Poultry trimmings and bones	n.a	22.4	68	15.4	4.9	11.42	n.a	n.a	[17]
Solid cattle meat and fat	n.a	88.5	96.5	0.3	76.2	1.9	n.a	n.a	[21]
Solid pig meat and fat	n.a	56.4	98.7	1.4	46.7	8.3	n.a	n.a	[21]
Pig stomach	n.a	18.2	98.3	1.2	8.7	6.7	n.a	n.a	[21]
Rumen content	n.a	11.6	93.1	0.1	1.8	0.8	n.a	n.a	[21]
Bovine slaughterhouse waste	n.a	53.2	98.8	0.6	46.1	3.5	n.a	n.a	[2]
Agrowaste									
Horse manure	n.a	81.5	75.8	1.7	1.6	11.0	49.2	n.a	[22]
Cattle manure	n.a	23	78.6	0.8	0.3	4.8	13.0	n.a	[22]
Swine manure	n.a	55	63.6	1.8	n.a	n.a	n.a	10.2	[23]
Mixture of animal manure	8.4	35	40	0.4	0.4	2.6	18	n.a	[20]
Sugar cane press mud	6.3	9.1	80.84	n.a	n.a	n.a	n.a	26.4	[24]
Rice husk	6.6	89.2	77.8	n.a	n.a	n.a	n.a	99	[25]
Rice straw	6.5	87.8	79.6	n.a	n.a	n.a	n.a	43	[25]
Maize crops	n.a	67.2	95.8	0.6	n.a	n.a	n.a	64.7	[23]
Various crops	4.2	24	90	0.3	0.2	2.1	28.7	n.a	[20]
Tomato processing waste	4.4	n.a	n.a	n.a	n.a	n.a	na	16.8	[18]
Potato pulp	3.7	13.5–17.8	96–97	n.a	n.a	n.a	n.a	42–60	[26]
Fruit and vegetable wastes	4.2	8.3	93	0.2	n.a	n.a	n.a	34.2	[27]

n.a., not available; * based on fresh matter; TS, total solid; VS, volatile solid (based on dry matter); C/N: carbon/nitrogen ratio.

Table 1. Characterization data on diverse animal waste and agrowaste fractions

2.1. The impact of final disposal of slaughterhouse residues and agrowastes

Taking into account that the food and agroindustries usually produce large amounts of wastes, in those places where suitable treatment systems are unavailable, the environmental problemsassociated to such waste streams became an emergency issue to solve. The slaughtering process in the meat industry is the major contributor to liquid waste [28]. Furthermore, large amounts of water is used in dairy plants and slaughterhouses counting up to approximately 40×10^6 m^3 $year^{-1}$, which is an equivalent of the demand of water required for 500,000 people. In general, the wastewater from the meat industry is very difficult to decontaminate due to its high content of organic, mineral, and biogenic matter and the irregular discharge [5]. In order to reduce adverse ecological effects, the direct disposal of both liquid and solid abattoir wastes is not permissible, and a waste treatment prior to landfill is essential.

Slaughterhouse wastewater is a concern from the epidemiological point of view since it can also contain disease-causing agents [29]. Together with the blood, the rumen, and the stomach contents, these are at the focus of the disposal problems. Even after the slaughter of healthy cattle, the rumens have been found to contain somewhat rare *Salmonella* types, as well as other bacteria, viruses, and parasites (e.g., worms) in concentrations that are alarming from epidemiological point of view [30, 31]. In order to diminish such negative environmental impacts, several technologies have been introduced around the world. Composting and bioremediation are alternatives to the disposal of untreated residues, taking into account that the materials are biodegradable and can provide nutrients to soil, if land application is considered [32].

In addition, agrowastes are one of the major contributors of greenhouse gas emissions. The necessity to reduce this adverse effect and to develop a reliable alternative to the fossil fuel-dependent economy has raised the interest in agrowastes as a renewable energy sources. When applying this concept, a double effect can be achieved: the reduction of fossil fuels' consumption together with solving the above-mentioned environmental problems [33, 34]. Therefore, anaerobic digestion of agricultural wastes should be considered as one of the main alternative for treating these types of waste streams in an environmentally friendly scheme. It is well known that AD technology is one of the most useful decentralized sources of energy supply, especially when considering that all substrates utilized are easily available in many farms. Moreover, the capacity of AD process to reduce the organic content of biowastes provides a low-CO_2 emission, taking into account the overall waste-to-energy transformation.

Accordingly, the AD process stands for a promising solution to the problem from both energy conservation and pollution control points of views [5]. Besides energy production, the AD process generates a pathogen-free effluent and produces a stabilized material to be utilized as fertilizer in land applications [35].

3. Anaerobic digestion

Biological transformations can generally be classified as either aerobic or anaerobic processes. Each organic waste has a constant ultimate biodegradable fraction, and the final outcome of its biodegradation is severely affected by different factors such as temperature, pH, alkalinity, nutrient requirements and bioavailability, digestion time (under anaerobic conditions), and particle size. Therefore, all aspects related to biodegradability should be taken into account to finally describe the degradation of different substrates and the performance of biological transformation processes [25, 36].

AD is a process by which the complex organic matter (proteins, lipids, and carbohydrates) are broken down by the action of different groups of microorganisms, i.e., *Bacterias* and *Archaeas* in the absence of oxygen, and a mixture of gases (mainly CH_4 and CO_2), called biogas, is produced. The final effluent with lower organic content can be utilized as a high-quality biofertilizer. The biodegradation process involves several serial and serial-parallel reactions in which each group of microorganisms is linked to another group and working together. The main steps of degradation are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the hydrolysis phase, the complex particulate materials are disintegrated by the action of several extracellular enzymes into amino acids, long chain fatty acids (LCFAs), and sugars. The activity of the main extracellular enzymes (i.e., proteinases, lipases, and cellulases) involved in this phase is dependent on the characteristic of the substrates to be degraded [14]. Further on, the soluble compounds, produced during the hydrolysis step, are converted to volatile fatty acids (VFAs) and alcohols with carbon chain units less than five by the action of facultative bacteria in the acidogenesis step. However, carbon dioxide, hydrogen, and ammonia are also produced [37]. During this step, the accumulation of some intermediate compounds, such as acetate, propionate, butyrate, or ethanol, may occur in the system depending on the hydrogen production [38]. Then, in the acetogenesis step, the previous intermediates are converted into acetic acid, hydrogen, and carbon dioxide. The last step of the process is called methanogenesis, and it is driven by methanogens. Additionally, in the presence of sulfate, it is possible to obtain H_2S, ranging from 1% to 2% v/v in the biogas, which is produced by the action of sulfate-reducing bacteria [39].

