Optimització i modelització de processos avançats de digestió anaeròbia

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Programa de doctorat de Ciències i Tecnologies del Medi Ambient

Optimització i modelització de processos avançats de digestió anaeròbia

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CERTIFICA QUE:

El treball d'investigació titulat "**OPTIMITZACIÓ I MODELITZACIÓ DE PROCESSOS AVANÇATS DE DIGESTIÓ ANAERÒBIA**" constitueix la memòria que presenta l'Enginyer Químic **Sergi Astals Garcia** per a aspirar al grau de Doctor per la Universitat de Barcelona. Aquesta tesi doctoral ha estat realitzada dins del programa de doctorat "Ciències i Tecnologies del Medi Ambient", en el Departament d'Enginyeria Química de la Universitat de Barcelona.

I perquè així consti als efectes oportuns, signo el present certificat a Barcelona, Abril 2013.

Dr. Joan Mata Álvarez
Director de la tesi doctoral

I hear and I forget. I see and I remember. I do and I understand.

Confucius

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Summary

Anaerobic co-digestion consists of the anaerobic digestion of a mixture of two or more substrates with complementary characteristics; so that, the methane production is enhanced. The improvement is mainly a consequence of the increase of the organic loading rate; however, when possible, it is important to choose the best co-substrate and blend ration with the aim of favoring positive interactions. Today, there is very little knowledge about interactions between substrates that may enhance or attenuate inhibition, rate, or potential. Moreover, little attention has been paid to the digestate quality, although both biogas and digestate have to be managed in appropriate ways in order to make anaerobic digestion plants sustainable in the long term.

In this thesis, several anaerobic mono- and co-digestion studies have been carried out to improve the knowledge about the interaction between wastes and to analyse the effect on the digestate quality after the addition of a co-substrate. Initially, the anaerobic digestion of sewage sludge was evaluated in order to develop a methodology which could enable the obtention of parameters, coefficients and state variables for anaerobic digestion modeling, based on the Anaerobic Digestion Model No.1. The comparision between the simulation and the experimental results showed the consistency of the developed methodology, although an underestimation of the solubilisation rate was detected. Secondly, the interaction between substrates during anaerobic co-digestion were evaluated and modeled. Pure substrates (cellulose, casein and olive oil) and slaughterhouse waste (paunch, blood and dissolved air flotation fat) were used to study the role of carbohydrates, protein and lipids in the co-digestion behaviour. It was concluded that mixing substrates lead to an improvement in kinetics for all mixtures, although the ultimate methane potencial is generally not affected. Next, co-digestion of sewage sludge or pig manure and glycerol was evaluated with the aim of identifying synergism and inhibitory mechanisms when glycerol is used as co-substrate. The results showed that glycerol is an ideal co-substrate for sludge and manure digestion, being overloading the main risk of process failure. Finally, pig manure and glycerol were co-digested at mesophilic and thermophilic conditions in a continuous reactor. The improvement of the biogas production in both cases was related with the increase of the digester organic loading rate, the balance of the carbon-to-nitrogen ratio and the reduction of the free ammonia concentration. The comparison between both digestates indicated that a lower stability is expected at thermophilic than at mesophilic conditions because of the higher accumulation of intermediate compounds in the digester medium. However, the thermophilic digestate was likely to fulfil the requirements of the European hygienisation legislation for unrestricted agricultural use.

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1. Introduction

- Mata-Alvarez J, Macé S, Astals S (2009). Codigestion of solid wastes: A review. International workshop on anaerobic digestion: An old story for today and tomorrow. Narbonne (France). 10^{th} – 11^{th} December of 2010
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1.1. Overview of the organic waste problem

Our present society consumption patterns leads to the production of huge and constant amounts of waste. Consequently, the waste management policy of the European Union (EU) is aiming, at long term, to turn Europe into a recycling society. The Waste Directive (Directive 06/12/EC) and the revision of the Waste Framework Directive (Directive 08/98/EC) constituted a modernised approach to waste management, where wastes are considered as a valued resource instead of an unwanted burden. The latter directive brings five hierarchy levels, where waste management must comply with the following priority: prevention, reuse, recycling, other forms of recovery, and disposal of waste in landfills (Fig. 1.1). Moreover, the implementation of the Landfill Directive (Directive 99/31/EC) is to minimise the adverse effects on water, soil and air related to organic matter landfill disposal, among others.

Fig. 1.1. Waste Framework Directive hierarchy management levels

The recovery of organic material should be included within the category of recycling (i.e. to feed a waste into a process to give a new use for that material) since the main organic matter processing technologies, composting and anaerobic digestion (AD), result in a stabilised product that can be used as fertiliser and/or soil conditioner. Recycling organic wastes reduce the amount of waste that ends up in incinerators or landfill, diminishes the amount of raw materials taken from the environment, and avoids several environmental phenomena. Moreover, the recycling of organic material through AD includes the production of energy from biogas.

A wide variety of organic materials can be used to produce energy, like wood and forest wastes, livestock wastes, agricultural wastes, energy crops, wastes from food and paper industries, municipal solid wastes and sewage sludge. In 2010, these biomass resources represent about the 67% of primary production of renewable energy; nonetheless, biogas only represented the 7% (Fig 1.2).

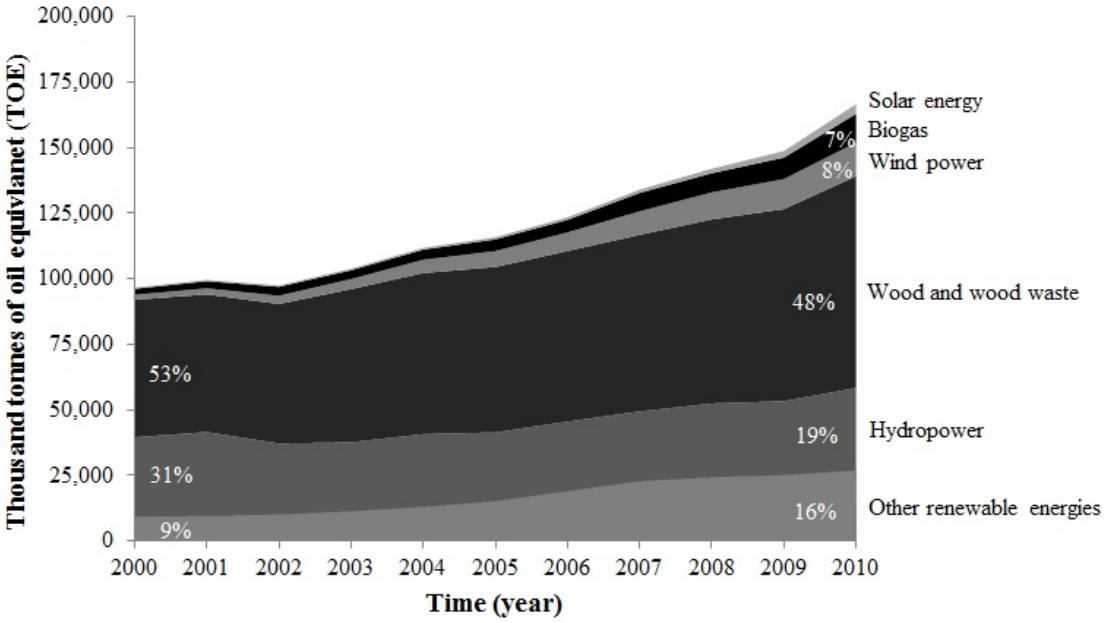


Fig. 1.2. Evolution of the different sources of renewable energy consumption in EU (adapted from Sturc, 2012)

Biomass renewable energy can basically be recovered using thermal or biological processes (Table 1.1). Incineration stands out as the most developed and widespread technology to recover energy from organic waste, while AD is the unique implemented biological technology with energy recovery. The application of one or other technology depends on the waste characteristics. Obviously, biological methods are more appropriate for highly biodegradable rich-moisture wastes. Sewage sludge, municipal solid wastes and farming wastes constitute an interesting source of renewable energy through AD since they can be found in almost every municipality. Nevertheless, those waste can also be stabilised though aerobic biological treatments, mainly composting.

Table 1.1. Main technologies to produce energy from organic wastes

Technologies	Thermal Conversion	Incineration
		Gasification/Plasma (in development)
		Pyrolysis and Liquefaction (in development)
	Biological Conversion	Anaerobic digestion
		Hydrogen fermentation

1.2. Biological technologies to stabilise biodegradable organic waste

Biodegradable organic wastes can either be treated by composting or by anaerobic digestion followed or not by composting. AD has the advantage of producing energy instead of consuming it, but the investment required as well as the process complexity is higher. The decision to adopt one or another solution depends, among other factors, on the quality and quantity of the organic stream and the availability and practice of the land where the digestate or compost is to be spread.

1.2.1. Composting technology

Composting is a process carried out in aerobic conditions in which the organic material is decomposed into CO_2, water, other minor emissions and a stabilised product; the latter has excellent conditions to be applied into the land. Composting technologies follow the stabilisation process that takes place in nature, accelerating it by supplying air and water in optimal conditions for the microorganisms responsible of the process. There are several technologies for composting organic waste, nevertheless, large and constant flow of waste are typically treated in tunnels, in which the composting process is continuously monitored and controlled (Diaz et al. 2007). Frequently, some material such as wood chips or other lingo-cellulosic products are added to give the necessary porosity to the composting pile, whereas in some cases, correction of the carbon-nitrogen ratio and/or moisture content is also carried out. In fact, free air space, oxygen and moisture should be adequately controlled, to provide a good composting media. Other important composting factors include temperature, nutrients and pH (Diaz et al. 2007).

1.2.2. Anaerobic digestion technology

Anaerobic digestion is the decomposition of organic matter in strict anaerobic conditions to produce biogas, a mixture of methane and carbon dioxide. The possibility of digesting biodegradable waste has been studied for more than a century, but it was not until the 1930's when anaerobic digesters, devoted to sewage sludge treatment, were built with energetic purposes (Metcalf and Eddy, 2003). Municipal and farming anaerobic digesters are also a mature application as they were developed in the 1970's and implemented during the 1980's (Cecchi et al. 1988). Today, it is possible to find urban, industrial or livestock AD plants around the world, even though most of them have been built in the EU. The large implementation of the AD in the EU is a result of the financial support for projects in the field of alternative energy sources and energy savings as well as the implementation of the Landfill Directive (Directive 99/31/EC). In recent years, a reduction of the number of projects has been observed due the difficult economic situation. Nevertheless, that does not change the future prospects of AD due to the significant advantage over others treatment and the great diversity of biogas applications. In the next section, a deep description of the process basis is given.

1.3. Basic principles of anaerobic digestion

Anaerobic digestion is a biochemical process which, in the absence of oxygen, decomposes biodegradable organic matter into biogas and a digestate, a mixture of partially degraded organic matter, anaerobic biomass and inorganic matter. The conversion of the organic matter into biogas is a process which involves several serie-parallel reactions and different groups of microorganisms (bacteria and archaea). The heterogeneity and structure of the organic matter present in solid and semi-solid wastes implies a complex metabolic pathway before the organic matter is transformed into biogas (Batstone et al., 2002). Additionally, the performance of the AD is highly related with the structure of its microbial community (Damirel and Scherer, 2008).

1.3.1. Steps of the anaerobic digestion process

The degradation of the organic matter has been classically divided in four steps, namely: (i) disintegration and hydrolysis, (ii) acidogenesis, (iii) acetogenesis and (iv) methanogenesis; where the starting point and degradation pathway depends on the nature of the organic matter (Fig. 1.3).

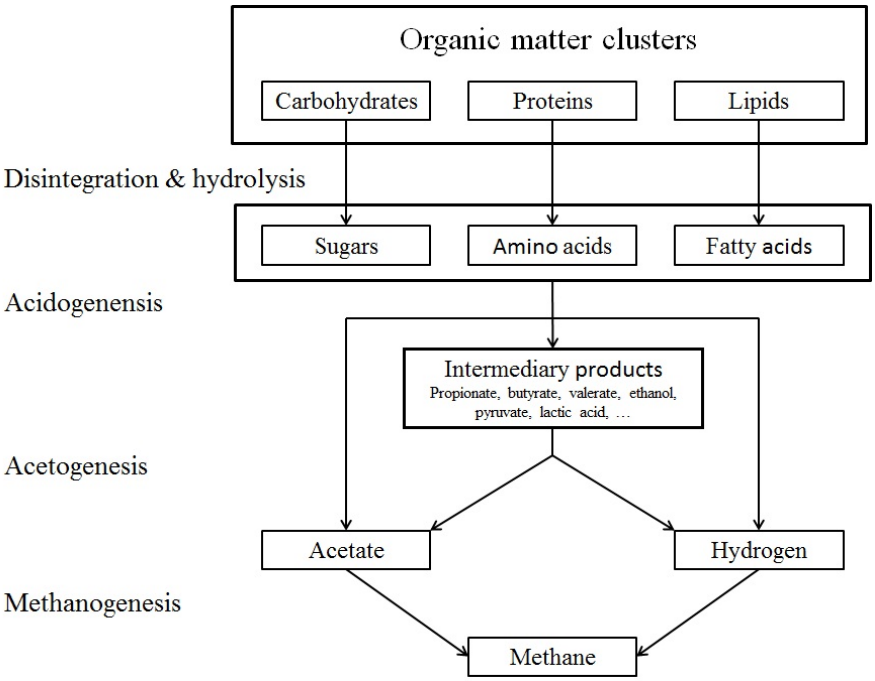


Fig. 1.3. Simplified scheme of the anaerobic degradation pathway (adapted from Batstone et al., 2002)

Disintegration and hydrolysis

Disintegration and hydrolysis step includes non-biological and extra-cellular biological processes mediating the breakdown and the solubilisation of complex organic matter to soluble compounds (Batstone et al., 2002). In this step, the organic matter clusters are disintegrated into macromolecules (i.e. carbohydrates, proteins and lipids) and then, those macromolecules are hydrolysed to soluble compounds. Specifically, the extra-cellular enzymes (cellulases, proteases and lipases) excreted by the fermentative bacteria solubilise carbohydrates, proteins and lipids to mono- and disaccharides (sugars), alcohols, amino acids and long chain fatty acids (LCFA) among others. Many studies have concluded, due to the large fraction of organic matter that must be solubilised before its methanisation, that the disintegration and hydrolysis step is the rate-limiting step of solid and semi-solid wastes (Sanders et al., 2002). However, the solubilisation rate is affected by several parameters such as particle size, pH, temperature, biomass concentration or the intrinsic substrate characteristics (Veeken and Hamelers, 1999).

Acidogenesis

Acidogenesis, also known as fermentation, is carried out by a large group of facultative fermentative bacteria. In this stage, the fastest of the AD process, the soluble compounds obtained from the disintegration and hydrolysis step are able to be transported inside the bacteria and then converted to volatile fatty acids (i.e. acetate, propionate, butyrate, valerate), lactic acid, ethanol, pyruvate, ammonia, hydrogen sulphide, hydrogen and carbon dioxide. It should be noted that the acidogenesis of sugars and amino acids is carried out without an electron acceptor or donor, whereas LCFA are oxidised using hydrogen ions as electron acceptors (Batstone et al., 2002). The main product of all acidogenesis reactions is acetate; however, the accumulation of hydrogen and/or acetate in the digester medium can promote the formation and accumulation of more reduced compounds such as propionate and butyrate.

Acetogenesis

The volatile fatty acids (VFA), excluding acetate, and other products from the acidogenesis stage are converted by obligate hydrogen-producing acetogens to acetate, hydrogen and carbon dioxide, which are appropriate substrate for the methanogenic biomass. It is well known that acetogenesis reactions are only thermodynamically possible when the hydrogen concentration in the digester medium is low. Consequently, the syntrophic relationship between acetongens and hydrogenotrophic methanogens (hydrogen degraders) is of utmost important to regulate the hydrogen concentration and, therefore, the whole digestion process (Batstone et al., 2002).

Methanogenesis

The last stage of the AD process is carried out by methanogenic archaea, which convert the end products of the previous reactions into biogas. The majority of the methane (~70%) is generated by the aceticlastic methanogens, which split the two carbons of the acetate; one is reduced to methane and the other is oxidised to carbon dioxide ($CH_3COOH \rightarrow CH_4 + CO_2$). Two different types of aceticlastic methanogens, mutually exclusive, dominate as function of the ammonia and VFA concentration in the digester medium. Methanosaeta, characterised by its filaments, dominate when the volatile fatty acid and the ammonia concentration are low whereas Methanosarcina, characterised by its clumps, dominate when the volatile fatty acids and the ammonia concentration are

high (Karakashev et al., 2005). Minor methane production (~30%) is produced by hydrogenotrophic bacteria, which used hydrogen as electron donor and carbon dioxide as electron acceptor to produce methane ($4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$). Finally, even been negligible, methyl groups can also be converted to methane ($CH_3OH + H_2 \rightarrow CH_4 + H_2O$).

1.3.2. Main anaerobic digestion environmental and operational factors

As a biological process, AD behaviour and efficiency is highly influenced by the environmental and operational conditions of the system.

Temperature

Temperature is one of the most important physical parameters in AD, since it directly affects the kinetics and the thermodynamics of the reactions as well as the growth and the metabolism of the biomass. As a result, temperature determines the degradation pathway and the biomass dynamics in the digester. Even though, AD can take place between 10 and 65 °C, most digesters are operated under the two optimal ranges: mesophilic (around 35°C) or thermophilic (around 55°C) conditions.

Due to the higher process stability and the lower energy requirements most digesters are, at the present time, operated at mesophilic conditions (Astals et al., 2012b). However, the need to improve the process feasibility, by means of increasing the biogas yield and improving digestate hygienisation has increased the interest on thermophilic conditions. Specifically, thermophilic anaerobic digestion (TAD) offers some potential advantages over the conventional mesophilic anaerobic digestion (MAD) (Duran and Speece, 1997; Appels et al., 2008): an increase of the biological and chemical reaction rates, an increase of the organic matter removal, a higher solubilisation of the particulate organic matter and a better hygienisation. Nevertheless, some drawbacks are unavoidable: an elevated energy requirement for heating the digester, a higher risk of process destabilisation, a poor digestate dewaterability and a higher odour potential.

Hydraulic retention time and organic loading rate

The hydraulic retention time (HRT), the solid retention time (SRT) and the organic loading rate (OLR) are usually used as digesters design parameters. Specifically, HRT

represents the average period of time during which the waste has remained in the digester, whereas SRT represents the average time that the anaerobic biomass has stayed in the system. In digesters without recirculation or supernatant withdrawal the HRT and SRT are equal. However, a recirculation of the digestate may increase the SRT and reduce the risk of biomass washout. The organic loading rate (OLR) is the amount of organic matter introduced in the digester per day and volume of digester. The OLR can be increased/decreased by reducing/increasing the HRT or increasing/reducing the organic matter concentration of the digester feedstock, respectively.