The end products of the previous phases are converted into CH_4 and CO_2 via the acetotrophic or hydrogenotrophic pathways. The acetotrophic pathway is well known to be responsible for about 70% of the methane produced [40]. The other 30% is produced by the hydrogenotrophic pathway, in which H_2 and CO_2 are converted to CH_4 by *Methanobacteriales* and *Methanomicrobiales* (order level). In this step, the hydrogen-consuming microorganisms play an important function in order to keep low hydrogen partial pressure in the system.

Many factors affect the AD process, and temperature is one of the most important physical parameters since it directly affects the kinetics of the degradation and the growth of the microorganisms. However, AD can be carried out in a wide range of temperatures (i.e., between 10°C and 65°C); for industrial applications, mesophilic (35°C-37°C) and thermophilic (50°C-55°C) temperatures are the most applied ones. Several biogas plants operate today under mesophilic conditions due to higher process stability and lower energy requirements [41]; however, when it comes to increase the reaction rates and to achieve higher reduction of pathogens, thermophilic conditions have got an increasing attention [42]. Nevertheless, the operation at thermophilic temperatures might result in a less stable process due to accumulation of inhibitory compounds [43].

Alkalinity and pH are also important factors to take into account since each group of microorganism has a different optimum pH range. In AD, acid-producing microorganisms live at pH<5.0, while most methane producers require neutral pH. Neutral and stable pH values in the reactor require high alkalinity values, which primarily depend on the presence of bicarbonate ions in equilibrium with carbon dioxide. Nevertheless, ammonia released from protein degradation can also provide alkalinity to the system as it often reacts with carbon dioxide and water to form ammonium carbonate. Usually, the anaerobic digestion of slaughterhouse waste results in higher alkalinity values in comparison with processes treating sewage sludge [44]. However, pH is not a good indicator to control the process. Relatively slight fluctuations in the pH values might lead to process imbalance and instability. In that sense, rather the use of VFA/alkalinity ratio to monitor the system will give a fast and good indicator to detect stress conditions in the process [45].

As a biological treatment, the biodegradability of the substrates and the efficiency of AD are strongly affected by several other environmental and operation conditions, which will not be fully discussed in this chapter. Nevertheless, prior information about how some of these parameters may affect the AD process can be obtained by the use of a biodegradability test. One of the most relevant and useful tools for assessing the biodegradability of wastes is the investigation of a parameter known as biochemical methane potential (BMP), also called "biomethanation" or "biomethane potential". It can be determined by an experimental assay called BMP test. Performing this assay will not only lead to the determination of the BMP value, which is the ultimate amount of methane produced under anaerobic conditions from a certain substrate, but will also give information about the kinetics of the degradation process. Both the yield of methane and the degradation rate are very important factors when designing and operating full-scale anaerobic digesters and give the basics for defining operation parameters, like organic loading rate (OLR) or hydraulic retention time (HRT). Other important results, which can be obtained during the BMP test, are the identification of microbial inhibition, overloading, and possible adaptation of the microbial community to certain conditions [46, 47].

3.1. Biodegradability of slaughterhouse and agrowaste

The proper development of the anaerobic digestion is highly dependent on the type and composition of the material to be digested [48]. The breakthroughs, when it comes to deal with reactor design and operating conditions, succeeded in overcoming the initial limits of AD implementation. Today, AD can be operated with shock loads and can deal with different feed compositions, sensitivity to possible toxicants, instability, and different temperature requirements [49]. Although biological treatments remove organic compounds and pathogens from the effluents using microorganisms, in the case of slaughterhouse residues, the AD treatment is often complicated due to the presence of particulates and fats [50]. Indeed, AD is still a promising alternative for the treatment of these materials, since, just because of their high protein and fat content, these types of residues have a high potential to produce biogas.

Slaughterhouse waste can be considered as a protein-rich waste. During the anaerobic digestion of such wastes, the concentration of ammonia nitrogen can considerably increase due to the degradation of proteins. Accordingly, ammonia toxicity represents a major problem during the anaerobic treatment of such wastes [7, 51]. With excess of ammonia concentrations (i.e., above 4 gN L^{-1}), the methanogenesis can be inhibited [52]. Furthermore, the digestion process becomes unstable, and the biogas production will drop as nitrogen concentration increases [10].

Due to the presence of higher amounts of lipids floating scum and the accumulation of long chain fatty acids (LCFAs), other problems during the AD of slaughterhouse wastes are presented [17, 53, 54]. Consequently, the methanogenesis will be inhibited, and the increased hydrogen levels will affect the propionate- and butyrate-degrading acetogens [55]. In general, the mechanisms responsible for the LFCAs accumulation are adsorption, precipitation with divalent ions, and entrapment in the flocculent structure of the sludge [5].

In the case of agrowastes, both cellulose and hemicellulose are the principal biodegradable components, which are linked with lignin in rigid lignocellulose complexes. On the one hand, due to the sheltering effect of lignin and the low biodegradability of lignin under anaerobic conditions, the degradation of these organic complexes is limited to yield of at most 50% of methane (<200 mL of CH_4 dry g^{-1}) compared to that produced from pure carbohydrates. On the other hand, several agrowastes can be degraded up to 80% of their fiber content, making them feasible for the AD treatment, e.g., paper [56] and rice residues from drying processes [25]. Nevertheless, at large-scale commercial farms, there is a lack of knowledge on biodegradability of agrowastes, and they are therefore not aware to utilize fruit and vegetable wastes for biogas production. However, a cost-effective operation, for example, using the codigestion of these residues, would drive the practical conditions to promote biogas technology.

3.2. Monodigestion of slaughterhouse wastes and agrowastes

In general, all sorts of biomass containing carbohydrates, proteins, lipids, cellulose, and hemicelluloses, as main components, are suitable to be used as substrates for biogas production. Among these residues, slaughterhouse wastes and agrowastes are of major importance due to both the amounts in which they are generated and the high organic content, as discussed before. The theoretical gas yield varies with the content of carbohydrates, proteins, and lipids, declared as the main volatile components that can be degraded under anaerobic conditions. The presence of carbohydrates and proteins provide faster conversion rates but lower gas yields, whereas the lipid content provides the highest biogas yield, however, with unfavorable kinetics of the overall process due to the requirement of a long retention time as a consequence of a slow biodegradability of lipids [56].

In the case of slaughterhouse wastes and agrowastes, diverse results are obtained whendigesting each of them alone as a sole substrate. Table 2 summarizes the results from severalresearch studies conducted either with slaughterhouse residues or agrowastes in batch orsemicontinuous operations.In most of the cases, the efficiency of VS reduction is low and is attributed to LCFA, protein, or lignocellulosic material (LCM) content of the different residues. In the next sections, the effect of LCFA, protein, and LCM content on the biodegradability of slaughterhouse wastes and agrowastes will be briefly discussed.

Substrates	T (C°)	Operation modeand conditions	Methane yield (Y_{CH4})	k_0 (d^{-1})	Degradation efficiency (%)	References
Solid cattle slaughterhouse waste	55	Batch (2L)	582 (mL gVS^{-1})	0.09	n.a	[19]
Solid cattle and swine slaughterhouse waste	35	CSTR (2L), HRT= 30 days	60 (mL gVS^{-1})	n.a	34.5 (VS red)	[1]
Iberian pig slaughterhouse waste	38	CSRT (2L), HRT=23.5 days	17.84 (m^3 m^{-3})	n.a	78.59 (COD red)	[18]
Fruit and vegetable wastes	35	CSTR (2L), HRT = 30 days	0.2 (mL gVS^{-1})	n.a	19.2	[1]
	55	ASBR (2L)	278 (mL gVS^{-1})	n.a	79 (VS red)	[27]
Horse manure	55	Batch (118 mL)	279 (mL gVS^{-1})	0.071	n.a	[22]
Cattle manure	55	Batch (118 mL)	250 (mL gVS^{-1})	0.041	n.a	[22]
Swine manure	35	Batch (250 mL)	357 (mL gVS^{-1})	n.a	n.a	[23]
	35	CSTR, HRT = 30 days, OLR = 1.2 kgVS m^{-3} d^{-1}	330 (mL gVS^{-1})	n.a	36.6 (VS red)	[23]
Organic fraction of municipal solid waste	55	Batch (2L)	537 (mL gVS^{-1})	0.33	n.a	[19]
Various crops	55	Batch (2L)	504 (mL gVS^{-1})	0.29	n.a	[19]
Sugar cane press mud	37	Batch (500 mL)	160 (mL gVS^{-1})	0.138	39 ($Y_{CH4}/Y_{CH4Theoric}$)	[24]
Rice straw	37	Batch (2 L)	226 (mL gVS^{-1})	0.078	62 ($Y_{CH4}/Y_{CH4Theoric}$)	[25]
	55	Batch (2 L)	281 (mL gVS^{-1})	0.168	74 ($Y_{CH4}/Y_{CH4Theoric}$)	[25]
	55	Batch (118 mL)	45 (mL g^{-1})	n.a	n.a	[25]
Rice husk	37	Batch (2L)	19 (mL gVS^{-1})	0.101	6 ($Y_{CH4}/Y_{CH4Theoric}$)	[25]
	55	Batch (2L)	44 (mL gVS^{-1})	0.111	11 ($Y_{CH4}/Y_{CH4Theoric}$)	[25]
Tomato processing waste	38	CSRT (2L), HRT = 8days	5.76 (m^3 m^{-3})	n.a	60.76 (COD red)	[18]
Potato pulp	35	Batch (5L)	n.a	0.073	80 (VS red)	[26]
Maize crops	35	Batch (250 mL)	350 (mL gVS^{-1})	n.a	n.a	[23]

n.a., not available; CSTR, continuous stirring tank reactor; ASBR, anaerobic sequence batch reactor; HRT, hydraulicretention time; COD, chemical oxygen demand; OLR:, organic loading rate; k0, the observed first-order kinetic constantof the overall process.

Table 2. Biodegradability of several substrates from agriculture/agroindustrial activities in anaerobic monodigestion

3.2.1. *Effect of LCFA on the biodegradability of slaughterhouse wastes and agrowastes*

Lipids are characterized as fats, liquid (oils), and solid (greases), commonly presented in slaughterhouse wastes and food wastes [57]. Lipids are quite attractive for biogas production since they have high theoretical methane potential due to the high number of C and H atoms present in their molecules. Nevertheless, the AD of lipids leads to several problems, such as their adsorption onto the cell wall of microorganisms and the inhibition of methanogenic consortium provoking sludge flotation and washout [58]. Usually, such inhibition problems occur when semicontinuous operation is applied.

Under anaerobic conditions, lipids are hydrolyzed into long chain fatty acids and glycerol by extracellular enzymes, i.e., lipases. Glycerol is easily degraded into biogas, while the degradation of LCFAs is more complicated. LCFAs are organic acids that contain long carbon chains of 8 to 18 units.

They are compounds, like lauric, myristic, palmitic, stearic, oleic, linoleic, caprylic, and capric acids, and usually occur in saturated or unsaturated fats (Table 3). Saturated fats are more difficult to degrade because of their high melting point compared with unsaturated fats. Their degradation is usually the rate-limiting step in the AD of solid slaughterhouse waste due to the slow growth of bacteria which consume LCFAs (i.e., with growth rate below 0.5 d^{-1}) in thermophilic reactors [59].

Fatty acids	**Carbon units**	**Number of double bonds**	**Saturated/unsaturated**
Caprylic	8	0	Saturated
Capric	10	0	Saturated
Lauric	12	0	Saturated
Myristic	14	0	Saturated
Palmitic	16	0	Saturated
Palmitoleic	16	1	Unsaturated
Stearic	18	0	Saturated
Oleic	18	1	Unsaturated
Linoleic	18	2	Unsaturated

Table 3. Common long chain fatty acids present in anaerobic digesters (adapted from [60])

The pathway for catabolism of LCFAs is referred as ß-oxidation (Figure 1) because the oxidation occurs at the ß-carbon (C-3) once LCFAs go into the cell [61]. In general, fatty acids can be found with an odd number of carbons or even number of carbons. The odd numbers of carbon are quite abundant in lipids of plants and some marine animals, while the even numbers of carbon are found in the rumen of cows. The oxidation of even or odd chains takes place in similar manner, but the final products are different. When even chains of carbon are oxidized, two carbon units of acetyl-CoA are released in each cycle; meanwhile, propionyl-

CoA is the final product when odd chains of carbon units are oxidized (Figure 1). In that sense, acetate and hydrogen or acetate, hydrogen, and propionate are produced [61], which are further converted to methane and carbon dioxide in the AD process. The degradation of the propionic acid released is thermodynamically dependent on low H_2 partial pressure in the reactor and hence on the activity of H_2 consuming methanogens. This means that if these compounds are not efficiently degraded, their accumulation can cause several disturbances and will lead to an instable process. Acetogenic microorganisms degrading LCFAs are closely related to *Syntrophomonadaceae* and *Clostridiaceae* families living together with H_2 consuming methanogens [62, 63], although few species are described to use LCFAs with more than 12 carbon units [64].