Nutrients

There are many substances, organic and inorganic, which are indispensable for the anaerobic biomass growth and metabolism. Not considering the obvious presence of organic carbon, there is the requirement of nitrogen, phosphorous and sulphur (macronutrients) and several metals (micronutrients) like iron, nickel, cobalt, magnesium, calcium, sodium, selenium, copper, etc. It is important to highlight that the presence of micronutrients is small quantities can stimulate the activity of the anaerobic biomass. However, if a certain limit concentration is surpassed their presence can slow down the growth or even cause severe inhibition (Chen et al., 2008).

pH

Each group of microorganisms, responsible of a different anaerobic step, has a different optimum pH range. Fermentative bacteria can survive in a wide range of pH (4 - 9), although the optimum pH is reported to be around 5 - 6. In contrast, the methanogenic biomass present a narrow survival pH range (6.0 - 8.5), with and optimum around the neutrality (Appels et al., 2008). Since the methanogens are the most sensitive and the key microorganisisms of the process, the digester are design and operated to achieve a pH between 7 and 8. It should be taken into account that a drop in the pH or the accumulation of inhibitory/toxic compounds mainly affects the methanogens activity, whereas the acid-forming bacteria, much more resistant, can still degrade more organic matter. As a result, the acid concentration increases and the methanogens become even more inhibited. This phenomenon can lead to digester failure.

Alkalinity and volatile fatty acids

The alkalinity, or buffer capacity, is the capacity of the digester medium to neutralise the acids formed during the process and, therefore, to mitigate pH changes. The alkalinity of a digester is mainly given by few acid-base pairs, mainly carbon dioxide - bicarbonate, ammonium - ammonia, dihydrogen phosphate - hydrogen phosphate and unionised - ionised volatile fatty acids. Volatile fatty acids, which typically include acetate, propionate, butyrate and valerate, are the main intermediates of the AD process. Therefore, its concentration and evolution is very important to monitor digester performance and stability.

1.3.3. Inhibitors of the anaerobic digestion process

There are many substances that at a given concentration inhibit the anaerobic biomass activity, especially methanogens. However, the reported threshold values vary significantly from one study to another. The differences can be attributed to the characteristics of the substrates, the anaerobic biomass origin, the environmental conditions and the adaptation periods.

Oxygen

Oxygen is an inhibitory compound for acetogens and methanogens, which are strictly anaerobic microorganisms but not for the fermentative bacteria, which are facultative bacteria (i.e. can live either in aerobic or anaerobic conditions). Unintentionally, all AD are exposed to low oxygen doses. However, the fermentative bacteria protect the strictly anaerobes from oxygen exposure since they become aerobic when oxygen is present (oxygen reactions are more energetically favourable) and switch back fermentative when the oxygen level is negligible.

Substrate competition

Sulphate and nitrate in the digester medium are used as electron acceptor by sulphate-reducing and the nitrate-reducing bacteria, respectively. Both groups of bacteria compete with the methanogens and the acetogenic biomass for the substrate, which is converted to carbon dioxide instead of methane. Moreover, sulphate-reducing bacteria convert sulphate to hydrogen sulphide, which is inhibitory for all the microorganisms involved in the anaerobic process.

Ammonia

Ammonia is produced, by biological degradation of the nitrogenous organic matter, in the acidogenesis step and remains in the digester medium in two forms, acid (NH_4^+) and basic (NH_3), which are in equilibrium depending mainly on temperature and pH. Although both forms have been reported as inhibitors of the methanogenic activity, the capacity to diffuse into the cell, causing proton imbalance and/or potassium deficiency made NH_3 the most harmful form (Kayhanian, 1999). However, this inhibition did not lead to a process instability, since the interaction between NH_3, VFA and pH led the AD to an ‘‘inhibited steady state’’, which is a condition where the process is running stable but with lower methane yields (Angelidaki and Ahring, 1994).

Volatile and long chain fatty acids

High VFAs concentrations can cause inhibition to anaerobic microorganisms. Normally, the VFA inhibition is coupled with low pH inhibition, since the undissociated species are the more toxic because of its capacity to diffuse into the cell. Therefore, VFA inhibition is linked with the pH and the alkalinity of the system. Moreover, high acetate concentration inhibits propionate and butyrate acetogenesis as well as aceticlastic methanogenesis, whereas propionate is known to inhibit methanogenesis. Saturated and unsaturated LCFA (such as palmitic, stearic and oleic acids) are inhibitor of the methanogenesis step, mainly affecting acetoclastic archaea. LCFA inhibition is likely to occur when treating fatty wastes. Specifically, LCFA accumulates on the surface of the cell, leading to the cell membrane no longer been able to perform important functions, such as protecting the cell and transportation of materials in and out of the cell (Chen et al., 2008).

Other compounds

There are many other inhibitory compounds, such as cations, heavy metals or xenobiotic compounds. Cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and some heavy metals are indispensable micronutrients for the anaerobic biomass; however, they are inhibitory at high concentrations. Cadmium, chromium, cobalt, copper, iron, lead, mercury, nickel and zinc are reported to be the most toxic heavy metals. Xenobiotics (complex organic compounds), which are released in large quantities due to human activities, includes surfactants (detergents: AES and LAS), solvents (alcohols, ketones, benzene and

toluene), phenols, pesticides (halogentedphenols and nitrophenols), phthalates esters (added to plastics) and medicines (antibiotics) among others (Chen et al., 2008).

1.3.4. Methods for improving anaerobic digestion yields

As aforementioned, many parameters influence the performance of an AD. However, the yields of the process are mainly related with the biomass characteristics, the enzymatic activity and the physical characteristics of the substrate, which manifest themselves through three measurable parameters (Fig. 1.4): methanogenic activity, biodegradability and solubilisation rate (Sanders et al. 2002).

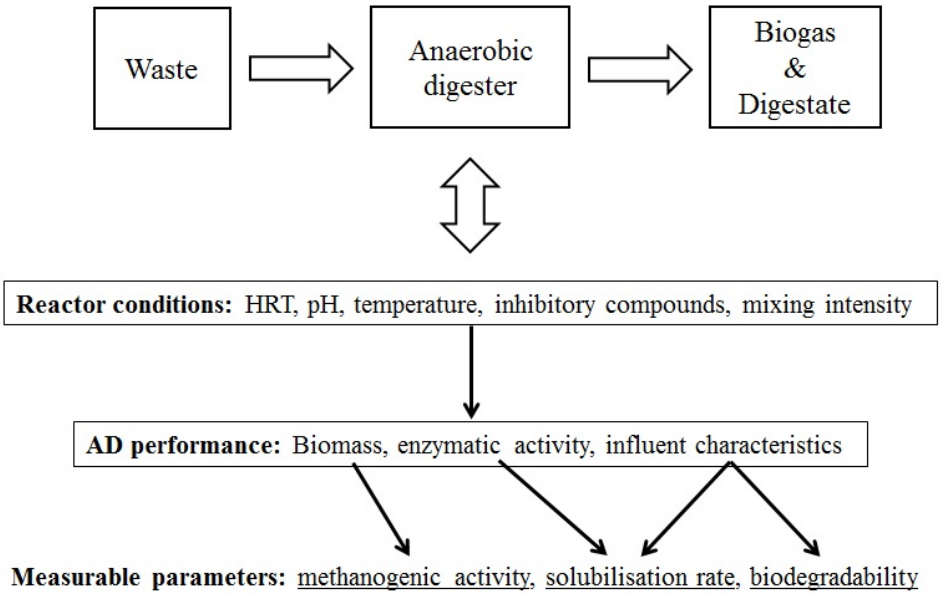


Fig. 1.4. Schematic diagram of the relationship between the reactor conditions, digester performance and measurable parameters (adapted from Sanders et al. 2002)

Therefore, enhancing the AD process performance can be done through the analysis and optimisation of one or more of those three parameters. To be specific, three options are currently available: (i) improve the AD working conditions, (ii) pre-treatments that favour waste biodegradability and solubilisation rate, and (iii) anaerobic co-digestion. The present thesis is devoted to anaerobic co-digestion, a technology that has grown in the last 10 years and that now can be considered the most relevant topic within anaerobic digestion research.

1.4. Anaerobic co-digestion review

Anaerobic co-digestion (AcoD) consists of the anaerobic digestion of a mixture of two or more substrates with complementary characteristics, so that biogas production is enhanced through their joint treatment. Thus, it is not simply the digestion of a mixture of substrates (such as primary and secondary sludge), or of different types of wastes in a municipal solid waste (MSW) digester. When possible, it is very important to choose the best blend ratios in order: (i) to favour positive interactions, i.e. positive synergisms, macro- and micronutrient equilibrium and moisture balance; (ii) dilute inhibitory and/or toxic compounds, (iii) optimise methane production and (iv) enhance digestate stability (Mata-Alvarez et al., 2000; Astals et al., 2011; Alburquerque et al., 2012a). Consequently, the AD process becomes more economically feasible through the application of co-digestion.

Potential inhibition of methanogenesis by ammonia is a well-known problem when digesting wastes with high nitrogen content. For instance, it has been shown that optimum values for the carbon-to-nitrogen (C/N) ratio fall within the range of 20 to 70 for the AD process (Burton and Turner, 2003) but even much lower values (12 to 16) have also been reported (Mshandete et al., 2004). A wide range of inhibiting total ammonia nitrogen (TAN) concentrations have been reported, where differences can be attributed to the characteristics of the substrates and the inoculum, the environmental conditions (mainly temperature and pH) and the adaptation periods (Chen et al., 2008; Cuetos et al., 2008). In any case, the level of methanogenic activity decreases with increasing concentrations of ammonia (Angelidaki and Ahring, 1993; Chen et al., 2008; Hansen et al., 1998). Therefore, the main issue for the AcoD process lies in balancing the C/N ratio, but, as aforementioned, the right combination of several other parameters in the co-substrate mixture, such as macro- and micronutrients, pH and alkalinity, inhibitors and toxic compounds, biodegradable organic matter and dry matter, is also relevant (Hartmann et al., 2003). This more balanced operation achieved by AcoD not only enhances biogas production, but also results in a more stable process (Monou et al., 2008; Cuetos et al., 2008). Other advantages include the possibility of cost-sharing, since the equipment and general infrastructures can be used for several wastes (Macias-Corral et al., 2008); minor modifications are usually required in the AD plant (reception

tank, piping and dosing bomb) to include the majority of co-substrate in the digester feed supply (Fig. 1.5).

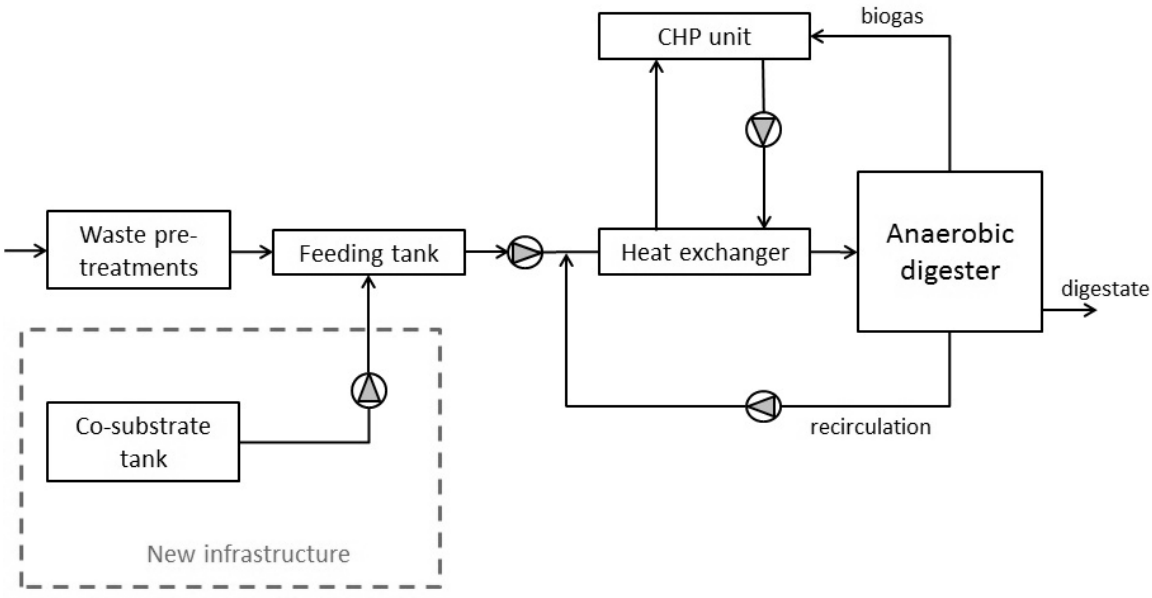


Fig. 1.5. Modifications needed to implement a co-substrate in an existing AD plant

AcoD offers several potential ecological, technological and economic advantages, resulting in improved organic waste treatment through AD. For instance, it is very important for plants treating manures to produce as much biogas as possible, in order to increase the economic viability of the plant. In many cases, biogas production from mixed substrates is higher than the sum of biogas production from substrates digested separately. AcoD can easily increase the methane production of manure digesters by up to 200%, depending on the operating conditions and the characteristics and amount of co-substrates used (Murto et al., 2004; Amon et al., 2006; Ferreira et al., 2007; Soldano et al., 2007; Arhoun et al., 2013). Finally, in addition to the production of renewable energy, using AD to treat organic waste produces greater reductions in greenhouse gases (GHG) emissions than the aerobic options.

1.4.1. Anaerobic co-digestion scenario

Co-digestion is not a new concept. Early references to this procedure using sewage sludge and the organic fraction of municipal solid waste (OFMSW) appeared in the late seventies (Miller et al., 1978), and first reports in the area of agricultural wastes also date from the same period (Hills, 1979; Fujita et al., 1980; Hills and Roberts, 1981;

Fisher et al., 1983; Hashimoto, 1983). Later on, various authors studied the behaviour of different substrate blends (Llabrés-Luengo and Mata-Alvarez, 1987 and 1988; Lo et al., 1988; Kumar et al., 1988; Mtz-Viturtia et al., 1989; Robbins et al., 1989). The number of papers on AcoD published in referred journals has recently grown exponentially (Fig. 1.6).

An analysis of published papers on co-digestion reveals the distribution of the main substrates used: sewage sludge (27%), manures (25%), OFMSW (21%), industrial wastes (13%), crops (5%), agricultural wastes (4%) and animal and meat industry wastes (4%). Fig. 1.7 shows the interrelationship between co-substrates, and in particular, the C/N ratio (the most important parameter in AcoD) together with the percentage of papers which included these co-substrates. As can be seen, the highest percentage of articles on co-digestion dealt with sewage sludge, and these have also become much more frequent in the last years. Many of the authors analysed lab-scale digesters, assessing digestibility of co-digestates, process performance, design aspects, the inhibitory effect of co-digestates and the effect of temperature (Alatriste-Modragon et al., 2006).

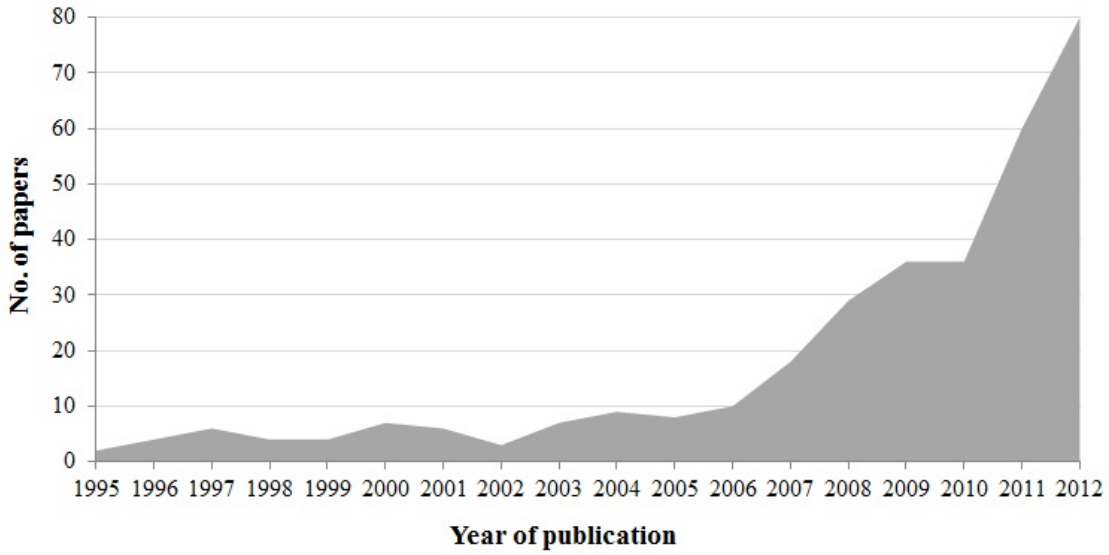


Fig 1.6. Evolution of the number of papers with the word ‘co-digestion’ or ‘codigestion’ in the title per year of publication

The remainder of this review is divided into three parts: The first deals with sewage sludge and the most common co-substrate, the OFMSW. The second part examines the

co-digestion of manures, especially with regard to energy crops and OFMSW. These two parts cover most of the published co-digestion papers. Finally, the third part explores the issue of AcoD modelling.

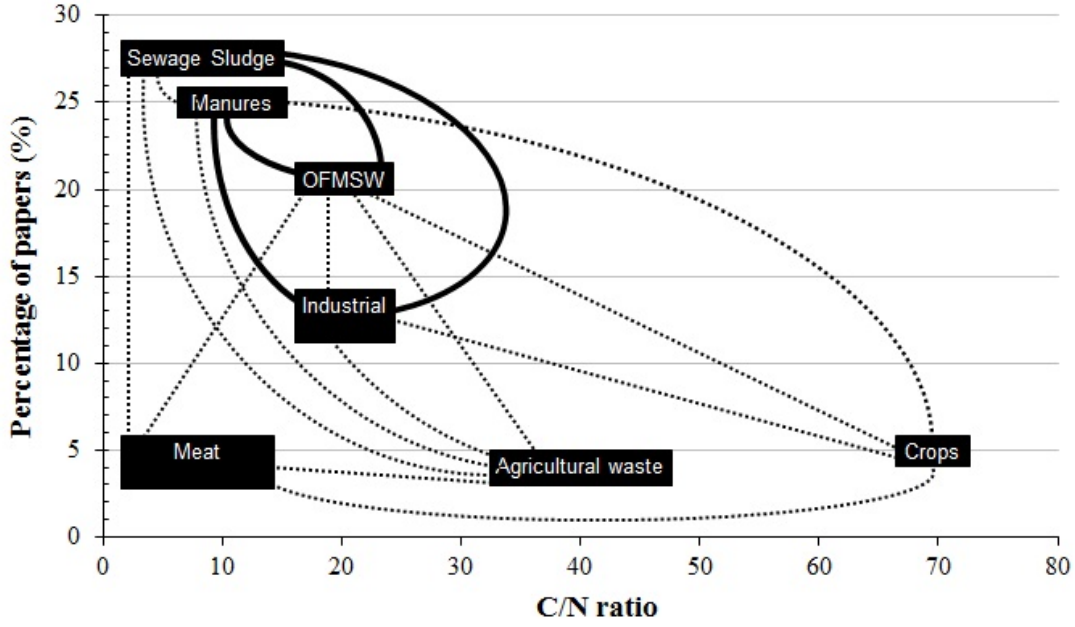


Fig 1.7. Distribution of papers dealing with anaerobic co-digestion as a function of the substrate percentage of use and its C/N ratio. Solid lines link the most reported mixtures while dotted lines link other mixtures

1.4.2. Co-digestion of sewage sludge and OFMSW

Research into co-digestion of sewage sludge (SS) and OFMSW has a relatively long history and, as shown in Fig. 1.7, is the most popular co-digestion research subject, representing 27% of all AcoD papers. Early references concerning this subject date from the seventies and eighties (Ghosh and Klass, 1976; Diaz et al., 1980), and looked at ways to enhance biogas production from landfill cells. Recently, this issue has been reconsidered, looking at the use of SS as a co-substrate, or other wastes with a similar high humidity level such as mixed industrial sludge (Agdad and Sponza, 2005 and 2007) or septic tank sludge (Valencia et al., 2009), to fit the nutrient balance. One of the first and most comprehensive demonstrations that the co-digestion process could be successfully implemented in existing wastewater treatment plants (WWTP), to improve digester performance and thus energetic balance, was carried out by Cecchi et al.

(1988). These authors published a pilot scale study, comparing the performance of different types of OFMSW co-digested with sewage sludges and confirming the interest inherent in this approach. From this point onwards, many papers were published which described different configurations on either a laboratory or pilot scale. For example, Schmit and Ellis (2001) and Sosnowski et al. (2003) focused on the operational advantages of a two-stage and temperature-phased system, while Caffaz et al. (2008) tested two different kinds of source-sorted OFMSW - fruit and vegetable waste and kitchen food waste in a pilot digester. In the OFMSW-SS system, both basic components of the sewage sludge play an important role in co-digestion: the N content of secondary sludge can supplement a possible deficit of nutrients in the other co-substrate (OFMSW), whereas the higher biodegradability of the primary sludge provides an additional contribution to the increase in biogas production potential.

The influence of the mixing regime on AcoD performance of SS and OFMSW has been studied by different authors. It seems that for AcoD, good contact among co-substrates is necessary in order to balance nutrients and other parameters. For instance, Gómez et al. (2006) carried out experiments on high mixing conditions (200 rpm), low mixing conditions (80 rpm) and static conditions. As expected, the results showed a reduction in biogas yield when the reactors were run under static conditions. However, no differences were found when the reactors were run under high or low mixing conditions. This latter study was complemented by that of Stroot et al. (2001) who, using the same co-substrates, demonstrated that reducing the level of mixing improved digester performance, and that therefore continuous mixing was not necessary for good performance and could even be inhibitory at higher loading rates. They concluded that a reduction in mixing levels could stabilise digesters. It would appear that mixing inhibits the syntrophic oxidation of VFA, possibly by disrupting the spatial juxtaposition of syntrophic bacteria and their methanogenic partners (McMahon et al., 2001).

The nutrient problem

Biological nutrient removal is a major concern for WWTP managers. AcoD of SS with OFMSW, as well as with other wastes, increases the nutrient load of the plant. This effect was reported by Nowak et al. (2007), in a paper about the WWTP in Loewen (Austria). This plant is typical of many oversized digesters in WWTP. It has two

digesters (2,500 m^3 each) and one of them was not used. The digestion overcapacity was used by employing the second unit to digest organic wastes (as can be seen, this does not represent a true co-digestion approach). After operating the plant, they set balances for N and detected an increase in this nutrient. Namely, the supernatant of the organic wastes digester showed a level of ammonia which was twice that of the sewage sludge digester. In this case, this extra ammonia load was solved by using the primary settler as a nitrification/denitrification device for the reject water. In Treviso WWTP, the co-digestion of sewage sludge together with other organic substrates led to a digester supernatant rich in nutrients (approximately, 400 mg N L^{-1} of ammonia and up to 100 mg P L^{-1} of phosphates) which was recycled to the wastewater treatment line (Pavan et al., 1998 and 2000).

Many studies have reported a wide range of COD/N ratios required for satisfactory or complete denitrification processes, of between 4 and 15 g COD g^{-1} N. If the COD/N ratio in incoming wastewater to the WWTP is not sufficient for complete biological nutrient removal (BNR), an external carbon source is needed. This external carbon source can be chemical (acetic acid or methanol), primary settled hydrolysed sludge or the anaerobic fermentation products from OFMSW (Cecchi et al., 1994; Pavan et al., 1998 and 2000). In Treviso, the WWTP has a modified Johannesburg configuration, avoiding primary settling in order to preserve COD for nutrient removal. One drawback of this approach is that it creates a higher oxygen demand (5-10 %) and a greater amount of sludge to dispose of (from 2,700 to 4,300 kg day^{-1}). However, the advantages include increased biogas production and the treatment of 20 t day^{-1} of OFMSW.

1.4.3. Co-digestion in the agricultural area

The second most cited co-substrate is manure. As shown in Fig. 1.7, 25% of papers examine this substrate. In fact, the agricultural area is showing renewed interest in biogas technology as well as in other renewable energy sources, due to the need to reduce GHGs, and because of the sector's decentralised nature. However, the real driving force behind this development has been the income it represents through the sale of electricity. Ideal co-substrates for manures (substrate with a high N content and high alkalinity) are agricultural and crop wastes (substrates with a lower alkalinity and a high C/N ratio); although the main co-substrate reported in publications is OFMSW. In many

cases, the reduced biogas yield of manures does not justify the high capital costs for farm-scale plants of manure-only digestion. However, biogas productivity can be dramatically increased by adding energy-rich co-substrates to the anaerobic digester, namely C-rich wastes and especially energy crops (Pavan et al., 2007). Biogas yield from manure digestion typically ranges from 10 to 20 $m^3 t^{-1}$, while the operation is only profitable when biogas yields higher than 30 $m^3 t^{-1}$ of treated material can be achieved (Angelidaki and Ellegaard, 2003).

Co-digestion of manures and energy crops has developed very rapidly in some countries such as Germany or Austria. This precipitate growth has led to inaccurate plant design in the opinion of Lindorfer et al. (2008). To correct this extra capacity, the load of a full-scale two-stage digester (2,000 + 1,850 m^3) was doubled, resulting in a proportional increase in biogas production. The authors also warned of an increase in the residual methane potential of the digestate and higher VFA values. Cavinato et al. (2010 and 2013) reported the importance of the operating temperature in agricultural co-digestion. They recorded an increase from 0.45 to 0.62 $m^3 kg^{-1}$ VS at a full-scale digester in Italy treating a mixture of cattle manure (solid and liquid), maize and fruit-processing wastes, with total solids at the inlet of between 10-12%. This increase was due to an operating temperature correction, changing the temperature from 47 ºC to 55 ºC, which also had a positive effect on biogas methane content (increasing from 52 to 61%). The authors also studied the economics of this type of digester and concluded that with the present rates for electricity in Italy (green certificates) of €0.22 per kWh, the investment return period (AD only) was around 2.5 years, rising to 3.5 years if a nutrient removal step was included due to N restrictions in the soil.

The most frequently reported waste in co-digestion with manures is OFMSW (Ahring et al., 2001; Møller et al., 2004; Mladenovska et al., 2004; Hartaman and Ahring, 2006; Park et al., 2008). This is due to the high biodegradability of OFMSW and its relatively high proportion of solid contents, which enables the digesters to operate at higher OLR. In this way, the low productivities obtained with cattle manures (between 150-240 L CH_4 kg^{-1} VS) or pig manures (in the range of 280-360 L CH_4 kg^{-1} VS), can be increased dramatically (Møller et al., 2004).

One of the countries which pioneered the application of co-digestion at farm level was Denmark, where presently there are around 20 centralised AD plants, treating approximately 1.5 million tons per year of manures, most of them together with other organic wastes, preferably in the thermophilic range (Nielsen and Angelidaki, 2008; Angelidaki and Ellegaard, 2003). Other countries, such as Sweden, also have a significant number of co-digestion plants, with a total of around 200, 10 of them centralised. Co-substrates for manure in these centralised plants come mainly from the food-processing industries, whereas in individual plants they come from crop residues (Lantz et al., 2007). In Sweden, as well as in Germany, it seems that the full utilisation of this energy potential in centralised plants presents difficulties, and therefore individual AcoD plants are more common (Svensson et al., 2005). The problem now seems to be that the volume of easily degradable substrates is not sufficient to satisfy demand, and consequently some plants have begun to import organic waste with high biogas potential. In addition, research has begun into other types of biomass which require more expensive pre-treatment in order to increase biodegradability, such as wet explosion (Wang et al., 2009).

1.4.4. A review of anaerobic co-digestion modelling

Much research has been carried out with the aim of understanding AcoD technology and establishing the effect of mixing two or more individual wastes in a digester. Achieving a successful combination of different types of waste requires careful management, since random or heuristic decisions on the ratio between waste streams or feedstock to full-scale plants often lead to process disturbance and significant reductions in methane production (Zaher et al., 2009). Consequently, the need has arisen for accurate modelling of the anaerobic degradation of waste (Angelidaki et al., 1993). The power of models lies in their capacity to reproduce empirical behaviour on a computer, in a clear and quantifiable manner, where the mathematical equations are able to simulate the physical, chemical and biological processes (Esposito et al., 2008; Galí et al., 2009). Pioneering papers dealing with AcoD modelling appeared in 1996, 1997 and 1999, although the majority were published in recent years, all of the latter been based on ADM1 (Table 1.2).

Table 1.2. Evolution of co-digestion modelling

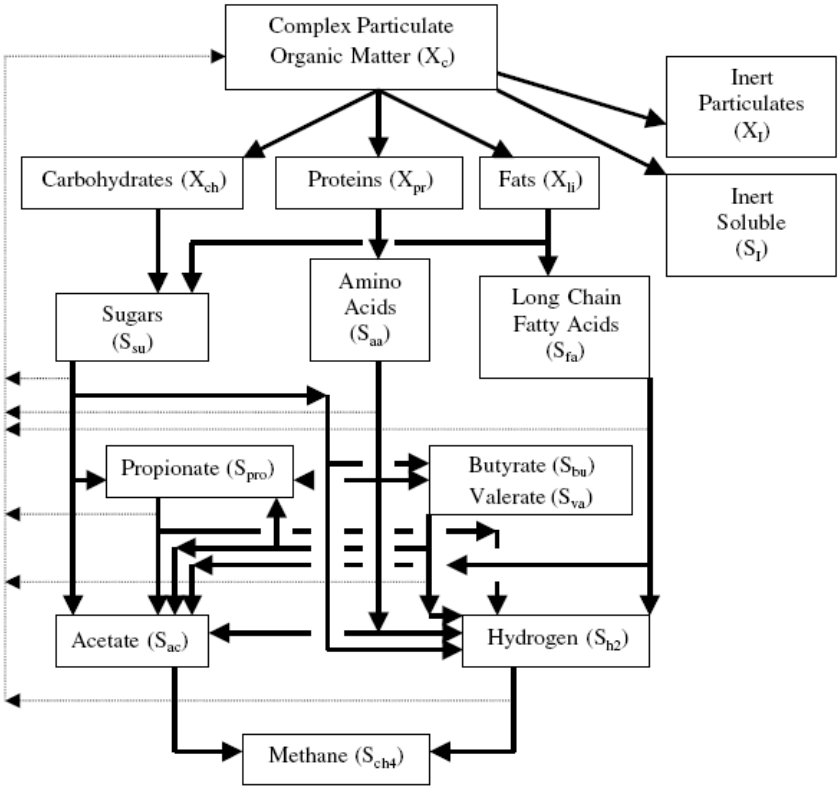
Year	Author	Model	Waste stream
1996	Bozinis et al.	- One Monod kinetics - Many parameters	- Industrial Wastewater
	Gavala et al.	- Four step pathway Three bacterial groups	- OMW, PM, Dairy WW
1997	Kiely et al.	- Two step pathway Two inhibitions: ammonia and acetic acid	- OFMSW and primary sludge
	Angelidaki et al.	- One enzimatic step Six bacterial groups Two inhibitions: ammonia and acetic acid	- OMW and Cattle manure.
1999	Angelidaki et al.,	- One enzimatic step Six bacterial groups Two inhibitions: ammonia and acetic acid	- Manure and glycerol trioleate - Manure with gelatine Manure with proteinous WW
2007	Lübken et al.	- ADM1 including bacteria and methanogens in the inflow	- Cattle manure and energy crops
2008	Fezzani and Ben Cheikh,	- ADM1 including total VFA amount inhibition in the acetate uptake.	- OMW and OMSW
	Espostio et al.	ADM1 including surface based kinetics at hydrolysis step	- OFMSW and sewage sludge
2009	Derbal et al.	- ADM1	- OFMSW and sewage sludge
	Fezzani and Ben Cheikh,	- ADM1 including phenolic compound degradation	- OMW and OMSW
	Galí et al.	- ADM1 including sulphide inhibition in the acetate uptake.	- Combinations of agricultural waste
	Zaher et al.	- ADM1	- Combinations of solid waste

OMSW stands for olive mill solid waste; pig manure; PM stands for pig manure; WW stands for waste water.

Today, some 6% of papers on AcoD address modelling aspects. The first co-digestion modelling study was performed by Bozinis et al. (1996), using an operation model based on a simple uninhibited Monod kinetic model depending on composition of the waste (lipids, proteins and carbohydrates). The authors included an engineering model but many constants were necessary to produce viable results. Another pioneering model was developed by Gavala et al. (1996) for the co-digestion of olive mill wastes (OMW), pig sludge and dairy wastewaters. To carry out the degradation process, this model considers a four step pathway (hydrolysis, acidogenesis, acetogenesis and methanogenesis) and three bacterial groups. Meanwhile, the wastes were defined by a simplified composition: carbohydrates (soluble and insoluble), proteins (soluble and insoluble) and VFA. However, the model could not predict pH and biogas composition nor did it take into account the inhibitory effect by low pH values, high VFA concentration or a shortage of ammonium nitrogen (Fezzani and Cheikh, 2008). Kiely et al. (1997) modelled the results of co-digestion of OFMSW and primary sludge using a two-step model (hydrolysis/acidogenesis and methanogenesis) which introduced ammonia inhibition, affecting the specific growth rate of methanogenic biomass and acetic acid inhibition in the methane production rate. Later on, a more complete model was developed for the degradation of complex organic material. At first, development of this model focused on the AcoD of OMW and cattle manure at thermophilic conditions in a continuous stirred tank reactor (Angelidaki et al., 1997), and involved one enzymatic process, six bacterial groups and three inhibition mechanisms. Further development of the model was based on the AcoD of manure with glycerol trioleate or manure with gelatine (Angelidaki et al., 1999), and evolved to include two enzymatic processes, eight bacterial groups and six inhibition mechanisms. Furthermore, it considered the hydrolysis process, the intermediate compounds derived from the degradation of complex substrates and the digestion inhibition mechanism as key factors for achieving a successful simulation.

A more recent and sophisticated model, the Anaerobic Digestion Model No.1 (ADM1), was developed in 2002 by the International Water Association (IWA) task group for mathematical modelling, where complex substrates are described by their complete organic and inorganic composition (Batstone et al., 2002). Fig. 1.8 shows the ADM1

pathways, with an indication of the number of variables, equations and other parameters of the model.



Process equations	**Inhibitions**	**Variables**
19 Biochemical	- pH	12 particulate (X_i)
- 4 for particulate matter degradation	- Lack of inorganic nitrogen	18 soluble (S_i)
- 8 for soluble matter degradation	- H_2 to fatty acids degradation	3 Gases
- 7 for biomass concentration	- H_2 to butyric/valeric degradation	
6 Equilibrium acid/base	- H_2 to propionic degradation	
3 Gas transfer (CH_4, CO_2, H_2)	- NH_3 to acetate degradation	

Fig 1.8. ADM1 biochemical processes (adapted from Batstone et al., 2002)

The ADM1 was designed to be easy and extendible; as a result of the improvements, additional functions work very well, and are easily documented (Batstone et al., 2006). Since its development in 2002, these advantages have been demonstrated in practice. The ADM1 has been tested and used on different substrates, as reflected in the large number of related research papers reported in the literature (Derbal et al., 2009). Nevertheless, the next papers to address co-digestion using the already established

ADM1 were published more than five years after its appearance. Furthermore, all of them considered the two following premises when the model runs under co-digestion conditions: (i) the ADM1 model component for composites cannot be used as an inflow fraction, and substrate characterisation should be in terms of carbohydrates, proteins and lipids (Lübken et al., 2007; Fezzani and Cheikh, 2008; Galí et al., 2009; Zaher et al., 2009), and (ii) the disintegration/hydrolysis step is generally considered the rate-limiting step during the degradation of particulate organic matter (Lübken et al., 2007; Derbal et al., 2009; Galí et al., 2009; Zaher et al., 2009).

Lübken et al. (2007) applied ADM1 to simulate energy production by co-digesting cattle manure and energy crops. The model inflow characterisation included the content of proteins, lipids, carbohydrates, inert particles and biomass, where the active biomass content in the fed manure was 4.2% of total COD for anaerobic bacteria and 0.05 of total COD for methanogens. This last value, and the different hydrolysis constant for each single fraction, had a negligible influence on achieving the higher biogas production predicted. Other papers dealing with co-digestion modelling were published by Fezzani and Cheikh (2008), who reported a satisfactory simulation, after optimising some of the kinetic and stoichiometic parameters and using OMW and olive mill solid waste (OMSW) as substrates in a semi-continuous tubular digester at mesophilic (Fezzani and Cheikh, 2009a) and thermophilic conditions (Fezzani and Cheikh, 2009b). Their model included a slight modification to predict reactor failure at short HRTs. More precisely, the inhibition factor applied to the rate of acetate uptake was modified to take into account inhibition of the methanogenic step by the total amount of VFA. Moreover, in 2009 the same authors published an extension of ADM1 to include phenol compound biodegradation (Fezzani and Cheikh, 2009a and 2009b). Incorporating the phenol degradation process, first into benzoate and then into acetate, in ADM1 required the addition of five state variables, six phenol conversion processes and the corresponding inhibitory effect of phenolic compounds on the rate of acetate uptake.

ADM1 has been applied in two studies to simulate the co-digestion of OFMSW with sewage sludge at a WWTP (Esposito et al., 2008; Derbal et al., 2009). Esposito et al. (2008) focused their research on the disintegration process. More specifically, sludge degradation was modelled according to ADM1, while surface-based kinetics was used

to simulate the OFMSW disintegration process, in order to reproduce the particle size distribution effect on the process. Derbal et al. (2009) reported a unique ADM1 co-digestion simulation case using a full-scale reactor. The data obtained from their research indicated that ADM1 is a useful tool for assisting in system operation and controlling a full scale anaerobic digester.