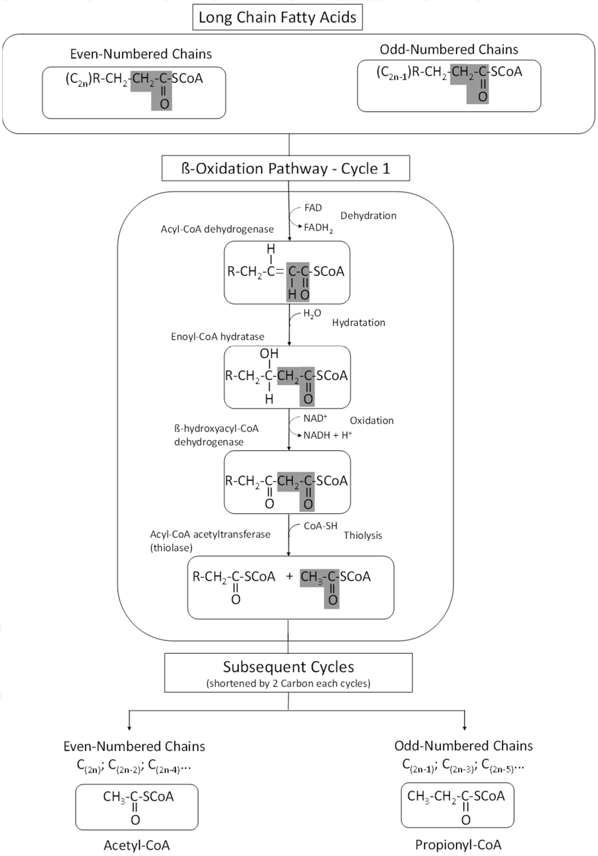

Figure 1. The degradation pathway for long Chain fatty acids (adapted from [61]).

The accumulation of LCFAs is well known to inhibit the methanogenic activity of both acetotrophic and hydrogenotrophic methanogens causing several operational and microbiological problems in biogas plants treating slaughterhouse waste [65-67]. The mechanism of inhibition has been attributed to the adsorption onto the cell wall creating a physical barrier and affecting the transport of nutrients, substrates, and products [68]. This is strongly dependent on the type of microorganisms present in the system, the specific surface area of the sludge, the length and complexity of the carbon chain (i.e., the number of double carbon bonds), and the adaptation of the biomass [69]. Anaerobic sludge with higher specific surface area, i.e., suspended and flocculent, was observed to be more susceptible to inhibition than granular sludge [69]. In addition, process failure has been observed in up-flow anaerobic sludge blanket reactors fed with a mixture of LCFAs at concentrations below of the toxicity level [70] because of the washout of the anaerobic biomass [64]. Sludge flotation is caused by the adsorption of LCFAs on the cell surface and mainly depends rather on the loading rates of LCFAs than on their concentration [70]. Concentrations over 50-75 mg L^{-1} for oleic acid have been reported inhibitory [65, 69], while inhibitory levels of 1 100 mg L^{-1} and 1 500 mg L^{-1} where reported for palmitic [68] and stearic [71] acids, respectively. However, other studies have proved that the inhibition is a reversible process and the system may recover after a lag phase and adaptation period [72].

Another problematic associated with lipids' degradation is their high tendency to form floating aggregates and foam in biogas reactors leading to severe operational problems, such as obstruction of the piping gas collection and pump failure. Recent studies have linked the foam formation with substrate composition, especially in cases of fatty and protein-rich materials [73-75]. Furthermore, specific microorganisms, such as *Dialister*, *Pseudonocardia*, *Thermoactinomyces*, *Pseudomonas*, and *Thermotoga*, were found to increase their abundance in foaming biogas reactors overloaded with lipid-rich substrates [75]. During the metabolic activity of these microorganisms, natural biosurfactant products are released to the medium decreasing the surface tension and contributing to foaming. Intermittent feeding operations have been proposed as an effective strategy to allow biological degradation of LCFAs coupled with inhibition phenomenon [76].

3.2.2. Effect of proteins on the biodegradability of slaughterhouse wastes and agrowastes

Slaughterhouse wastes as well as other agrowaste residues, such as swine and poultry manure, are protein-rich materials. Proteins, like fats, are also energy-rich materials that provide high gas production. Proteins are composed of long chains of amino acids joined together by peptide bonds each containing an amino group ($-NH_2$) and a carboxyl group (-COOH) [60]. In the course of decomposition, proteins are first hydrolyzed by extracellular enzymes, i.e., proteases, into amino acids. One particular element to consider, when analyzing protein-rich wastes, is the low C/N ratio in contrast to the high organic content and the high biological oxygen demand (BOD) [77, 78].

During degradation of proteins, the nitrogen is released in the form of ammonia (NH_3) or ammonium (NH_4^+). Acetate and butyrate are also produced. NH_3 and NH_4^+ are present in equilibrium with each other, and the predominance of which will depend on prevailing conditions inside the digester, such as pH and temperature. The described inhibitory specie is NH_3 rather than NH_4^+. So far, the most accepted mechanism of inhibition has been attributed to the fact that uncharged NH_3 may easily diffuse into the cell, where it is further converted to NH_4^+ consuming hydrogen ions and causing proton, sodium and potassium imbalance (Figure 2) [79]. As a consequence, the cell must then use energy to recover the transmembrane ion gradient, which is used for various energetic purposes and to drive several biochemical reactions [80-82]. Some studies have shown that acetoclastic methanogens are more affected by ammonia than hydrogenotrophic methanogens [52, 53]; however, the reported inhibitory concentrations vary in a wide range depending on the source of inoculum and the adaptation of the microorganisms, as well as on substrates' characteristics, pH, and temperature [79]. It was reported previously that free ammonia inhibits methanogenesis in nonadapted sludge at concentrations ranging from 0.1 to 2.0 gN L^{-1} [52, 53, 83-85]. However, in the presence of adapted biomass, higher inhibitory concentrations, i.e., up to 4 gN L^{-1}, have been observed [52]. The adaptation of methanogens to arising ammonia concentrations has been reported [86, 87] and explained by the growth of new biomass rather than the metabolic changes in the methanogens [52]. However, differing from LCFAs' inhibition, ammonia inhibition does not lead to failure and instability of the biogas process. The interaction that takes place between volatile fatty acids, pH, and ammonia will lead to a so-called "inhibited steady state," in which the process is running under apparent stable condition but with lower methane production [88].

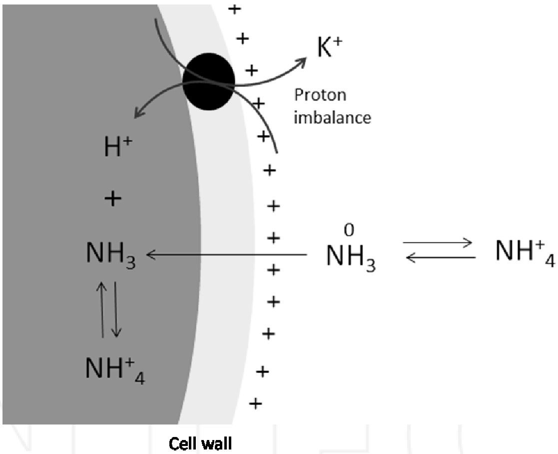

Figure 2. The mechanism of ammonia inhibition in nonionized form NH_3 can easily enter the cell causing proton imbalance (adapted from [79]).