Finally, two powerful simulation tools for the AcoD of multiple waste combinations and based on ADM1 have recently been reported by Galí et al. (2009) for agricultural wastes and Zaher et al. (2009) for numerous solid wastes. Both models were developed in MATLAB/SIMULINK, where the practical information is taken from Excel files, although the Simulink schemes are slightly different. On the one hand, the model developed by Galí et al. (2009) can be operated for one or two stirred reactors in series, which can be filled separately either continuously or semi-continuously, and the number of substrates included can be chosen indiscriminately for each of the reactors. On the other hand, the model developed by Zaher et al. (2009) divides the solid anaerobic degradation process into an enzymatic hydrolysis phase and an uptake phase of the hydrolysis product in ADM1 node. Moreover, the Galí et al. (2009) model implemented H_2S in liquid and gas phases and the inhibitory effect of this compound on the rate of acetate uptake, whereas the Zaher et al. (2009) model only needed eleven characteristics as model inputs.

1.5. Digestate final destination

As mentioned before, the EU legislation is trying to promote the recycling of the organic matter through biological process and diminish the amount incinerated or dumped in a landfill. Currently, the combination of AD and composting seems the best option to recover energy and material from the organic wastes. The present scenario is a result of the AD plants operation, where the prevalence of efficiency criteria for biogas production instead of digestate stability, lead to low HRT of the material in the digester and consequently a digestate that is not completely exhausted in terms of easily biodegradable organic matter. The quality of the digestate and, therefore, its recycling options are a result of three main factors: feedstock origin, digestion process and digestate post-treatment (Holm-Nielsen et al., 2009).

The addition of a co-substrate in the feedstock is an important factor to take into account, since it represents a decrease of the HRT or an increase of the OLR and, therefore, it is likely to obtain a less stabilised digestate (Astals et al., 2012a). In this field, industrial wastes, like food processing or pharmaceutical wastes, can dilute the heavy metals and pathogen concentration in the digestate, whereas the addition of sewage sludge or animal manure can raised the need for effective sanitation procedures during the operation of AD or composting plant. At the present time, there is not an EU unified legislation for AcoD digestates; even though the 2nd draft of the biological treatment of biowaste and the the 3rd draft for the unrestricted use of sludge in agriculture (Environment DG, EU, 2000 and 2001) can be used as reference.

The use of digestate/compost derived from the anaerobic digestion into the soil may depend on: (i) Chemical properties; although the only restriction limit is the one stabilised by the nitrate directive (Directive 91/676/EEC), from an agricultural point of view is very interesting to consider other parameters like pH, conductivity, density, nutrient content, etc. (ii) Stability and maturity: the use unstable compost/digestate can cause N-immobilisation and/or oxygen exhaustion because of an excessive increase in soil microbial activity. However, there is little agreement about which methodology (dynamic or static respirometric index, dissolved organic carbon or VFA concentration, residual biogas potential) should be used to determine digestate stability. (iii) Hygienisation: because most wastes are known to contain pathogens (Salmonella, E.coli, etc.), the digestate must be safe for people and animals in order to be recycled. Otherwise, new ways of transmission of pathogens between people and animals could be established (Sahlstrom, 2003). Thermophilic digestates are known to fulfil the EU higienisation requirements, whereas mesophilic digestates have to be pasteurised or composted prior its use in land (Astals et al., 2012b). (iv) Heavy metals and inerts: heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) concentration can limit or even prohibit the use of a digestate on land, since they accumulate on plants, animals and soil. The presence of inerts (sand, glass, plastics, etc.) must be avoided.

2. Objectives and thesis structure

2.1. Motivation and objectives

As stated in Chapter 1, the implementation of the Landfill Directive and the Waste Framework Directive among others, created a favourable scenario for the growth and further development of the anaerobic digestion in the European Union. At the present time, anaerobic digestion can be considered a completely mature technology. Since anaerobic digestion plants economic feasibility is directly linked with the biogas potential of the treated waste, many research efforts have been done in order to improve biogas yields and/or mitigate inhibitory mechanisms. Anaerobic co-digestion, the anaerobic digestion of two or more wastes, has stood out during the last years as the most relevant option to improve digester yields and plants incomes through electricity sells. However, at present, there is very little knowledge about interactions between substrates that may enhance or attenuate inhibition, rate, or potential. Knowledge about the interaction between wastes can be used to better understand the AcoD and therefore, to improve co-substrate selection and dose. Additionally, little attention has been paid to digestate quality, although both biogas and digestate have to be managed in an appropriated ways in order to make plants sustainable in the long term. Finally, anaerobic co-digestion modelling is required to predict, in a clear and quantifiable manner, the effect of mixing two or more wastes in a digester and remove potentially negative impacts from mixing based on random or heuristic decisions. Moreover, models can also be used to estimate important biochemical parameters such as biodegradability, degradation rate and inhibition constant, which are critical in AD design, performance and troubleshooting.

These considerations are the motivation of the present thesis, which deals with the evaluation, optimisation and modelling of the anaerobic co-digestion of some urban, farming and industrial wastes. To reach this general objective, the following specific goals were proposed:

- To perform an extended physico-chemical characterisation of the wastes under study as well as determining their biodegradability and degradation kinetic.

- To develop a methodology to provide parameters, coefficients and state variables for anaerobic digestion modelling based on the Anaerobic Digestion

Model No.1, developed by the IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes.

- To identify the interactions (synergisms and antagonisms) between wastes that take place during anaerobic co-digestion, focusing on process kinetics and the anaerobic biodegradability of the wastes.

- To determine the influence of the co-substrate addition in a continuous digester in terms of process performance and digestate stability.

- To compare the feasibility (biogas and digestate) of an anaerobic co-digester at mesophilic or thermophilic conditions when using the same main substrate and co-substrate.

- To compare experimental and modelling results in order to check the robustness and the accuracy of the developed models.

2.2. Thesis structure

Chapter 1: Introduction

This chapter provides a general introduction regarding the main issues included in the thesis. Firstly, a description of the current scenario within the European Union. Secondly, a description of the anaerobic digestion basic concepts. Finally, a review about anaerobic co-digestion research from 1980s until today.

Chapter 3: Materials and Methods

In this chapter, the biological reactors (discontinuous and continuous) and the analytical methods used to perform the experimentation are detailed.

Chapter 4: Anaerobic digestion of sewage sludge: a biodegradability and modelling study

In this chapter, several mixed sewage sludges were evaluated in order to clarify the literature uncertainty with regard to the sewage sludge characterisation and biodegradability. Moreover, a methodology is provided to determine the Anaerobic

Digestion Model No. 1 parameters, coefficients and initial state variables as well as a discussion about the accuracy of the first order solubilisation constant.

Chapter 5: Identification of synergistic impacts during anaerobic co-digestion of organic wastes

In this chapter, the interaction (synergisms and antagonisms) that take place between substrates during anaerobic co-digestion was evaluated. To be specific, the chapter evaluates the role of carbohydrates, protein and lipids in co-digestion behaviour separately, and together. Modelling was used in order to show in a clear and quantifiable way the conclusions.

Chapter 6: Co-digestion of pig manure and glycerol: experimental and modelling study

In the present chapter, the feasibility of co-digesting pig manure and synthetic glycerol as well as to define the effect originated by the nitrogen limitation when large amounts of glycerol were supplied was evaluated. Finally, a modified model based on Anaerobic Digestion Model No. 1 was used to simulate the methane production profiles for the mixtures tested and compare both experimental and modelling results.

Chapter 7: Co-digestion of sewage sludge and glycerol: synergism and inhibition mechanisms

Similarly to chapter 6, the synergism and inhibitory mechanisms when sewage sludge and crude glycerol are co-digested were determined. Additionally, in the present chapter, nonlinear parameter estimation was used in order to estimate biodegradability, kinetic and inhibition parameters and to better support the conclusions.

Chapter 8: Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: biogas and digestate

In this chapter, pig manure and crude glycerol were co-digested at mesophilic conditions (37ºC) in a continuous stirred tank reactor. Co-digestion results were compared with the ones obtained in the reference digester, only fed with pig manure. However, not only standard parameters were monitored but also protein, lipids,

carbohydrates and fibers. Finally, the stability of both digestates was evaluated through a respirometric assay.

Chapter 9: Thermophilic co-digestion of pig manure and crude glycerol: process performance and digestate stability

Similarly to chapter 8, pig manure and crude glycerol were co-digested in a continuous stirred tank reactor but, this study at thermophilic conditions (55ºC). Co-digestion results were also compared with the ones obtained in the reference digester, only fed with pig manure. Finally, a comparison between mesophilic and thermophilic pig manure and crude glycerol co-digestion was done in order to compare both processes viability.

Chapter 10: Conclusions and recommendations

In this chapter, the general conclusions extracted from this work are compiled. Moreover, recommendations for further research are proposed.

3. Materials and methods

3.1. Analytical methods

Analyses were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005) in the laboratories of the Department of Chemical Engineering of the University of Barcelona (UB), the scientific-technical services of the University of Barcelona and the Advanced Water Management Centre (AWMC) at the University of Queensland. The total fraction analyses were determined directly from the raw samples, whereas for the analyses of the soluble fraction, the samples were centrifuged (1,252 x g for 10 minutes at the UB and 9,500 x g for 5 minutes at the AWMC) before the supernatant was filtered through a 0.45 μm filter.

Analytical procedures at the University of Barcelona

The analyses carried out at the Department of Chemical Engineering were:

- Total solids (TS) and volatile solids (VS) were determined following the guidelines given by the Standard Methods 2540G, whereas the Standard Methods 2540D and 2540E were used to determine total suspended solids (TSS) and volatile suspended solids (VSS), respectively.
- Total chemical oxygen demand (CODt) and soluble chemical oxygen demand (CODs) were determined following 5220D Standard Methods procedure. Particulate chemical oxygen demand (CODp) is the difference between CODt and CODs.
- The 5-day biochemical oxygen demand (BOD_{5d}) was determined, with a WTW Oxitop® measuring system, following the 5210D Standard Methods procedure.
- Dissolved organic carbon (DOC) and inorganic carbon (IC) were measured by means of a Shimadzu 5055 TOC-V_{CSN} TOC analyser.
- Total (TA) and partial (PA) alkalinity were determined, using a Crison 5014T pH probe, by a titration method at pH 4.3 and at 5.75, respectively. The intermediate alkalinity (IA) was the difference between TA and PA (2320B - APHA, 2005).
- Individual VFAs (acetate, propionate, iso-butyrate, n-butyrate, iso-valerate and n-valerate) were analysed by a HP 5890-Serie II gas chromatograph equipped with a capillary column (Nukol™) and a flame ionisation detector.

- The anions (F^-, Cl^-, PO_4^{3-}, SO_4^{2-}) and the cations (Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) were determined in an 863 Advanced Compact Metrohm ionic chromatograph using Metrosep columns.
- The sample conductivity was measured by a Crison 5070 conductivity probe.
- Total ammonia nitrogen (TAN), i.e. free ammonia ($N\text{-}NH_3$) plus ammonium ($N\text{-}NH_4^+$), and the total Kjeldahl nitrogen (TKN) were measured according to Standard Methods procedures 4500-NH_3D and 2500-NorgB, respectively. The free ammonia concentration was calculated by means of Anthonisen et al., 1976, which considers TAN concentration, temperature, and pH.
- Protein was estimated by multiplying the organic nitrogen (TKN minus TAN) by 6.25 (Galí et al., 2009).
- Lipids were analysed following Standard Methods procedure 5520E.
- Carbohydrates were estimated by subtracting the amount of protein, lipids and from VS (Galí et al., 2009), while fibers (i.e. cellulose, hemicellulose and lignin) were determined according to the Goering and Van Soest (1970) procedure.
- The capillary suction time (CST), used to determine the sample dewaterability, was determined with a Tritron Electronics Ltd. 304M CST analyser.
- Biogas composition was analysed by a Shimadzu GC-2010+ gas chromatograph equipped with a thermal conductivity detector.

The analyses carried out at the scientific-technical services of the University of Barcelona were:

- Elemental organic components of the particulate fraction were determined with an 1108 CHNS-O from Carlo Erba Instruments equipped with a thermal conductivity detector.
- The sludge particle size distribution was determined by a LS 13 320 Beckman Coulter laser diffraction particle size analyser, which can detect particle size from 0.02 up to 2,000 μm. The particle size distribution was obtained from the second run after an elapsed time of 60 seconds; the instrument pump was set at 70%.

Analytical procedures at the Advanced Water Management Centre

- TS and VS were measured, as at the UB, according to standard methods procedures 2540G (APHA, 2005).
- CODt and CODs were measured using Merck COD Sprectroquant® test, range 500-10000 mg L^{-1}, and by a SQ 118 spectrophotometer (Merck, Germany).
- VFAs (acetic, propionic, butyric and valeric) and ethanol were analysed by an Agilent 7890A gas chromatograph equipped with a Phenomenex ZB-FFAP column.
- Ions (Cl^-, NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-}) were determined in an Dionex ICS-2000 ion chromatograph using a IonPac® AS18 column.
- Total Kjeldahl nitrogen (TKN) and phosphorous (TKP) were determined, using a Lachat Quik-Chem 8000 flow injection analyser.
- Total and soluble protein was determined using the bicinchoninic acid method (Smith et al., 1985).
- Oil and grease were determined by a Wilks Enterprise, Inc. InfraCal TOG/TPH analyser, where S-316 was used as extraction solvent.
- Total and soluble carbohydrates were analysed by the anthrone method (Herbert et al., 1971).
- Total metals where analysed by an inductively coupled plasma optical emission spectrometer (ICP-OES) Perkin Elmer Optima 7300 DV.
- Biogas composition was determined using a PerkinElmer Autosystem 1022 Plus gas chromatograph equipped with a thermal conductivity detector.

3.2. Experimental devices

Two types of anaerobic assays have been carried out: (i) discontinuous assays or biomethane potential (BMP) tests, and (ii) continuous assays in laboratory stirred tank reactors (CSTR).

3.2.1. Biomethane potential test

In this study, three different performances have been used to carry out BMP test (two at the UB and one at the AWMC). However, all of them were done following the stages defined by the German Standard Procedure VDI 4630 (2006) and by Angelidaki et al.

(2009). A detailed description of each performance is given in the correspondent chapter. Next, the current UB procedure is described.

The BMP tests were carried out in 115 mL serum bottles. The bottles were filled in with 50 mL of inoculum, the amount of sewage sludge that met a $VS_{substrate}$-to-$VS_{inoculum}$ ratio of 0.5, and deionised water, used to adjust the same effective volume for all tests (80 mL). The blank assay, only filled with inoculum and deionised water, was used to determine the background effect of the inoculum. In order to deplete the residual biodegradable organic matter the inoculum was degasified at 37 ºC during 1 week (Angelidaki et al., 2009). Later on and before closing the bottles, all digesters medium were flushed with nitrogen for one minute (3L min^{-1}). The bottles were closed with PTFE/Butyl septums, which were fixed by an aluminium crimp cap. Finally, the digesters were placed in a water bath set at mesophilic conditions (37±1 ºC) and mixed twice a day.

Fig. 3.1. BMP serum bottles and vacumeter used in this study

The biogas production during the running test was measured, after discarding the overpressure generated during the first hour, by using a vacumeter (Ebro – VAM 320). At each sample event, the methane content of the biogas accumulated in the bottle headspace was analysed by a GC analyser. The methane production in the course of time was obtained by multiplying the biogas production, once subtracted the vapour

pressure and converted at standard temperature and pressure conditions (i.e. converted to 0 ºC and 1 atm), by the percentage of methane in the biogas. All tests and blanks were carried out in triplicate, and all error bars indicate 95% confidence in the average of the triplicate.

3.2.2. Continuous stirred tank reactor

Two identical 5.5-L continuous stirred tank reactors with a working volume of 4-L were used. Both reactors were equipped with a pH probe (Metter Toledo HA405). The biogas production was measured and recorded with an on-line biogas measuring device (Ritter MGC-1). Biogas production was converted to standard temperature and pressure conditions (0 °C, 1 atm). The operational temperature, 37 ºC at mesophilic conditions and 55 ºC at thermophilic conditions, was ensured by circulating water from a heated water bath (HUBER 118A-E) through a jacket surrounding the reactor. The digester medium was continuously stirred at 60 rpm. The hydraulic retention time (HRT) was set at 20 days at mesophilic conditions and at 15 days at thermophilic conditions. Under steady-state conditions, the reference digester (D1) was fed only with pig manure while the co-digestion digester (D2) was supplied with a mixture of pig manure and glycerol, on a wet-basis (w/w). The digesters were manually fed and purged once a day, and the co-digestion mixture was daily prepared before the feeding in order to avoid uncontrolled degradation.

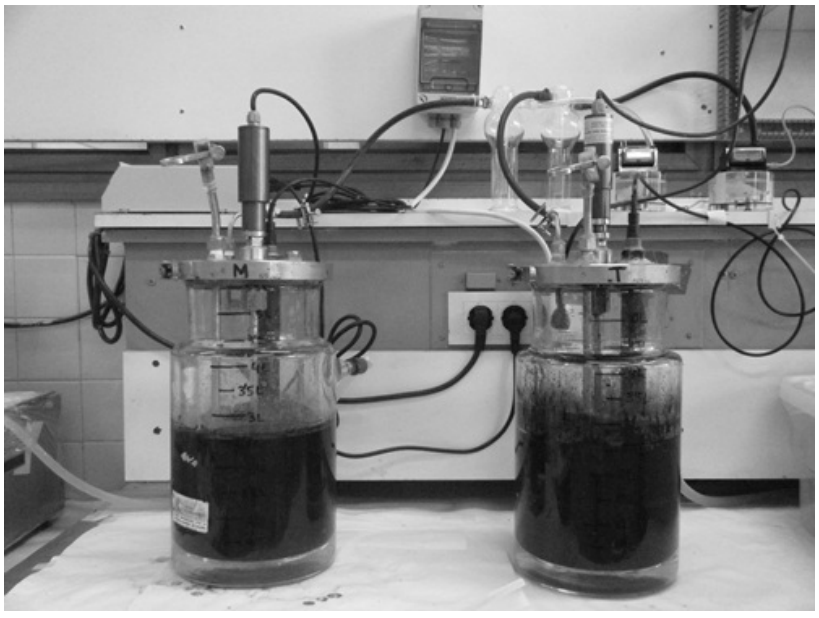


Fig. 3.2. Laboratory continuous stirred tank reactors used in this study

3.3. University of Barcelona anaerobic digestion model

The model developed by the Environmental Biotechnology Group of the University of Barcelona (UB-model) to simulate the anaerobic digestion process is based in the ADM1 (Gali et al., 2009). The UB-model, which was developed within the framework of the Agrobiogas project (The sixth framework programme of the European Comission), was modified to simulate AD and AcoD of agricultural and industrial wastes. The present model is formed by 32 processes (20 biological processes, 8 equilibrium processes and 4 gas transfer processes) instead of the 28 process included in the ADM1 (Table 2.1). Furthermore, 41 dynamic state compounds, divided into 24 soluble (S_i), 13 particulate (X_i) and 4 gas compounds (G_i) are considered and, therefore, simulated (Table 2.2). Fig 2.1 presents the Petersen matrix where all the processes (Table 2.1) and compounds (Table 2.2) are detailed. As can be seen in Fig. 2.1, the model is build up in order to guarantee total elemental mass (i.e. C, N, P) and charge continuity for all transformation involved in the anaerobic digestion process. As a result, some components act as source-sink or compensation terms, which allow a better monitoring of alkalinity, phosphates, nitrogen compounds and pH (de Gracia et al., 2006). When proceeding like this, it is possible to control and follow in a continuous way the alkalinity, the phosphates concentration, the TKN, and finally the pH with the proton concentration (Gali et al., 2009).

Model implementation

The UB-model is developed in Matlab/Simulink, with the code written in C language. The kinetic and intrinsic waste data, such as substrate characterisation, stoichiometry and kinetics, is taken from a Microsoft Excel file which, afterwards, is reported to Matlab. Simulink acts as flow sheet diagram software where the different units (reactors) are connected with the influent flow-rates. Fig. 2.2 show the scheme of the model when it is operated by one or two indistinct stirred reactors. Furthermore, both reactors can be filled separately in a continuous or semi-continuous way and the number of substrates included can be chosen indistinctly for both reactors. The code, which is written in C language, includes the different differential equations used to solve the evolution of each compound or variable. At the same time, the variables of the model and the important engineering aspects (COD removal, biogas production, etc.) are defined and connected with Matlab through Simulink. In order to perform the

connection, the C files must be compiled and converted to MEX files. The latter files, which include the simulation results, can be easily read with Matlab/Simulink and/or copied to an excel file.

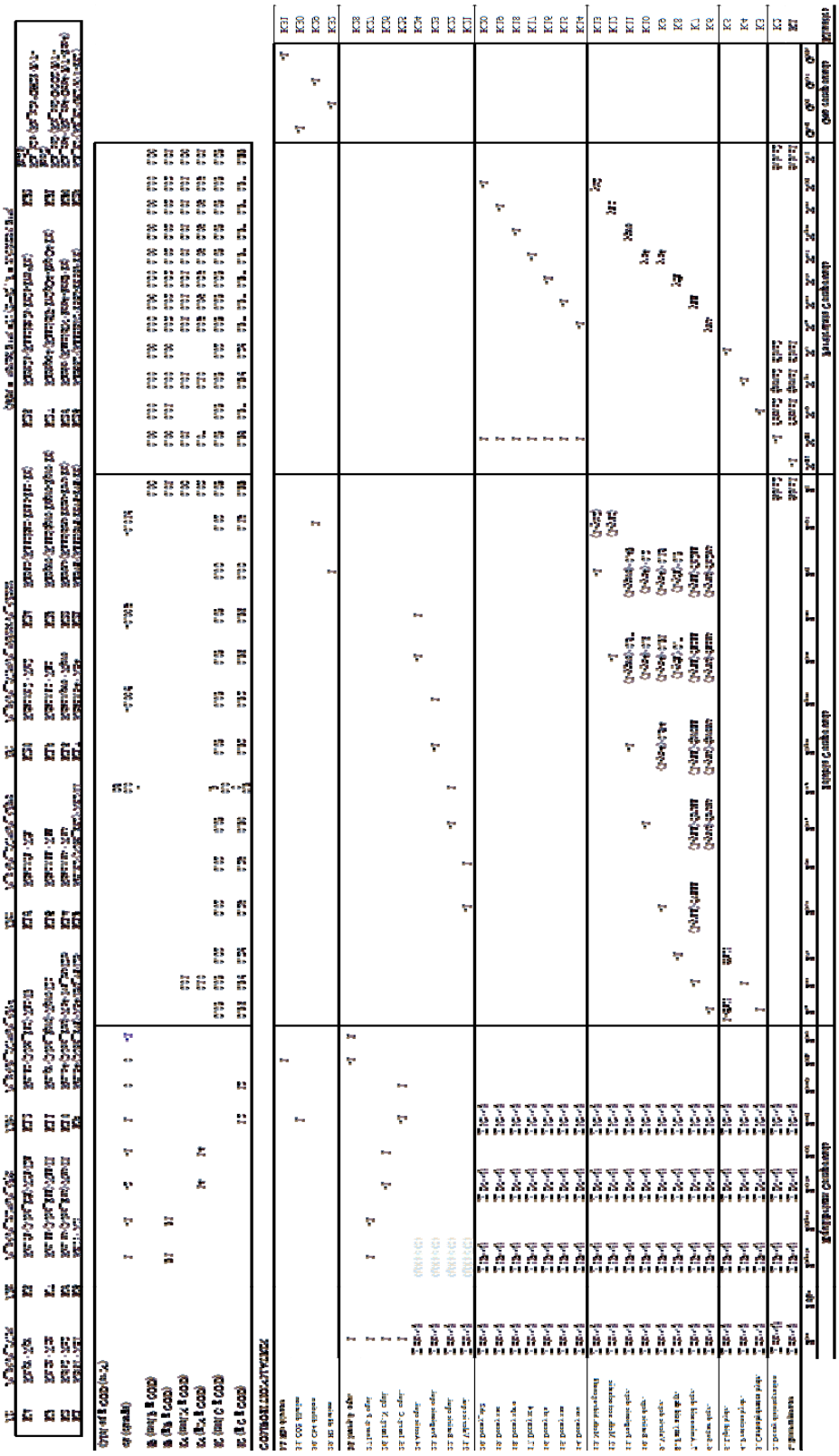


Fig. 3.3. UB-model Petersen matrix (Gali et al., 2009)

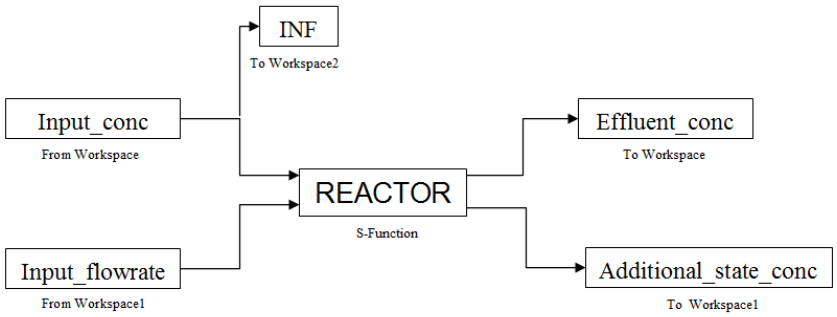
Table 3.1. Process included in the UB-model

Process	Description
1	Disintegration of the particulate materials from waste
2	Disintegration of the particulate materials from death biomass
3	Hydrolysis of carbohydrates produced in disintegration
4	Hydrolysis of proteins produced in disintegration
5	Hydrolysis of lipids produced in disintegration
6	Fermentation of sugars
7	Fermentation of amino acids
8	Fermentation of long chain fatty acids
9	Degradation of the valeric acid
10	Degradation of the butyric acid
11	Degradation of the propionic acid
12	Conversion of acetate to methane (Acetotrophic methanogenesis)
13	Conversion of hydrogen to methane (Hydrogenotrophic methanogenesis)
14	Lysis of sugars degraders biomass
15	Lysis of aminoacids degraders biomass
16	Lysis of long chain fatty acids degraders biomass
17	Lysis of valeric and butyric degraders biomass
18	Lysis of propionic degraders biomass
19	Lysis of acetic degraders biomass
20	Lysis of hydrogen degraders biomass
21	Equilibrium of valeric acid
22	Equilibrium of butyric acid
23	Equilibrium of propionic acid
24	Equilibrium of acetic acid
25	Equilibrium of inorganic carbon
26	Equilibrium of inorganic nitrogen
27	Equilibrium of inorganic phosphorus
28	Equilibrium of inorganic sulphur
29	Dissolution of hydrogen
30	Dissolution of methane
31	Dissolution of carbon dioxide
32	Dissolution of hydrogen sulphide

Table 3.2. Compounds considered in the UB-model

Compound	Units	Description
S_{h+}	kmole m^{-3}	Proton concentration
S_{oh-}	kmole m^{-3}	Hydroxyl concentration
S_{hpo42-}	kmole m^{-3}	Hydrogen phosphate concentration
S_{h2po4-}	kmole m^{-3}	Dihydrogen phosphate concentration
S_{nh4+}	kmole m^{-3}	Ammonium concentration
S_{nh3}	kmole m^{-3}	Free ammonia concentration
S_{co2}	kmole m^{-3}	Bicarbonate concentration
S_{hco3-}	kmole m^{-3}	Carbon dioxide concentration
S_{h2s}	kmole m^{-3}	Sulphide acid
S_{hs-}	kmole m^{-3}	Hydrogen sulphide
S_{su}	kg COD m^{-3}	Soluble sugar concentration
S_{aa}	kg COD m^{-3}	Soluble aminoacids concentration
S_{fa}	kg COD m^{-3}	Soluble large chain fatty acids concentration
S_{hva}	kg COD m^{-3}	Valeric acid concentration
S_{va-}	kg COD m^{-3}	Valerate concentration
S_{hbu}	kg COD m^{-3}	Butyric acid concentration
S_{bu-}	kg COD m^{-3}	Butyrate concentration
S_{hpro}	kg COD m^{-3}	Propionic acid ammonia concentration
S_{pro-}	kg COD m^{-3}	Propionate concentration
S_{hac}	kg COD m^{-3}	Acetic acid concentration
S_{ac-}	kg COD m^{-3}	Acetate concentration
S_{h2}	kg COD m^{-3}	Hydrogen concentration
S_{ch4}	kg COD m^{-3}	Methane concentration
S_i	kg COD m^{-3}	Soluble inert concentration
X_{c1}	kg COD m^{-3}	Composite concentration from waste
X_{c2}	kg COD m^{-3}	Composite from death biomass concentration
X_{ch}	kg COD m^{-3}	Carbohydrate concentration
X_{pr}	kg COD m^{-3}	Protein concentration
X_{li}	kg COD m^{-3}	Lipid concentration
X_{su}	kg COD m^{-3}	Sugars degraders concentration
X_{aa}	kg COD m^{-3}	Amino acids degraders concentration
X_{fa}	kg COD m^{-3}	LCFA degraders concentration
X_{c4}	kg COD m^{-3}	Valerate and butyrate degraders concentration
X_{pro}	kg COD m^{-3}	Propionate degraders concentration
X_{ac}	kg COD m^{-3}	Acetate degraders concentration
X_{h2}	kg COD m^{-3}	Hydrogen degradation concentration
X_i	kg COD m^{-3}	Particulate inert concentration
G_{co2}	kg COD m^{-3}	Carbone dioxide gas concentration
G_{h2}	kg COD m^{-3}	Hydrogen gas concentration
G_{ch4}	kg COD m^{-3}	Methane gas concentration
G_{h2s}	kg COD m^{-3}	Hydrogen sulphide concentration

A



B

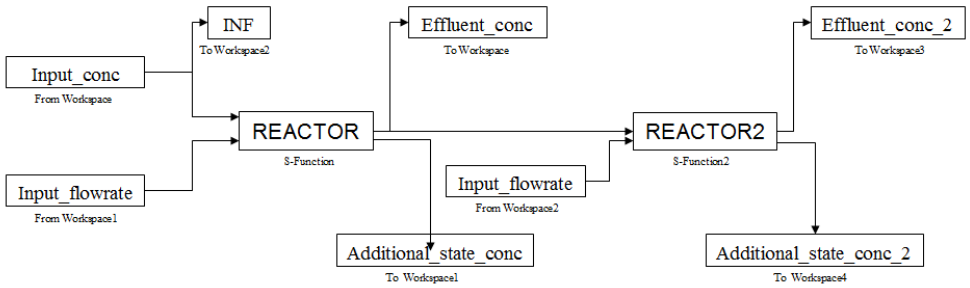
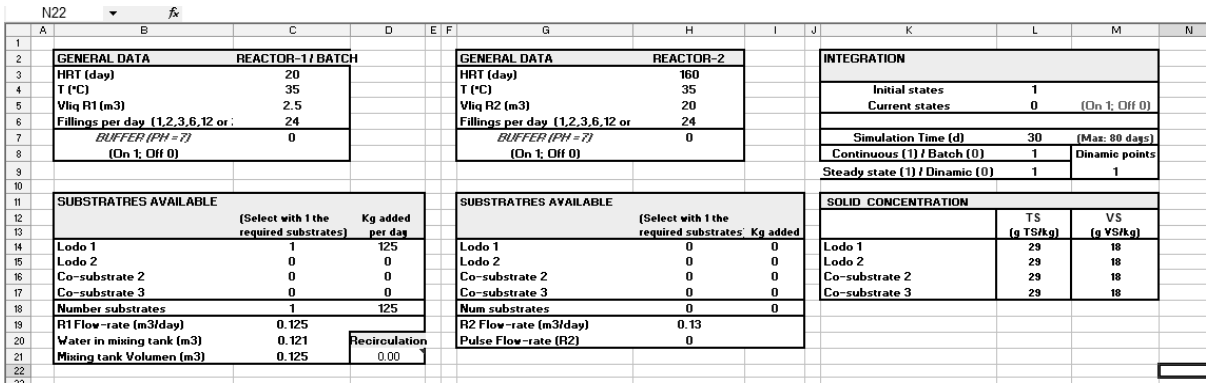


Fig. 3.4. UB-model flow sheet scheme in Matlab/Simulink. (A) one reactor (B) two reactors

Model inputs and outputs

The UB-model lead you select a main substrate and up to three co-substrates, where the kilograms per day of each substrate must be specified (Fig. 2.3). Moreover, to start the simulation procedure and before running the model it is indispensable to introduce: (i) the hydraulic retention time of the reactor, (ii) the operational temperature, (iii) the volume of the liquid part of the reactor, (iv) the number of times that the reactor is filled per day (select 1, 2, 3, 6, 12 or 24), (v) continuous or discontinuous mode, and (vi) activate or deactivate the pH control.



GENERAL DATA	REACTOR-1 / BATCH
HRT (day)	20
T (°C)	35
Vliq R1 (m3)	2.5
Fillings per day (1,2,3,6,12 or :	24
BUFFER (PH = 7) (On 1; Off 0)	0

SUBSTRATRES AVAILABLE	(Select with 1 the required substrates)	Kg added per day
Lodo 1	1	125
Lodo 2	0	0
Co-substrate 2	0	0
Co-substrate 3	0	0
Number substrates	1	125
R1 Flow-rate (m3/day)	0.125	
Water in mixing tank (m3)	0.121	Recirculation
Mixing tank Volumen (m3)	0.125	0.00

GENERAL DATA	REACTOR-2
HRT (day)	160
T (°C)	35
Vliq R2 (m3)	20
Fillings per day (1,2,3,6,12 or	24
BUFFER (PH = 7) (On 1; Off 0)	0

SUBSTRATRES AVAILABLE	(Select with 1 the required substrates	Kg added
Lodo 1	0	0
Lodo 2	0	0
Co-substrate 2	0	0
Co-substrate 3	0	0
Num substrates	0	0
R2 Flow-rate (m3/day)	0.13	
Pulse Flow-rate (R2)	0	

INTEGRATION		
Initial states	1	
Current states	0	(On 1; Off 0)
Simulation Time (d)	30	(Max: 80 days)
Continuous (1) / Batch (0)	1	Dinamic points
Steady state (1) / Dinamic (0)	1	1

SOLID CONCENTRATION	TS (g TS/kg)	VS (g VS/kg)
Lodo 1	29	18
Lodo 2	29	18
Co-substrate 2	29	18
Co-substrate 3	29	18

Fig. 3.5. UB-model operating screen

In addition to all the compounds listed in Table 2.2, the engineering parameters listed in Table 2.3 are going to be followed and calculated in the reactor modelling. The results can be plotted in Matlab or in an Excel file.

Table 3.3. Additional engineering parameters obtained from the model simulation

Parameter	**Units**
Chemical Oxygen Demand	kg COD m^{-3}
Alkalinity	kmole m^{-3}
Total Nitrogen	kg N m^{-3}
Solids (total and suspended)	kg m^{-3}
pH	-
Gas production	m^3 day^{-1}
Gas composition (CO_2, CH_4, H_2)	%
COD removal	%
Solids removal	%
Organic loading rate	kg VS m^{-3} day^{-1}

4. Anaerobic digestion of sewage sludge: a biodegradability and modelling study

Abstract

Seven mixed sewage sludges from different wastewater treatment plants were evaluated in order to clarify the literature uncertainty regarding sewage sludge characterisation and biodegradability. Moreover, a methodology is provided to determine the Anaerobic Digestion Model No. 1 parameters, coefficients and initial state variables as well as a discussion about the accuracy of the first order solubilisation constant, which was obtained through the biomethane potential test. The results of the biomethane potential tests showed ultimate methane potentials from 188 to 214 mL CH_4 g^{-1} COD_{fed}, COD removals between 58 and 65% and two homogeneous groups for the first order solubilisation constant: (i) the lowest rate group from 0.23 to 0.35 day^{-1} and (ii) the highest rate group from 0.27 to 0.43 day^{-1}. However, no statistically significant relationship between the ultimate methane potential or the disintegration constant and the sewage sludge characterisation was found. Next, a methodology based on the sludge characterisation before and after the biomethane potential test was developed to calculate the biodegradable fraction, the composite concentration and stoichiometric coefficients and the soluble COD of the sewage sludge; required parameters for the implementation of the Anaerobic Digestion Mod el No. 1. The comparison of the experimental and the simulation biomethane potential test results proved the consistency of the developed methodology. Nevertheless, an underestimation of the first order solubilisation constant was detected when the experimental results were simulated with the solubilisation constant obtained from the linear regression experimental data fitting. The latter phenomenon was related to the accumulation of intermediary compounds during the biomethane potential assay. Finally, the developed methodology was validated with a sewage sludge continuous laboratory digester.

- Astals S, Esteban-Gutiérrez M, Fernández-Arévalo T, Aymerich, E, García-Heras JL, Mata-Alvarez J. Anaerobic digestion of seven different sewage sludges: a biodegradability and modelling study. Submitted to Water Research.
- Astals S, Venegas C, Peces M, Jofre J, Lucena F, Mata-Alvarez J (2012). Balancing hygienization and anaerobic digestion of raw sewage sludge. Water Res 46:6218-6227

4.1. Introduction

Anaerobic digestion (AD), a multistep biological process, has been used to stabilise the sewage sludge (SS) produced by wastewater treatment plants (WWTP) for more than a century. Within this framework, mathematical models have been developed to reproduce on a computer the physical, chemical and biological processes involved in AD (Donoso-Bravo et al., 2011; Mata-Alvarez et al., 2011). Since laboratory experiments are expensive and time consuming, the development and use of models is very important in AD design, performance and troubleshooting as they are both time and money efficient (Batstone et al., 2009; Galí et al., 2009).