3.2.3. *Effect of Lignocellulosic Materials (LCM) on the biodegradability of slaughterhouse wastes and agrowastes*

Carbohydrates are the main components of organic wastes from anthropogenic activities including the organic fraction of municipal solid waste (MSW) from households and markets. In addition, agrowaste with a high content of lignin, cellulose, and hemicellulose are produced in several activities from the agriculture sector. Typical substrates are green residues of fruits and vegetables remaining after the harvest, leaves of sugar beets, energy crops, straw from animal feeding, and animal manure consisting a large fraction of straw, among others. As it was point out earlier, the composition of feedstock used for biogas production is highly important, as the biogas yield will be strongly linked with its biological degradability. Obtaining high biodegradability will also lead to a better final disposal of such residues, as well as higher energy production per unit of mass of substrates [25].

LCM is a carbohydrates-rich substrate mainly composed of cellulose (40%-50%), hemicellulose (25%-35%), and lignin (15%-20%) [89]. Lignin protects the lignocellulosic structure and is linked with cellulose and hemicellulose by different chemical bonds, making this structure extremely resistant to enzymatic digestion. The rate and the biodegradability (extension) of lignocellulosic substrate are one of the most important factors to be considered, when applying them for anaerobic digestion. Depending on the ratio between the rate of acidification and the rate of methanogenesis, the anaerobic degradation can be considered to be successful or not. VFAs tend to be accumulated as a result of a faster acidification step than methanogenesis step, provoking a drop in pH, which leads to inhibition of the methanogenic activity [90].

Since carbohydrates vary in their nature, they are anaerobically converted at different rates. A fast biodegradation gives more methane per unit of feed biomass per time and also reduces the reactor's size, making the process economically more attractive. However, the easily biodegradable fraction present in fruit and vegetable wastes with high moisture content may be converted too fast, increasing the volatile fatty acid content in the digester. This is a major limitation of treating these kinds of wastes, as they are very prone to acidify the system, decreasing the pH and making the process unstable [91, 92]. On the other hand, complex agrowaste fractions, such as straw, contain a large amount of recalcitrant structures that hamper the degradation [22, 25]. Straw is a cellulose-rich substrate. Cellulose consists of linear polymer chains of glucose molecules linked by β-glucosidic bonds, which makes this structure hard to digest. Hence, hydrolysis has proved to be the rate-limiting step during anaerobic degradation because of this complex structure of cellulosic materials [92, 93]. Enzymes involved in the hydrolysis of cellulose-rich materials have difficulty to access the structure, especially in the case of lignocelluloses, making the process slow. In order to improve the accessibility providing higher methane yield and increasing biodegradation rate, some pretreatments (chemical, physical, and enzymatic) have been used to open up the structure and to disrupt cellulose crystallinity [24, 94-99]. For example, methane yields reaching up to 88% of the theoretical values have been achieved after a chemical pretreatment using *N*-methylmorpholine-*N*-oxide pretreatment on barley straw [100].

3.3. Anaerobic codigestion (AcoD) of slaughterhouse waste and agrowaste

Codigestion is not a new concept in anaerobic processes. It has been applied within research and practice for more than 20 years, especially in Europe [101]. Codigestion appears to be "the solution" to obtain an increase in methane production, to avoid inhibition, and to operate profitable biogas plants. In order to achieve these objectives, an appropriate mixture of substrates, containing proper percentages of different kinds of organic matter to be degraded, must be determined. A lot of studies have been investigating such factors [102-108]. This chapter will give a brief summary of these results.

The beneficial effect of the codigestion has been widely studied from different points of views, as the nutrient balance, mainly when mixing nitrogen-rich wastes with carbon-rich ones, as well as pH, the presence of inhibitors/toxic compounds, biodegradable organic matter, and dry matter [109]. Hence, at early stage of different studies and applications related to codigestion the principal variable was the C/N ratio.

Codigestion can be defined as the treatment of a mixture of at least two different waste streams under anaerobic condition with the intention of improving the efficiency of the anaerobic process. Additional benefits of AcoD are the dilution of toxic compounds if present or developed during the process, the supply of required buffer capacity, as well as the adjustment of the moisture content and the augmentation of bacterial strains taking part in the process [108, 110].

Thus, why is the codigestion of slaughterhouse waste and agrowaste preferable to apply? As we described it above, several technological and economic advantages are established when codigestion is performed. Due to these benefits, an increasing number of full-scale codigestion plants treating manure and industrial organic wastes are in operation, mainly in Denmark and in Germany [111, 112]. Higher biogas output and therefore better anaerobic process performance and profitability can be attained by codigesting of, for example, animal manure or sewage sludge with 10% to 20% of solid waste fractions from agroindustry and food industry, i.e., slaughterhouse, pharmaceutical, kitchen, fermentation, or municipal wastes [49]. In this way, an increase of 50% to 200% in the methane (CH_4] production of manure digesters can be achieved [10, 113, 114]. Manure is widely accepted as the basic substrate in codigestion. This substrate is easily available in many farms all over the world; however, the low biogas yield of manure usually does not justify the investment costs for farm-scale plants. Nevertheless, by introducing energy-rich cosubstrates, this aspect can overcome.

The effect of temperature on codigestion has also been widely studied due to the problems that are faced in case of temperature fluctuations, which provoke instability and disturbances in all the other main parameters of the process [115, 116] with corresponding decreasing levels of yields. As a result of several experiences, the thermophilic operation is preferred over the mesophilic one, mainly due to its kinetics improvements and sanitization capability [117].

Still, the optimal operational conditions in terms of mixture composition must be investigated for each specific case [78, 118-120]. In addition, the amounts of wastes available in site should also be evaluated. The amounts of residues with high organic content that are generated at a particular location usually are not enough for a cost-effective anaerobic process. If codigestion concept is applied, designing a proper mixture with other locally generated residues might improve the economy of the overall process.