Among all the existing models, the Anaerobic Digestion Model No.1 (ADM1), developed by the IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes, was designed to reach a common basis for AD model development and validation studies (Batstone et al., 2002). However, the large numbers of model components and the detailed substrate characterisation required are the major drawbacks of ADM1 (Kleerebezem and Van Loosdrecht, 2006). In order to solve this problem, many studies used most of the kinetic and stoichiometric parameters suggested by ADM1 as default values (de Gracia et al. 2006; Grau et al. 2007; Galí et al., 2009). Although the full identification of the kinetics, parameters and compounds concentration is not generally possible, at least not on a regular basis, an adequate inflow characterisation is still needed to obtain realistic simulations (Kleerebezem and Van Loosdrecht, 2006; de Gracia et al. 2011). In this vein, if the solubilisation (disintegration and hydrolysis) could be considered the rate-limiting step of the overall AD process, good model results were obtained when the composite fractionation and the solubilisation rate were characterised (de Gracia et al. 2009 and 2011; Galí et al., 2009; Aymerich et al., 2010; Mata-Alvarez et al., 2011). The solubilisation rate is typically estimated from the cumulative methane production curve of the biomethane potential (BMP) test and then implemented as disintegration rate, while a default non-limiting value is given to the carbohydrates, protein and lipids hydrolysis rate (Batstone et al., 2002; de Gracia et al., 2009; Galí et al., 2009; Aymerich et al., 2010; Astals et al., 2011; Girault et al., 2012). In contrast, there is not a sound method to determine the composite stoichiometric coefficients, where most procedures are based on

biodegradability fraction and/or the influent characterisation of the waste under study (Lubken et al., 2007; Galí et al., 2009; Girault et al., 2012).

In ADM1, the disintegration step was included to represent the pool of composite organic matter and to allow the implementation of different types of SS and wastes; in which composite (X_C) is divided into particulate carbohydrates (X_{ch}), protein (X_{pr}), lipids (X_{li}), inerts (X_i) and soluble inerts (S_i) from their respective stoichiometric coefficients ($f_{Xch,XC}$, $f_{Xpr,XC}$, $f_{Xli,XC}$, $f_{Xi,XC}$, $f_{Si,XC}$) (Batstone et al., 2002). The disintegration step was implemented as a first order kinetic, an empirical approach that reflects the cumulative effect of a multi-step process (Eastman and Ferguson, 1981; Pavlostathis and Giraldo-Gomez, 1991; Batstone et al., 2002; Vavilin et al., 2008). Finally, it is important to note that in batch digesters modelling establishing the initial conditions of the model state variables (particulate and soluble compounds) and parameters (kinetics and coefficients), as well as estimating initial biomass concentration and distribution, are a much bigger limitation since they represent the unique system input and, therefore, they exert great influence in model results (Batstone et al. 2004; Grau et al., 2007; Donoso-Bravo et al. 2011).

The aim of the present work was to assess sewage sludge anaerobic digestion, where the experimental and the modelling results of seven mixed sewage sludge from different WWTPs were evaluated and compared. To achieve this objective the research sought to: (i) determine sludge biodegradability and solubilisation kinetics through BMP tests; (ii) develop a methodology to provide ADM1 parameters, coefficients and initial state variables; and (iii) compare discontinuous and continuous experimental and model results in order to check the robustness of the developed methodology.

4.2. Materials and Methods

Biomethane potential test

The BMP tests were performed as described in section 3.2.1.

Structure of the model

The modified version of the ADM1 (Batstone et al., 2002) designed by the CEIT (de Gracia et al. 2006) was used to model the results of the BMP tests, in which the WEST® (www.mikebydhi.com) platform was used as a simulation software tool. The model,

which uses a set of differential and algebraic equations, is built up in order to guarantee total elemental mass (C, H, O, N and P) and charge continuity for all transformation involved in the anaerobic digestion process (de Gracia et al. 2006). As a result, some components acted as source-sink or compensation terms, avoiding C, H, O, N and P imbalances (Grau et al., 2007). In the present model, the composite is decoupled in two forms to avoid elemental mass discrepancies: composites from the influent (X_{C1}) and composites from the dead biomass (X_{C2}); both were implemented as first order kinetic (Grau et al., 2007; Aymerich et al., 2010). Most of the stoichiometric and kinetic parameters used in the present model were ADM1 default values. However, for each evaluated SS, the fractionation of the influent particulate material ($f_{Si,XC1}$, $f_{Xi,XC1}$, $f_{Xpr,XC1}$, $f_{Xli,XC1}$, $f_{Xch,XC1}$) and its disintegration rate ($k_{dis,XC1}$), as well as soluble organic and inorganic compounds were estimated from the SS characterisation and the BMP results. The following hypotheses were set with the aim of simplifying the model calculations: (i) the influent sludge did not contain active and/or dead biomass and (ii) biomass chemical composition ($C_5H_{6.9}O_2NP_{0.1}$) and biomass disintegration stoichiometric coefficients ($f_{Xch,XC2}$ = 0.103, $f_{Xpr,XC2}$ = 0.413; $f_{Xli,XC2}$ = 0.285, $f_{Xi,XC2}$ = 0.184 and $f_{Si,XC1}$ = 0.015) were taken by default (de Gracia et al., 2009; Aymerich et al., 2010).

Mixed sewage sludges and inoculum origin

Seven different mixed SS from six different municipal WWTPs were used in the present study. The SS were obtained from WWTPs of the Barcelona Metropolitan Area (October 2011), which have anaerobic digesters in operation. However, the characteristic of the wastewater (origin and amount) and the technology used to treat it, prior to anaerobic digestion, vary from plant to plant (Table 4.1). Specifically, sludges A, B and C were collected as mixed sludge, whereas for sludges D, E, F and G primary sludge (PS) and waste activated sludge (WAS) were separately collected and subsequently mixed, keeping the WWTP mixture of the sampling day (Table 4.1). It should be noted that sludges F and G were obtained from the same WWTP. Sludge G, which was collected three months earlier than the other sludges (July 2011), was stored at 4 ºC until its utilisation. The inoculum used in these assays was obtained from a stable laboratory mesophilic sewage sludge digester operated at a hydraulic retention time of 15 days. The characterisation of all SS and the inoculum is given in Table 4.2.

Table 4.1. Characteristics of the WWTPs and its mixed sewage sludge

Sewage sludge	WWTP IE	Plant Technology		Sludge Composition (% in wet basis)	
		PS	WAS	% PS	% WAS
A	80,000	Primary clarifier Gravity thickener	CAS Secondary clarifier Flotation thickener	67	33
B	135,000	Primary clarifier Gravity thickener	CAS Secondary clarifier Mechanical thickener	70	30
C	295,000	Primary clarifier Gravity thickener	MBR Secondary clarifier Flotation thickener	60	40
D	375,000	Primary clarifier Centrifugal thickener	CAS Secondary clarifier Centrifugal thickener	50	50
E	385,000	Primary clarifier Centrifugal thickener	IFAS / MBR Secondary clarifier Centrifugal thickener	50	50
F	2,275,000	Primary clarifier Centrifugal thickener	CAS Secondary clarifier Centrifugal thickener	68	32
G	2,275,000	Primary clarifier Centrifugal thickener	CAS Secondary clarifier Centrifugal thickener	60	40

IE stands for inhabitant equivalent; CAS stands for conventional activated sludge; MBR stands for membrane bioreactor; IFAS stands for integrated fixed-film activated sludge.

Table 4.2. Characterisation of the influent mixed sewage sludges and inoculum

Parameter	**Units**	**SS_A**	**SS_B**	**SS_C**	**SS_D**	**SS_E**	**SS_F**	**SS_G**	**Inoculum**
Density	kg L^{-1}	0.98	0.97	0.97	0.98	0.98	0.97	0.97	0.98
TS	g L^{-1}	38.3	18.4	32.7	35.2	48.4	39.9	31.3	23.0
VS	g L^{-1}	27.9	14.3	25.7	26.3	40.5	29.6	20.4	13.1
TSS	g L^{-1}	33.4	15.6	27.1	30.0	42.9	34.5	26.3	21.6
VSS	g L^{-1}	24.7	12.1	21.7	23.1	37.0	25.9	16.3	12.2
CODt	g O_2 L^{-1}	50.3	25.3	46.6	46.4	70.8	51.0	33.3	29.3
CODs	g O_2 L^{-1}	3.7	1.9	3.5	3.3	4.0	4.0	4.2	0.2
DOC	mg C L^{-1}	1,259	682	1,224	1,086	1,299	1,285	1,377	361
pH	-	6.1	6.3	5.8	5.9	5.7	6.1	6.6	7.5
Partial Alk.	mg CaCO3 L^{-1}	299	208	5	95	0	445	588	2,732
Total Alk.	mg CaCO3 L^{-1}	4,118	1,644	1,964	3,469	2,921	3,753	3,635	4,561
IC	mg C L^{-1}	83	64	52	70	77	77	118	588
VFA	mg L^{-1}	2243	1340	1620	1657	1774	2006	2444	35
- Acetic acid	mg L^{-1}	1101	817	936	888	889	1059	989	23
- Propionic acid	mg L^{-1}	670	288	444	486	541	426	681	n.d.*
- i-Butyric acid	mg L^{-1}	65	44	40	45	37	63	117	12
- n-Butyric acid	mg L^{-1}	263	102	113	147	195	283	355	n.d.
- i-Valeric acid	mg L^{-1}	87	58	47	55	32	100	197	n.d.
- n-Valeric acid	mg L^{-1}	58	31	40	37	81	75	105	n.d.
TAN	mg N L^{-1}	229	161	142	193	152	151	327	995
NTK	g N L^{-1}	1.89	1.15	2.18	2.33	2.32	2.32	1.62	1.90
Protein	g L^{-1}	10.39	6.20	12.73	13.35	13.54	13.54	8.11	5.67
Carbohydrates	g L^{-1}	10.32	4.51	7.56	7.43	17.22	9.57	6.98	6.83
Lipids	g L^{-1}	4.93	2.23	3.81	3.82	7.96	4.47	2.87	0.58
Sodium	mg L^{-1}	535	195	182	491	335	382	172	232
Potassium	mg L^{-1}	207	71	217	258	280	187	171	192
Calcium	mg L^{-1}	396	192	303	360	389	410	317	154
Magnesium	mg L^{-1}	123	37	109	163	134	136	95	62
Fluoride	mg L^{-1}	115	74	90	98	93	109	115	n.d.
Chloride	mg L^{-1}	511	189	167	566	387	465	191	267
Phosphate	mg L^{-1}	690	128	870	879	787	553	272	68
Sulphate	mg L^{-1}	n.d.	n.d.	n.d.	10	31	39	n.d.	13
CST	s	124	156	262	144	278	115	90	55

* n.d. non-detected (< 10 mg L^{-1})

4.3. Results and discussion

4.3.1. Biomethane potential experiments

The BMP is the most widespread test to evaluate the biodegradability, i.e. the ultimate methane potential per mass of substrate (B_0) and the matter removal (in terms of TS, VS and/or COD), and kinetic studies of organic substrate in anaerobic processes (Angelidaki and Sanders, 2004; Angelidaki et al., 2009; Jensen et al., 2011).

Biodegradability of sewage sludge

The profiles of the methane production against time of the seven SS under study, after removing the inoculum production, are presented in Fig. 4.1. The cumulative methane profile presented a similar trend in all SS; that was a first-order kinetics plot without lag phase, in which the higher methane rates were recorded during the first 5 days. Despite these similarities each SS presented a different biodegradability, where B_0 and COD removals ranged from 188 to 214 mL CH_4 g^{-1} COD_{fed} and from 58 to 65%, respectively (Table 4.3).

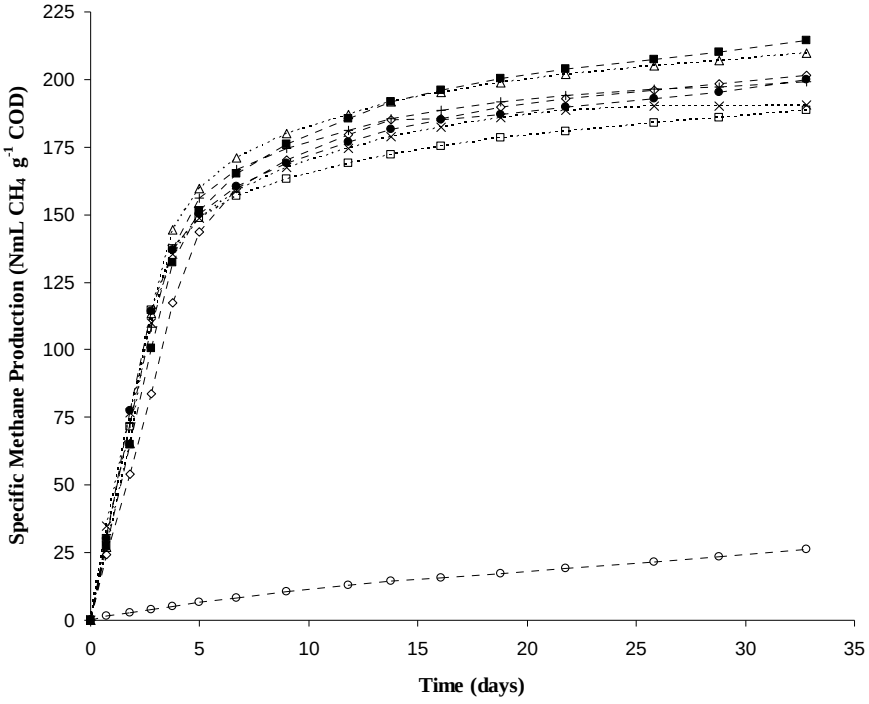


Fig. 4.1. Cumulative methane production curve of each sewage sludge: SS_A (◊), SS_B (■), SS_C (+), SS_D (×), SS_E (▲), SS_F (□), SS_G (●) and blank (○).

However, eq. 4.1 does not consider the amount of COD for bacterial growth and maintenance even though it is the most common comparison criteria within reported tests (Buffiere et al., 2006; Raposo et al., 2012). A methodology which takes into account the COD used for the cell growth is the one reported by Field et al. (1988), where three indexes, i.e. acidogenesis (%A), methanogenesis (%M) and biodegradability (%BD), are considered (eq. 4.2 – 4.4).

$$COD_{removal} = 100 \cdot \frac{(COD_0 - COD_f)}{COD_0} \quad \text{(eq. 4.1)}$$

$$\%A = 100 \cdot \frac{(COD_{CH_4} + COD_{VFA_f})}{COD_0} \quad \text{(eq. 4.2)}$$

$$\%M = 100 \cdot \frac{COD_{CH_4}}{COD_0} \quad \text{(eq. 4.3)}$$

$$\%BD = \%A + \frac{Y_A}{1 - Y_A} \cdot \left(\%A - 100 \cdot \frac{COD_{VFA_0}}{COD_0} \right) + \frac{Y_M}{1 - Y_M} \cdot \%M \quad \text{(eq. 4.4)}$$

where Y_A is the yield of the acidogenic microorganisms (0.050 g COD g^{-1} COD) and Y_M is the yield of the methanogenic archaea (0.029 g COD g^{-1} COD).

The COD removals obtained with the Field et al. (1988) procedure were similar to the one obtained with eq. 4.1, with values ranging from 58 to 66% (Table 4.3). In this method, the difference between %A and %BD is related to the amount of COD used for cell growth (Field et al. 1988), whereas the short difference between %A and %M was like that due to the negligible VFA concentration at the end of the BMP tests (below 20 mg L^{-1} in all tests). Regardless of the methodology applied, some uncertainties about SS biodegradability have been found in the literature, since reported B_0 and COD removals range from 80 to 220 mL CH4 g^{-1} COD_{fed} and from 36 to 63%, respectively (Jih-Gaw et al., 1999; Wang et al., 1999; Yeom et al., 2002; Bougrier et al., 2006; Davidsson et al., 2008; Aymerich et al., 2010; Qiao et al., 2011). Finally, a set of simple and multi regressions were carried out between sewage sludge macro-compounds (carbohydrates, protein and lipids) and B_0 in order to find an equation which based on a SS characterisation, could allow a quick estimation of the SS B_0. The toughest relationship was obtained when the model used the three compounds ($R^2 \sim 0.83$); nonetheless, the p-

value was lower than 0.05 in any scenario. It was concluded that there is no statistically significant correlation, with a confidence level of 95%, between the sludge characterisation and its methane potential. Although no relationship was found, the reported results highlight the variability of the SS characterisation and biodegradability as well as the high influence of the wastewater origin and treatment.

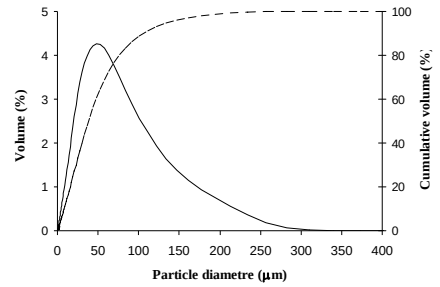
Table 4.3. Ultimate methane production and matter removal of each sewage sludge

	Units	SS_A	SS_B	SS_C	SS_D	SS_E	SS_F	SS_G
Experimental ultimate methane potential								
$B_{0, COD}$	mL CH_4 g^{-1} COD_{fed}	201.3	214.2	199.0	190.2	209.8	188.3	200.0
$B_{0, VS}$	mL CH_4 g^{-1} VS_{fed}	362.7	379.7	360.6	335.7	366.9	324.5	326.0
$B_{0, TS}$	mL CH_4 g^{-1} TS_{fed}	263.9	295.5	284.0	250.6	306.6	240.7	212.8
Experimental removal efficiency								
$COD_{removal}$	%	62.1	62.7	62.2	58.6	64.7	58.4	59.1
$VS_{removal}$	%	52.1	52.3	55.2	44.0	59.1	51.1	49.7
$TS_{removal}$	%	41.3	45.7	45.1	34.6	56.0	40.5	34.5
Field et al.(1988)biodegradability parameters								
Acidogenesis	%	57.5	61.2	56.9	54.4	59.9	53.8	57.2
Methanogenesis	%	57.6	61.3	56.9	54.4	60.0	53.9	57.2
Biodegradability	%	62.0	66.0	61.3	58.7	64.7	58.1	61.3

Disintegration rate of sewage sludges

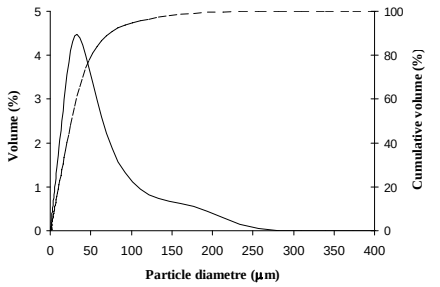
Many reports have concluded, due to the large fraction of organic matter that must be solubilised before its methanisation, that the disintegration/hydrolysis step is the rate-limiting stage of the overall sewage sludge AD process (Eastman and Ferguson, 1981; Apples et al., 2008; Burgess and Pletschke, 2008; Yasui et al., 2008). The SS presented high amounts of particulated organic matter as shown by the high VSS/VS ratio and the low CODs/CODt ratio, which ranged from 0.91 to 0.80 and from 0.06 to 0.13, respectively. The analysis of the particle size distribution showed that the SS under study mainly had particles with a diameter between 10 and 250 μm, with mean diameter varying from 40 to 60 μm. Nevertheless, SS_D presented a wider range of particles diameter distribution (10-400 μm) and a much higher mean value (114 μm). Furthermore, the SS_C, SS_E, and SS_G also presented a high concentration of particles around 150 - 200 μm (Fig. 4.2).