3.3.1. Effect of the C/N ratio on the biodegradability of slaughterhouse wastes and agrowastes

As explained before, during the codigestion process, protein-rich wastes can provide the buffering capacity and a wide range of nutrients, while carbon-rich wastes provide a high carbon content. This will balance the C/N ratio for all substrates decreasing the risk for inhibition [84]. The C/N ratio is one of the most controlled parameter for an efficient biodegradation of residues. Higher or lower values than the optimal ones of this parameter diminish the reaction rate of microorganisms involved in each step of degradation, and in some cases, the anaerobic process can be inhibited. As carbon and nitrogen are the main macronutrients for microorganisms, established levels of those provide certain insurance for the nutrient supply during the process. Nevertheless, a wide range of values is reported in the literature, showing the proposed optimal range, and in some cases with inconsistency in the boundaries of these values. The bioavailability of the carbon seems to play an important role in this apparent contradiction. Numerous investigations found that the C/N ratio values ranging from 20 to 35 are optimal for the anaerobic process [79, 99, 121-123]; meanwhile, optimal intervals as between 12 and 16 [123] and between 20 and 70 [124] have also been suggested. Moreover, lower values of C/N ratios (i.e., 6-9) have been also reported as suitable values for the anaerobic digestion of nitrogen-rich wastes [123, 125]. Some authors revealed the relevance of temperature not only for the nitrogen balance [88] but also for the carbon bioavailability [25]. The biodegradation capability of certain substrates will also influence the C/N ratio due to the release of NH_4^+ [107]. As demonstrated, there is no a unique criteria for C/N boundaries to define an optimal ratio for the anaerobic process. Hence, substrate characteristics as well as operational parameters should also be considered in order to ensure a proper development of the anaerobic process.

Generally, we can conclude that the codigestion concept is widely applied, and in most cases, it is related to positive effects and rarely with negative interactions for the process itself. Modeling of anaerobic codigestion has been therefore a focus of many researchers in order to predict the expected methane yield [126, 127]. However, the mathematical tools are still unable to properly predict synergy and antagonisms effects [128]. The necessity to clarify adverse effects when mixing substrates and cosubstrates has not gained enough attention yet, as it can be seen from the topics in recent literature [129].

3.4. Recent developments in AcoD of slaughterhouse waste and agrowaste mixtures

As it was pointed out in the previous chapter, using a codigestion concept will lead to several advantages. However, it is important to access the mixture interactions affected by the waste composition in order to determine the responses and optimize the process. The composition of substrate will influence the activity of the microbiological population [21, 130], which in turn will largely affect long-term process stability, the degradation rate of the solids, and consequently the biogas yield. When larger yields than the predicted ones can be detected, it is usually because of synergy occurring in the mixture, while lower yields are caused by antagonism. The recent developments in AcoD of slaughterhouse waste and agrowaste mixtures were therefore focused on the evaluation of these synergistic or antagonistic interactions [20]. To be able to investigate the mixture interactions between several fractions of substrates, it is preferable to use a statistically designed experimental setup. Meanwhile synergy and antagonism can be detected using this methodology; the actual explanation of why those effects are presented cannot be explained by a simple statistical evaluation. In this section, the biological influence of different substrate compositions on AcoD of solid cattle slaughterhouse wastes mixed with different residues from agricultural activities will be discussed.

The use of slaughterhouse waste for biogas production gained an increasing attention in Europe during the past years; however, it is still not very common in Latin America and in developing countries. As it was earlier mentioned, this waste fraction can increase the economic feasibility of biogas production in codigestion plants due to its high methane potential. Nevertheless, slaughterhouse waste is a complex material, which cannot be easily treated because of its high potential to inhibit the process owing to ammonia and long chain fatty acids' accumulation [9, 21, 121]. Therefore, the use of this waste fraction in codigestion processes was investigated in different studies, and several surveys have proved that this concept will improve the biological degradation [1, 19, 20]. However, it is still a little knowledge available when it comes to its biodegradation and mixture interactions using several cosubstrates from agricultural activities. The decision of which cosubstrates should be used for the codigestion had been simplified so far, based on determining an optimal C/N ratio together with a balanced lipids/proteins/carbohydrates composition; however, the bioavailability of these materials must also be considered.

A brief summary on the investigations and the obtained results considering the use of different slaughterhouse waste fractions in codigestion processes are shown in Table 4. When investigating codigestion processes, most of the studies are focused so far on manure and sewage sludge as the main raw materials used in agricultural and agroindustrial sector [129], and two components have been typically used in the codigestion processes. Considering slaughterhouse waste as the main substrate used in the mixtures, poultry and swine animal wastes are the most reported waste fractions found in the literature [2, 18, 131].

Substrates	**Mixture ratio (%)**	**T (°C)**	**Operation mode and conditions**	**Methane yield (Y_{CH4})**	***k*0 (d^{-1})**	**Degradation efficiency (%)**	**References**
Binary mixture combinations							
Solid cattle/swine slaughterhouse waste + fruit/vegetable waste	50:50 (wet basic)	35	CSTR (2L), HRT = 30 days	40 (mL gVS^{-1})	n.a	53.8 (VS red)	[1]
Solid cattle/swine slaughterhouse waste + solid cattle/swine manure	50:50 (wet basic)	35	CSTR (2L), HRT = 30 days	0.26 ($m^3 kgVS^{-1}$)	n.a	51.7 (VS red)	[1]
Solid cattle slaughterhouse waste + animal manure (pig, cow, horse)	64:36 (VS basic)	55	Batch (2L)	613 (mL gVS^{-1})	n.a	n.a	[20]

Substrates	**Mixture ratio (%)**	**T (°C)**	**Operation mode and conditions**	**Methane yield (Y_{CH4})**	***k*0 (d^{-1})**	**Degradation efficiency (%)**	**References**
Solid cattle slaughterhouse waste + organic fraction of municipal solid waste	62:38 (VS basic)	55	Batch (2L)	647 (mL gVS^{-1})	n.a	n.a	[20]
Solid cattle slaughterhouse waste + various crops (straw and fruit/vegetable waste)	53:47 (VS basic)	55	Batch (2L)	461 (mL gVS^{-1})	n.a	n.a	[20]
Liquid poultry slaughterhouse waste + organic fraction of municipal solid waste	17:83 (wet basic)	34	CSTR (3L), HRT = 50 days, OLR = 1.85 kgVS m^{-3} d^{-1}	400 (mL gVS^{-1})	n.a	80.6 (VS red)	[134]
Abattoir waste + fruit/ vegetable waste	70:30 (wet basic)	35	ASBR (2L), HRT = 20 days, OLR = 1.2 gVS L^{-1} d^{-1}	191 (mL gVS^{-1})	n.a	84 (VS red)	[27]
Abattoir waste + fruit/ vegetable waste	70:30 (wet basic)	55	ASBR (2L), HRT = 20 days, OLR = 1.28 gVS L^{-1} d^{-1}	453 (mL gVS^{-1})	n.a	86.2 (VS red)	[27]
Slaughterhouse waste (hog and cow stomach content) + sewage sludge	25:75 (wet basic)	37	CSTR ($2m^3$), HRT = 17 days, OLR = 2.9 kgTS m^{-3} d^{-1}	0.23 (m^3 $kgTS^{-1}$)	n.a	n.a	[136]
Cattle/swine slaughterhouse waste + sewage sludge	1:7 (wet basic)	35	CSTR (3L), HRT = 50 days, OLR = 1.85 kgVS m^{-3} d^{-1}	430 (mL gVS^{-1})	n.a	38 (VS red)	[137]
Ternary mixture combinations							
Solid cattle slaughterhouse waste + cattle manure + various crops (straw, fruit/vegetable waste, animal feed)	25:37.5:37.5 (wet basic)	55	Batch (2L)	499 (mL gVS^{-1})	0.32	n.a	[19]
Solid/liquid cattle slaughterhouse waste + cattle manure + various crops	25:37.5:37.5 (wet basic)	37	Batch (500 mL)	208 (mL gVS^{-1})	0.169	n.a	[4]

Substrates	**Mixture ratio (%)**	**T (°C)**	**Operation mode and conditions**	**Methane yield (Y_{CH4})**	***k*0 (d^{-1})**	**Degradation efficiency (%)**	**References**
Solid cattle/swine slaughterhouse waste + solid cattle/swine manure + fruit/vegetable waste	67:17:17 (wet basic)	35	CSTR (2L), HRT = 30 days	270 (mL gVS^{-1})	n.a	67.3 (VS red)	[1]
Solid cattle slaughterhouse waste + various crops + organic fraction of municipal solid waste	40:35:25 (VS basic)	55	Batch (2L)	614 (mL gVS^{-1})	n.a	n.a	[20]
Slaughterhouse waste + pig manure + a mixture of industrial waste	12:71:17 (wet basic)	35	CSTR (3L), HRT = 28 days, OLR = 3.1 kgTS m^{-3} d^{-1}	624 (mL gVS^{-1})	n.a	n.a	[48]
Quaternary mixture combinations							
Solid cattle slaughterhouse waste + cow manure + various crops + organic fraction of municipal solid waste	25:25:25:25 (wet basic)	55	Batch (2L)	664 (mL gVS^{-1})	0.20	n.a	[19]
Solid cattle slaughterhouse waste + cow manure + various crops + organic fraction of municipal solid waste	22:22:45:11 (wet basic)	55	Batch (2L)	491 (mL gVS^{-1})	0.34	n.a	[19]
Slaughterhouse waste + pig manure + vegetable waste + various kinds of industrial waste	12:66:5:17 (wet basic)	35	CSTR (3L), HRT = 36 days, OLR = 2.6 kgTS m^{-3} d^{-1}	682 (mL gVS^{-1})	n.a	n.a	[48]

n.a., not available; CSTR, continuous stirring tank reactor; ASBR, anaerobic sequence batch reactor; HRT, hydraulic retention time; COD, chemical oxygen demand; OLR, organic loading rate; k_0, the observed first-order kinetic constant of the overall process.

Table 4. Biodegradability of slaughterhouse waste in anaerobic codigestion with residues from agricultural/agroindustrial activities

Taking a look into the literature, there are only few studies investigating the codigestion of solid cattle slaughterhouse waste with residues from agriculture activities in ternary or quaternary mixtures (Table 4). It is proved that a considerable improvement in methane yield can be achieved, when treating several waste fractions together at the same time, due to the positive synergistic interactions that will lead to an increase in the biogas yield [132].

In addition, temperature also plays an important role in increasing the methane yield, probably due to higher bioavailability of carbon and nitrogen sources and a better hydrolysis performance at higher temperatures [19, 20, 27].

Recent investigations in anaerobic codigestion have evaluated the biodegradability in binary, ternary, and quaternary mixture combinations of solid cattle slaughterhouse waste with agrowastes in terms of methane yield and the specific methane production rate [20]. The experiments were performed in batch assays at thermophilic conditions (55°C±1°C) using a four factor mixture design to evaluate the two and three factor mixture interactions (i.e., synergy or antagonism). The biodegradability of every individual fraction was also assessed. As shown in Figure 3, the response variables, i.e., methane yield (Y_{CH4}) and methane production rate (r_{sCH4}), can be predicted as a function of any component in the blend. These results show that high methane yield can be attained with the presence of slaughterhouse and municipal solid wastes in the mixture. On the other hand, the presence of manure and various crops, even though they do not contribute with high values of methane yield (Figure 3A), is needed for a proper balance of macro- and micronutrients. This was proved when analyzing the biodegradability in terms of specific methane production rates (Figure 3B), in which the optimal result was found when the slaughterhouse waste was codigested with various crops, manure, and municipal solid wastes. The mixture that includes all of the four substrates resulted in an increase in methane yield by 31%, compared to the expected yield, which was calculated on the basis of the methane potential of each individual fraction. This clearly demonstrates a synergistic effect. Moreover, when combining cattle slaughterhouse waste in ternary mixtures, an increment of 15% in the methane yield was achieved compared to that in binary mixtures [20]. Mixtures in similar combinations were also investigated in another studies. When slaughterhouse waste was codigested with pig manure, vegetable waste and food industrial waste a biogas yield of 0.9 to 1 m^3 $kgVS^{-1}$ was obtained together with a stable operation [48]. Furthermore, anaerobic digestion of mixtures of rumen, stomach/intestinal content, food waste, and manure showed also stable performance working at OLR exceeding 2.5 kgVS m^{-3} d^{-1} with a hydraulic retention time of less than 40 days under mesophilic conditions (37ºC) [133].

The binary mixture combination of liquid poultry slaughterhouse waste and MSW has shown to give a feasible process after an acclimatization period comparing with the results observed when these fractions were digested individually [134]. HRT could be decreased from 50 to 25 days with the corresponding increase in OLR up to 3.70 kgVS m^{-3} d^{-1}, and an increase of volatile fatty acid reduction efficiency from 80.6% to 82.6% was also found [134]. Moreover, the codigestion of slaughterhouse waste (i.e., either cattle or swine) with animal manure (i.e., either pig or cow) has also proved to be successful [1, 20, 135]. High methane yields and stable process performance have been observed for these mixtures both during thermophilic and mesophilic