SS_A



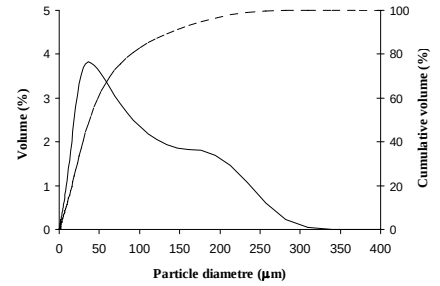
Mean	54 µm	d_{10}	9 mm
s	47	d_{90}	117 µm

SS_B



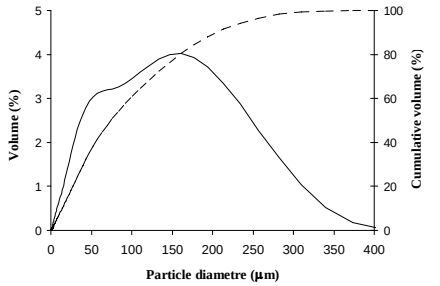
Mean	39 µm	d_{10}	6 µm
s	29	d_{90}	81 µm

SS_C



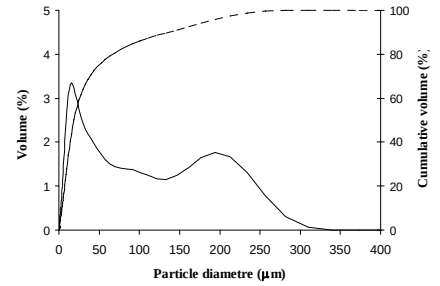
Mean	62 µm	d_{10}	9 µm
s	59	d_{90}	151 µm

SS_D



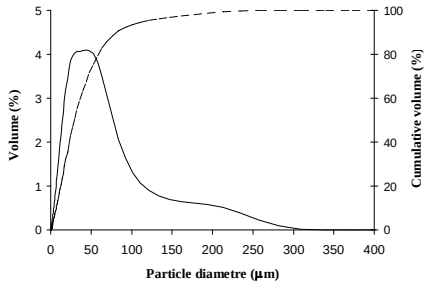
Mean	114 µm	d_{10}	16 µm
s	104	d_{90}	243 µm

SS_E



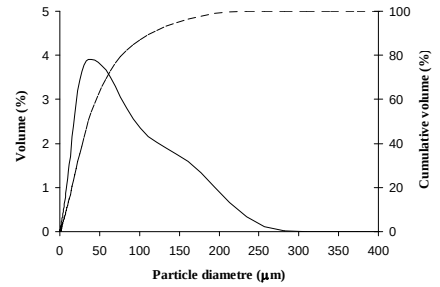
Mean	47 µm	d10	4 µm
s	62	d90	149 µm

SS_F



Mean	44 µm	d10	8 µm
s	43	d90	90 µm

SS_G



Mean	55 µm	d_{10}	9 µm
s	9 µm	d_{90}	126 µm

Fig. 4.2. Differential (solid line) and cumulative (dotted line, secondary axis) particle size distribution of the mixed sewage sludges under study. d_{10} and d_{90} stand for percentiles 10 and 90%, respectively.

When the solubilisation is considered as the limiting step of the substrate AD, the cumulative methane production curve of the BMP can be used to obtain the solubilisation rate (Angelidaki et al., 2009). Using first order kinetic as a model and assuming that there were no accumulation of intermediary products, the cumulative methane production in the course of time can be described by means of equation 4.5 (Veeken and Hamelers, 1999; Vavilin et al., 2008; Angelidaki et al., 2009; Galí et al., 2009).

$$B(t) = B_0 \cdot (1 - e^{(-k_{dis} \cdot t)}) \qquad \text{(eq. 4.5)}$$

where B (t) represents the cumulative methane production at a given time (mL), B_0 is the ultimate methane potential yield of the substrate (mL), k_{dis} is the first order disintegration rate (day^{-1}) and t means time (days).

The k_{dis} has generally been estimated by the first part of the methane curve. In summary, the exponential function is linearised by natural logarithm and then least squares regression is applied to the linear, own criteria, region (Angelidaki et al., 2009; Galí et al., 2009). Related to this results, other authors have estimated k_{dis} using the same methodology but taking the reciprocal of the time from the start of the BMP until B equalled $0.632B_0$ (Gunaseelan, 2004; Fountoulakis et al., 2008). It is clear that using this methodology, the k_{dis} value changed depending on the time used to estimate it. As an example, k_{dis} values of the SS_A were 0.26, 0.26 and 0.29 day^{-1} when the first 4, 5 and 7 days of the curve were used to estimate it ($R^2 > 0.97$ were obtained in all cases). However, if the 95% confidence interval (CI) was taken into account, no statistical difference was found between them, with true value ranging from 0.21 to 0.32 day^{-1}. Another option to estimate k_{dis} was to custom equation 5 and, then, to conduct a regression analysis. This performance allowed, not like the linearisation model, to estimate the k_{dis} with the entire curve, which was 0.25 day^{-1} for SS_A ($R^2 \sim 0.98$) and true value between 0.22 and 0.27 day^{-1}. Moreover, if the same regression analysis was carried out for the first 4, 5 and 7 days of the curve, the k_{dis} values were 0.26, 0.25 and 0.24 day^{-1} ($R^2 > 0.97$), with true value ranging from 0.19 to 0.29 day^{-1}. As it is shown, no statistical difference was observed when the k_{dis} of the SS_A was estimated by exponential custom or by linearisation; the same conclusions were obtained with the other sludges (k_{dis} and its CI were calculated with a 95% confidence level by Matlab

Curve Fitting Toolbox™). Next, the multiple range test of the obtained k_{dis} showed, with a confidence level of 95%, two homogeneous groups: (i) SS_A, SS_B and SS_E (0.23 - 0.35 day^{-1}) and (ii) SS_C, SS_D, SS_E, SS_F and SS_G (0.27 - 0.43 day^{-1}). In other words, no statistical difference is found within the k_{dis} of a group. In addition, since sludges with a similar particle size distribution presented different k_{dis}, it was clear that other parameters, like the structure of the composite, may also have influenced the solubilisation kinetic. Finally, a set of simple and multi regressions were carried out between the disintegration constant and the sludge properties; nevertheless, no statistically significant relationship was found with a confidence level of 95%.

Other researchers have used non-linear models to estimate k_{dis} and to identify parameters uncertainty and correlation (Batstone et al., 2009; Jensen et al., 2011). However, regardless the methodology used to estimate the k_{dis}, the values obtained in the present study (Table 4.4) are within the wide range of k_{dis} values reported for SS, between 0.1 and 0.6 day^{-1} (Pavlostathis and Giraldo-Gomez, 1991; Batstone et al., 2002; Batstone et al., 2009; Aymerich et al., 2010).

Table 4.4. Disintegration rate of the mixed sewage sludges

	Units	**SS_A**	**SS_B**	**SS_C**	**SS_D**	**SS_E**	**SS_F**	**SS_G**
mean	day^{-1}	0.26	0.28	0.39	0.38	0.31	0.38	0.37
min. CI	day^{-1}	0.23	0.26	0.36	0.34	0.27	0.34	0.35
max. CI	day^{-1}	0.29	0.30	0.43	0.42	0.35	0.43	0.38
R^2	-	0.98	0.99	0.99	0.98	0.97	0.97	0.99

4.3.2. Characterisation methodology for sewage sludge and inoculum in terms of model components

Determination of the composite concentration and stoichiometric coefficients

The present methodology was based, even being more time consuming, on the Aymerich et al. (2010) procedure, which characterises the substrates and the inoculum at the beginning and at the end of the BMP test. This was in contrast to the Galí et al. (2009) procedure, which only uses the initial characterisation of the waste. However, both methodologies work with COD balances, theoretical oxygen demand (ThOD or γi – gCOD g^{-1}compound) and mass conversion parameters (βi – gCOD g^{-1}element). Those

values were calculated using the Buswell's formula by means of the elemental composition, i.e. C, H, O, N and P, of the compound (Grau et al., 2007; de Gracia et al., 2006 and 2011).

Firstly, the COD and the VFA analytical results, obtained before and after the BMP, were used to split the COD_{XC1} into biodegradables (COD_{Xch}, COD_{Xpr} and COD_{Xli}) and inerts (COD_{Xi} and COD_{Si}). Equations 4.6 to 4.13 show the assumptions made to transform the COD results into stoichiometric coefficients. It should be noted that if the soluble inerts needs to be divided into soluble inerts from the soluble fraction and soluble inerts from the composite, a batch test of the soluble or the particulate fraction should be put into practice.

- COD initial substrate characterisation

$$tCOD_0 = COD_{XC1} + sCOD_0 - COD_{Si} \quad \text{(eq. 4.6)}$$

$$pCOD_0 = COD_{Xch} + COD_{Xpr} + COD_{Xli} + COD_{Xi} = COD_{XC1} - COD_{Si} \quad \text{(eq. 4.7)}$$

$$sCOD_0 = COD_{Ssu} + COD_{Saa} + COD_{Sfa} + COD_{VFA_0} + COD_{Si} \quad \text{(eq. 4.8)}$$

- COD final substrate characterisation

$$tCOD_f = COD_{Xi} + COD_{VFA_f} + COD_{Si} \quad \text{(eq. 4.9)}$$

$$pCOD_f = COD_{Xi} \quad \text{(eq. 4.10)}$$

$$sCOD_f = COD_{VFA_f} + COD_{Si} \quad \text{(eq. 4.11)}$$

where:

$$COD_{XC1} = COD_{Xch} + COD_{Xpr} + COD_{Xli} + COD_{Xi} + COD_{Si} \quad \text{(eq. 4.12)}$$

$$COD_{VFA} = COD_{TAc} + COD_{TPro} + COD_{TBu} + COD_{TVa} \quad \text{(eq. 4.13)}$$

Secondly, the COD_{Xpr} and the COD_{Xli} were obtained from the SS characterisation results. On the one hand, lipids were transformed into COD using its theoretical oxygen demand, 2.87 gCOD g^{-1}lipid since $C_6H_{97.9}O_6P_{0.1}$ was used as a compound formula (Grau et al., 2007). Then, the initial COD_{Xli} value was subtracted by the final COD_{Xli} value (see eq. 4.14). On the other hand, protein was transformed into COD using its mass conversion parameters, 0.105 gCOD g^{-1} N since $C_4H_{6.1}O_{1.2}N$ was used as a compound formula (Grau et al., 2007). After that, the initial COD_{Xpr} value was

subtracted by the final COD_{Xpr} value (see eq. 4.15). Next, the COD_{Xch} was obtained, as shown in eq. 4.16, by subtracting the other compounds to COD_{XC1}. Finally, the stoichiometric coefficients were set by normalizing the data (see eq. 4.12).

$$COD_{Xli} = [Lipids_0 - Lipids_f] \cdot \gamma_{li} \quad \text{(eq. 4.14)}$$

$$COD_{Xpr} = [(TKN - TAN)_o - (TKN - TAN)_f] \cdot \beta_{N,Xpr} \quad \text{(eq. 4.15)}$$

$$COD_{Xch} = COD_{XC1} - COD_{Xpr} - COD_{Xli} - COD_{Xi} - COD_{Si} \quad \text{(eq. 4.16)}$$

As indicated in Table 4.5, the stoichiometric coefficients of the composite biodegradable fraction presented a high variability within the SS under study, where $f_{Xch,XC1}$ ranged from 0.07 to 0.24, $f_{Xp,XC1}$ from 0.15 to 0.24 and $f_{Xli,XC1}$ from 0.20 to 0.31. Nevertheless, the biodegradable fraction ($D = f_{Xpr} + f_{Xli} + f_{Xch}$) of the composite showed a lower variability, with values ranging from 0.62 (SS_E) to 0.53 (SS_G). The D values were in agreement with Aymerich et al. (2010) and de Gracia et al. (2009) who reported a D of 0.54 and 0.62, respectively, and closer to 0.65, the default ADM1 value, and to 0.66 from Galí et al. (2009) (unpublished data; $f_{Xch,XC1} = 0.33$, $f_{Xpr,XC1} = 0.16$; $f_{Xli,XC1} = 0.17$, $f_{Xi,XC1} = 0.30$ and $f_{Si,XC1} = 0.04$).

Table 4.5. Individual, average and ADM1 values of the composite stoichiometric coefficients

	SS_A	**SS_B**	**SS_C**	**SS_D**	**SS_E**	**SS_F**	**SS_G**	**Avg. ± SD**	**ADM1**
$f_{Xch,XC1}$	0.19	0.24	0.20	0.17	0.14	0.07	0.10	0.16 ± 0.06	0.20
$f_{Xpr,XC1}$	0.15	0.15	0.19	0.18	0.17	0.21	0.18	0.17 ± 0.02	0.20
$f_{Xli,XC1}$	0.24	0.21	0.20	0.20	0.31	0.26	0.26	0.24 ± 0.04	0.25
$f_{Xi,XC1}$	0.41	0.40	0.41	0.44	0.38	0.45	0.46	0.42 ± 0.03	0.25
$f_{Si,XC1}$	0.008	0.005	0.005	0.007	0.002	0.009	0.008	0.006 ± 0.002	0.10

Determination of the sewage sludge soluble compounds

Sewage sludge soluble compounds were split in organic and inorganic compounds. The organic compounds considered in the model include: VFA basic and acid pairs (i.e. acetate (S_{ac-}), acetic acid (S_{hac}), propionate (S_{pro-}), propionanic acid (S_{hpro}), butyrate (S_{bu-}), butyric acid (S_{hbu}), valerate (S_{va-}), valeric acid (S_{hva})); and organic polymers, which were divided into sugars (S_{su}), amino acids (S_{aa}), fatty acids (S_{fa}) and inert (S_i) (Batstone

et al., 2002). As described above, the Si was calculated using the initial and the final COD measurement of the BMP test, whereas the VFA pairs were fractionated combining the VFA and the pH analysis with the corresponding equilibrium constant. Finally, the remaining biodegradable soluble COD (eq. 4.8) was divided in three equal parts (S_{su}, S_{aa}, S_{fa}) (Aymerich et al., 2010). It should be mentioned that the uncertainty of this latter approach will not greatly affect the model results as the sum of the three compounds range between 0.1 (SS_B) and 2.4% (SS_C) of the COD input. Similarly, Si represents less than 1% of the influent COD for all SS, with values ranging from 0.2 (SS_E) to 0.8% (SS_F). In contrast, values between 1.8 and 3.6 g COD L^{-1} made VFA the main organic soluble compound of the sewage sludge under study, being acetate and propionate the principal VFA.

With regard to the inorganic compounds, the model took into account the following ones: ion hydrogen (S_{h+}), ion hydroxyl (S_{oh-}), carbon dioxide (S_{co2}), hydrogen carbonate (S_{hco3-}), ammonium (S_{nh4+}), ammonia (S_{nh3}), dihydrogen phosphate (S_{h2po4-}) and hydrogen phosphate (S_{hpo4--}). The S_{h+} is obtained from the pH measurement and the S_{oh-} is calculated with the water equilibrium constant. The S_{co2} and S_{hco3-}, the S_{nh4+} and S_{nh3} and the $S_{h2po4--}$ and S_{h2po4-} were determined considering the analytical results, the pH and the respective acid/base equilibrium constant (pKa at 37 ºC: 6.29, 8.90 and 7.18 respectively). In the sewage sludge, the S_{hco3-} represented about the 90% of the IC, whereas more than the 95% of the inorganic nitrogen was as S_{nh4+}. Table 4.6 shows the initial concentration of the organic and inorganic soluble state variables.

Inoculum characterisation

In this study, the characterisation of the inoculum was related to the biomass concentration (X_{C2}), distribution and disintegration rate ($k_{dis,XC2}$). Firstly, a simulation of the system from which the inoculum came from was performed in order to estimate the distribution of the microbial population of the model (i.e. amino acids degraders (X_{aa}), sugar degraders (X_{su}), fatty acids degraders (X_{fa}), valerate and butyrate degraders (X_{c4}), propionate degraders (X_{pro}), acetate degraders (X_{ac}) and hydrogen degraders (X_{h2})). The results showed the following population mix: 25% X_{ac}, 20% X_{su}, 15% X_{aa} and X_{fa}, 10% X_{c4} and X_{h2}, and 5% X_{pro}. These values are similar to those reported by Girault et al. (2012), who estimated the biomass distribution of WAS using a similar strategy.

Afterwards, the biomass concentration and disintegration rate were optimised by a series of simulations in a previously calibrated sludge, where X_{C2} varied from 10% to 15% of the inoculum COD and $k_{dis,XC2}$ ranged from 0.15 used by Galí et al. (2009) to 0.70 day^{-1} used by de Gracia et al. (2011). A COD balance based on the ultimate methane production, without removing the inoculum production, was used to determine both parameters. The best fit was obtained when the X_{C2} represented the 12% of the inoculum COD and $k_{dis,XC2}$ was 0.50 day^{-1}. It should be pointed out that the biomass concentration had a greater influence in the COD balance than $k_{dis,XC2}$; however, no parameter had a significant influence in the methane percentage of the biogas. Biomass concentrations below 10% made the methane profile gain a sigmoid shape due the high accumulation of soluble compounds, situation where the methanogenesis is assumed to become the rate-limiting step (Vavilin et al., 2008).

Table 4.6. Concentration of the organic and inorganic soluble state variables

Parameter	Units	SS_A	SS_B	SS_C	SS_D	SS_E	SS_F	SS_G	Inoculum
Organic compounds									
S_{hac}	mg O_2 L^{-1}	4	2	4	4	2	3	3	0
S_{ac-}	mg O_2 L^{-1}	1174	872	997	946	949	1130	1055	25
S_{hpro}	mg O_2 L^{-1}	4	2	3	4	3	2	4	0
S_{pro-}	mg O_2 L^{-1}	1007	433	667	730	814	641	1025	0
S_{hbu}	mg O_2 L^{-1}	2	1	1	1	1	2	3	0
S_{bu-}	mg O_2 L^{-1}	596	264	277	347	421	628	855	21
S_{hva}	mg O_2 L^{-1}	1	1	1	1	1	1	2	0
S_{va-}	mg O_2 L^{-1}	293	182	177	187	229	356	615	0
S_{su}	mg O_2 L^{-1}	80	6	366	260	449	249	103	64
S_{aa}	mg O_2 L^{-1}	80	6	366	260	449	249	103	64
S_{fa}	mg O_2 L^{-1}	80	6	366	260	449	249	103	64
Inorganic compounds									
S_{h+}	mg H L^{-1}	$5.4\cdot10^{-8}$	$4.6\cdot10^{-8}$	$6.6\cdot10^{-8}$	$6.5\cdot10^{-8}$	$4.6\cdot10^{-8}$	$4.5\cdot10^{-8}$	$4.8\cdot10^{-8}$	$3.0\cdot10^{-8}$
S_{oh-}	mg H L^{-1}	$4.5\cdot10^{-7}$	$5.2\cdot10^{-7}$	$3.6\cdot10^{-7}$	$3.7\cdot10^{-7}$	$5.2\cdot10^{-7}$	$5.4\cdot10^{-7}$	$5.0\cdot10^{-7}$	$8.1\cdot10^{-7}$
S_{co2}	mg C L^{-1}	8	5	6	8	6	6	10	33
S_{hco3-}	mg C L^{-1}	75	59	46	62	70	71	108	555
S_{nh4+}	mg N L^{-1}	224	157	139	189	148	147	319	954
S_{nh3}	mg N L^{-1}	5	4	3	4	4	4	8	41
S_{h2p4-}	mg P L^{-1}	101	17	142	142	105	73	37	7
S_{hpo4-}	mg P L^{-1}	124	25	142	145	152	108	51	15

4.4. Modelling sewage sludge anaerobic digestion

4.4.1. Biomethane potential test

In the present model, as well as in ADM1, particulate organic matter breakdown and solubilisation is represented by the disintegration and the hydrolysis step, respectively (Batstone et al., 2002). The k_{dis} applied to model the BMP were the ones obtained from the experimental data fitting (Table 4.4), whereas the hydrolysis rate was fixed at 10 $days^{-1}$ for three particulate compounds (Galí et al., 2009, Vavilin et al., 2008; Batstone et al., 2002). Apart from these, the previously estimated state variables and parameters were also taken into account (Table 4.5 and 4.6).