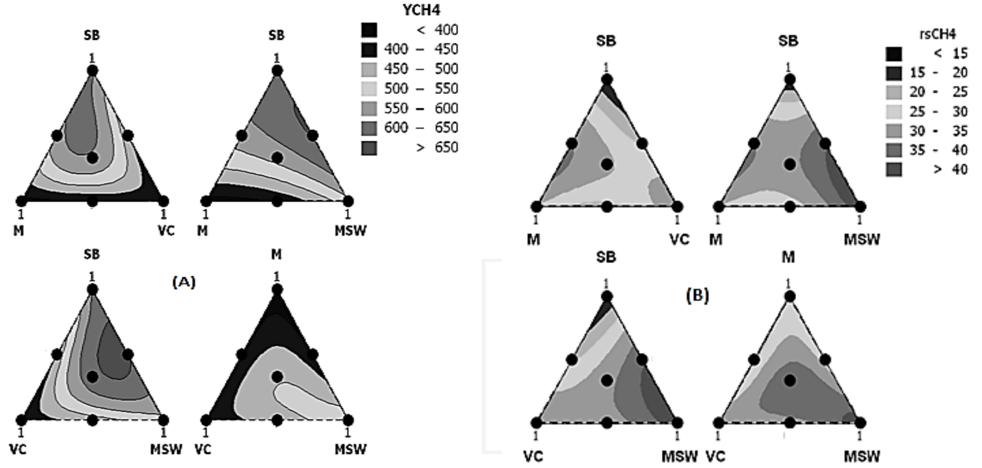

Figure 3. Mixture contour plots for methane yield (A) and for specific methane production rate (B). Letters on the apex of the triangle correspond to the following: SB, solid cattle slaughterhouse waste; M, a mixture of animal manure (pig, horse, and cow); VC, a mixture of fruit/vegetable waste and straw; MSW, the organic fraction of municipal solid waste (adapted from [20]).

semicontinuous operations [1, 135] (Table 4). This behavior has been mainly attributed to the characteristics of the animal manure itself (i.e., high fraction of fibers, nutrients, and good buffer capacity), which led to significant synergetic interactions during the codigestion process with solid cattle slaughterhouse waste [20].

In Figure 4, the performance of the semicontinuous anaerobic codigestion of slaughterhouse waste and manure is compared with the digestion of slaughterhouse waste as a single substrate. In accordance with the results attained previously in batch mode [20], the combination of slaughterhouse waste and a mixture of animal manure showed the best performance even during semicontinuous operation with an OLR of 3 gVS L^{-1} d^{-1} and HRT of 25 days. Meanwhile, the digestion of cattle slaughterhouse waste as sole substrate failed at much lower OLR (i.e., 0.9 gVS L^{-1} d^{-1}), and moreover, strong foam formation was also observed in this reactor (Figure 4A). When these results were compared with those found in the literature, a stable semicontinuous operation was reported for waste combinations of solid cattle/swine slaughterhouse waste and solid cattle/swine manure compared with that when slaughterhouse waste was digested with fruit and vegetable wastes at mesophilic conditions [1].

Similarly, the binary mixture combination of solid cattle slaughterhouse waste and various crops, including fruits/vegetables and straw, showed previously to be a blend with antagonistic interactions during the thermophilic batch assays [20], and this binary mixture combination led to unstable operation performance even during the semicontinuous operation with accumulation of VFAs leading to a drop in pH [1, 135]. The OLR of 2 gVS L^{-1} d^{-1} led to overloading with a consequent gradual decline in the methane production [135], probably due to the high biodegradability of fruit and vegetable wastes, leading to a fast acidification of the

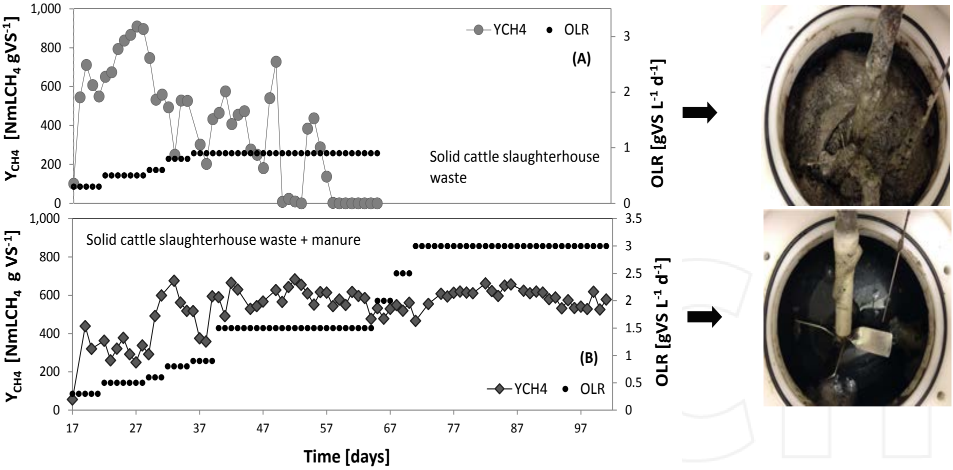

Figure 4. Daily methane yield (Y_{CH4}) and applied organic loading rate (OLR) during semicontinuous digestion of slaughterhouse waste as a single substrate (A) and its codigestion with animal manure (B) (adapted from [135]).

system [107]. Furthermore, on investigating kinetic parameters, this mixture composition showed the lowest degradation rate, resulting in the lowest value of methane production rate when compared with other mixtures of slaughterhouse waste and cosubstrates examined in batch assays [20].

These results clearly show that it is very important to choose the right cosubstrates' combinations and ratios in order to avoid failure at industrial level and get those technological, economical, and biological advantages of the codigestion technology mentioned above. Since the economic feasibility of AD plants is directly linked with the methane potential of the treated waste, it is important to investigate mixture interactions between substrates that may enhance or attenuate the degradation rate and the methane yield.

Hence, it is necessary to recall that the biodegradability of complex substrates, as slaughterhouse residues and agrowastes, is highly dependent on the relative quantities of fats, proteins, and LCM. The actual bioavailability of carbon and nitrogen sources, as well as temperature, organic loading rate, moisture content, pH and alkalinity, and many other parameters are interrelated and provoke either positive or negative effects when mixing the waste fractions in different ratios.

4. Conclusions

Agricultural residues, as slaughterhouse and agrowastes, are produced in large amounts with a high organic content holding an important potential for biogas production. As the organic composition of such residues can lead to the development of inhibitory effects during the anaerobic process, one possible way to overcome this problem is applying codigestion for an appropriate mixture of substrates. One aspect to be analyzed when designing a codigestion process is the availability of different waste fractions generated by local industries or communities. Nevertheless, the codigestion of slaughterhouse residues with agrowastes does not necessarily result in only positive effects in terms of methane yield and degradation rate. It has been shown that antagonistic effects can also be obtained with certain mixture ratios. It was also shown that applying BMP assays had provided a good way to detect synergy or antagonism in different mixtures, when the experiments were designed and the results were evaluated using statistical methods. However, it is essential to make further efforts to study the long-term effects of these interactions deeply and find possible impacts on the microbial community structure developed during the process. This will give the necessary information for engineers to develop and promote environmental-friendly technologies, such as biogas production, for the management of locally produced residues at or close to abattoir sites.

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