The comparison between the simulation results and the experimental data showed, for all SS, a good adjustment of the ultimate methane production. Nonetheless, the displayed curves were more flattened than the experimental results, even if the maximum k_{dis} CI values were used (Fig. 4.3). From these results it was concluded that the COD balance was consistent (i.e. biodegradable fraction, composite concentration, stoichiometric coefficients and soluble COD), while k_{dis} values were not accurate enough. Next, a series of simulations were carried out in order to find out the most precise k_{dis}; sum of squared errors were applied. The best fitted k_{dis} were (± 0.01): 0.32 day^{-1}, 0.35 day^{-1}, 0.41 day^{-1}, 0.63 day^{-1}, 0.40 day^{-1}, 0.55 day^{-1} and 0.45 day^{-1} (from SS_A to SS_G, respectively). These results highlight a 5 – 65% k_{dis} underestimation when the experimental data (Table 4.4) was compared to the best model fitting. k_{dis} underestimation, when the BMP experimental data was fitted through the first order kinetic, had also been reported in other papers. Specifically, similar underestimation range was reported by Aymerich et al. (2010) and by Jensen et al. (2011); whereas several orders of magnitude underestimation was reported by Batstone et al. (2009).

To support this point a simulation without the particulate organic matter was undertaken to distinguish the inner soluble compounds and the ones generated during organic matter solubilisation. After removing the soluble compounds from the SS simulation it was observed that the soluble compounds generated during composite solubilisation represented between 300 – 100 mg L^{-1}, being S_{fa} and S_{ac} the main compounds (SSB was used as an example but similar results were obtained for all SS) (Fig. 4.4B).

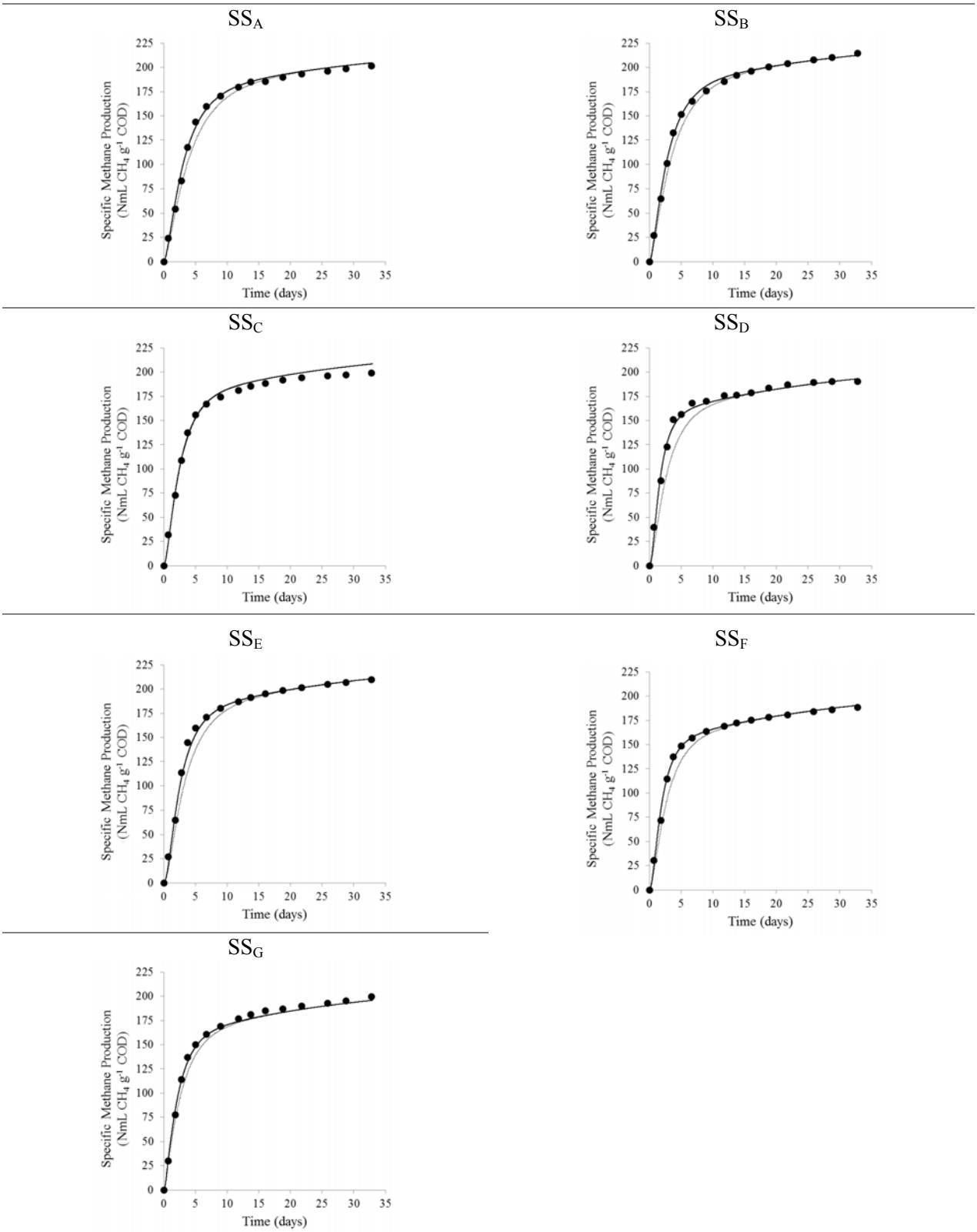
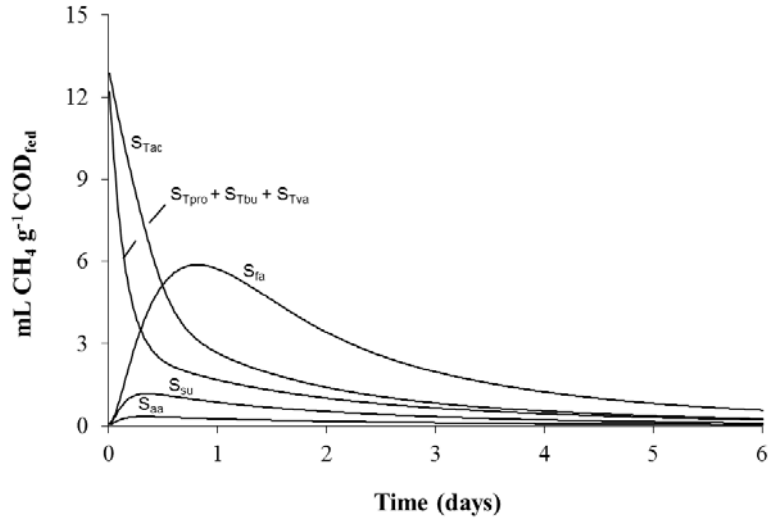


Fig. 4.3. Experimental and modelled cumulative methane production in the course of time of each sewage sludge: Experimental data (●), profile modelled with the mean disintegration constant obtained from the linear regression of the experimental data fitting (dotted line) and profile modelled with the disintegration constant best model fitting (solid line).

A



B

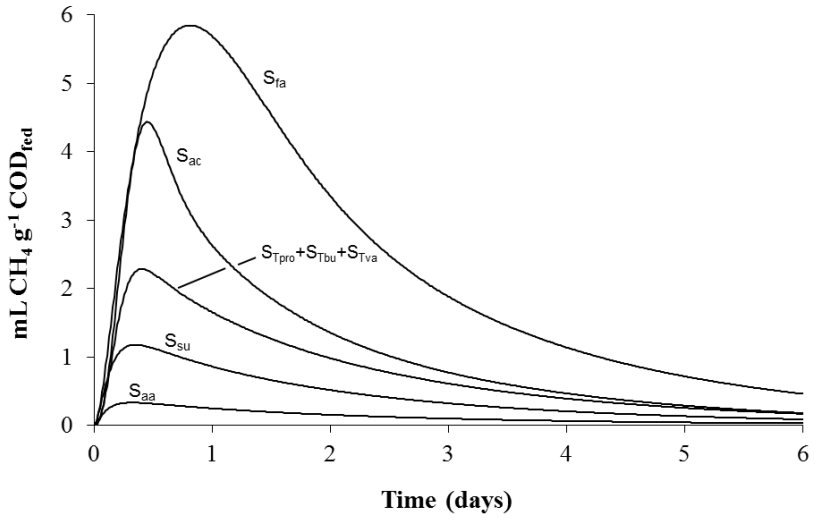


Fig. 4.4. Evolution of the soluble compounds during the first six BMP days of SS_B (A) from the total sewage sludge and (B) from the composite; k_{dis} = 0.35 day^{-1}

The accumulation of the intermediate compounds during the three first days of the test appeared in between 25 and 10 mL CH_4 g^{-1} COD_{fed}. The significant amount of methane, which had not been considered when the first order kinetics based on the methane production was applied, confirmed the k_{dis} underestimation. This conclusion is in agreement with Vavilin et al. (2008), who reported that first order kinetics was not accurate enough when dealing with complex wastes. However, the simplicity and the lack of a consensual, more reliable, approximation makes first order kinetics the most widespread method when solubilisation kinetic wants to be obtained from BMP experiments.

4.4.2. Lab-scale continuous digester

Once proved the consistency of the developed methodology and adjusted the disintegration constant through BMP test, a series of simulations were done in order to reproduce the performance of a sewage sludge lab-scale CSTR under steady state conditions. The SS digester, fed with mixed sewage sludge from where sludge F and G came from, was operated during steady state conditions for about 100 days. Table 4.7 summarises the working conditions of the digester (more details about digester configuration and performance can be found at Astals et al. 2012b).

Table 4.7. Average lab-digester operational conditions (Astals et al., 2012b)

	Units	
Digester volume	L	2.5
Feedings per day	-	1
HRT	days	20
COD	g O_2 L^{-1}	23.4
OLR	g VS L_R^{-1} day^{-1}	1.1
Temperature	ºC	35

In order to simulate the performance of the SS digester, the UB-model was loaded with the average characterisation of the mixed sludge. However, since not all the required compounds were determined during the operation of the CSTR (such as TKN or CODs), the characterisation of SS_F and SS_G were used to complete the required input data. Then, the model was run within a simulation period of 70 days. Figure 4.5 shows the simulation results, while Table 4.8 compares the average operational results and the output of the model at day 70.

The comparison between the experimental and the simulation results shows the robustness of the developed methodology. As shown by the CODt and COD removal, the model fits perfectly the COD balance. Moreover, the simulated CODs value is in agreement with the ones obtained in later experiments, which operated the same digesters under similar conditions (Peces et al. in preparation). The model also correctly reproduces the specific biogas production and the methane percentage (also in agreement with Peces et al. in preparation). However, TAN, total alkalinity and pH

were underestimated (Table 4.8). TAN, pH and alkalinity underestimation could be related with the underestimation of the TKN, inorganic carbon and/or phosphates.

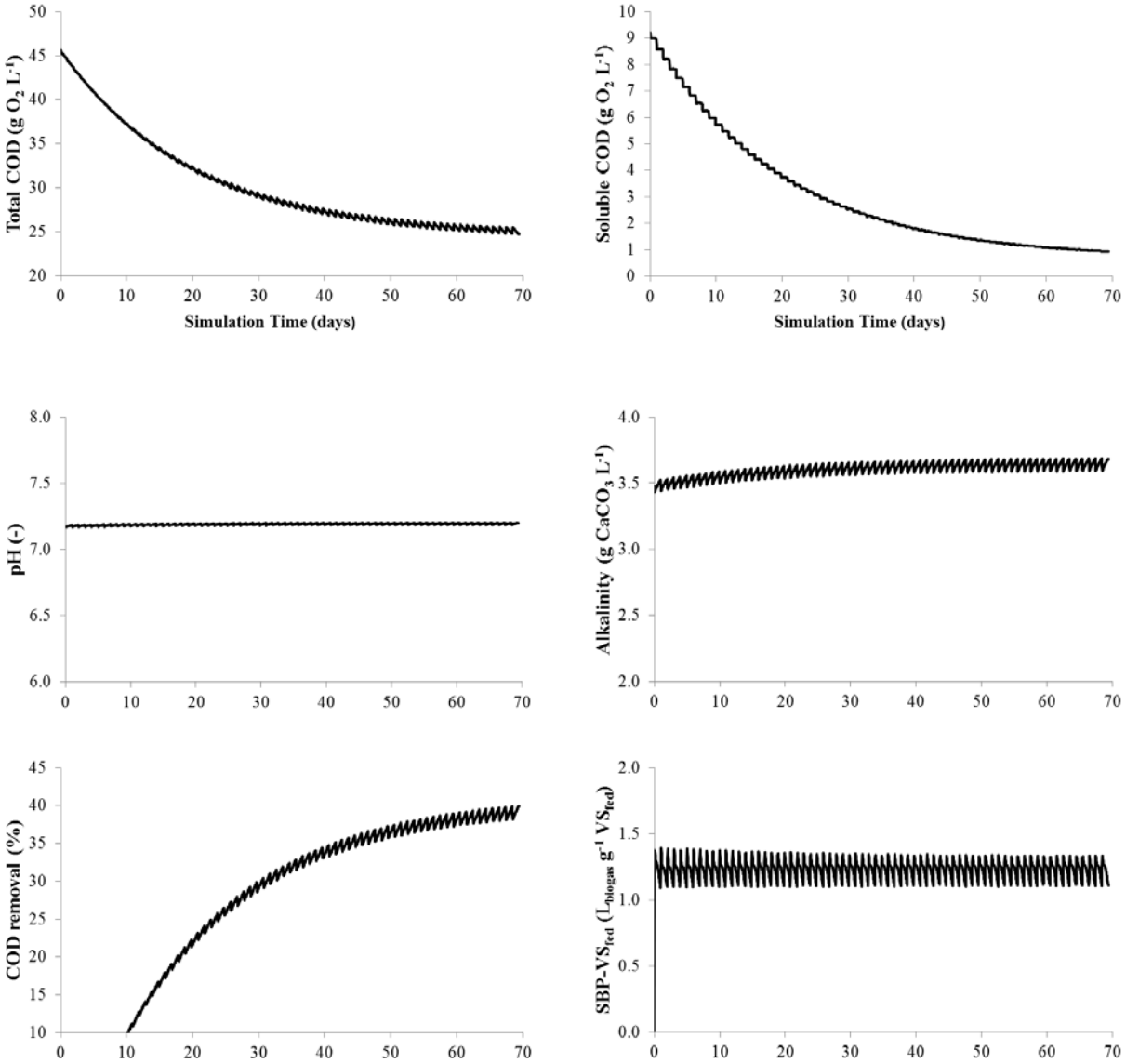


Fig. 4.5. Profiles obtained after 70 days of simulation

Table 4.8. Experimental and simulation outputs

	Units	Experimental	Simulation
CODt	g O_2 L^{-1}	23.7 (21.0 – 25.6)	24.6
CODs	g O_2 L^{-1}	n.r*	0.9
COD removal	%	42.4 (52.2 – 34.4)	40.3
pH	-	7.7 (8.0 - 7.5)	7.2
Total Alkalinity	g $CaCO_3$ L^{-1}	4.1 (4.4 – 3.8)	3.7
TAN	mg N L^{-1}	610 (580 – 627)	550
SBP-VS	L g^{-1} VS_{fed}	1.1 (1.3 – 0.8)	1.1
Methane content	%	n.r	68

*n.r. non-reported

4.5. Conclusions

In this paper, a research on mixed sewage sludge biodegradability and modelling was undertaken in order to clarify literature uncertainty with regard to sewage sludge biodegradability and to develop a methodology to determine ADM1 parameters, solubilisation kinetic and initial state variables. The main conclusions drawn from the study are summarised as follows:

- The ultimate methane potential of the sewage sludges ranged from 188 to 214 mL CH4 g^{-1} COD_{fed}, whereas the COD removals varied between 58 and 65%.

- The apparent first order solubilisation rate of the sewage sludges showed two homogeneous groups: (i) the lowest rate group from 0.23 to 0.35 day^{-1} and (ii) the highest rate group from 0.27 to 0.43 day^{-1}.

- No statistically significant relationship between the ultimate methane potential or the disintegration constant and the sewage sludge characterisation was found. Therefore, an empirical relationship based on sludge characterisation to estimate both values could not be established.

- A 5 – 65% solubilisation rate underestimation was found when the conventional first order rates, obtained from experimental data fitting, were compared with the best fit results of the model. The k_{dis} underestimation was

related to soluble compounds accumulation, mainly long chain fatty acids and acetate.

- The comparison between the simulation and the experimental results showed the consistency of the developed methodology, which is mainly based on the composite concentration and its stoichiometric coefficients.

5. Identification of synergistic impacts during anaerobic co-digestion

Abstract

Anaerobic co-digestion has been widely investigated, but there is limited analysis of interaction between substrates. The objective of this work was to assess the role of carbohydrates, protein and lipids in co-digestion behaviour separately, and together. Two sets of batch tests were done, each set consisting of the mono-digestion of three substrates, and the co-digestion of seven mixtures. The first was done with pure substrates -cellulose, casein and olive oil- whereas in the second slaughterhouse waste -paunch, blood and fat- were used as carbohydrate, protein and lipid sources, respectively. The batch assays and the modelling results clearly demonstrated a synergistic effect of mixing substrates. Co-digestion always led to an improvement of the process kinetics although, usually, without a change in ultimate degradability. Moreover, co-digestion substantially mitigated the effect of inhibitory compounds.

- Astals S, Batstone DJ. Mata-Alvarez J, Jensen P. Identification of synergistic impacts during anaerobic co-digestion of organic wastes. Submitted to Bioresource Technology

5.1. Introduction

Cattle slaughterhouses process meat for human consumption, animal by-products (e.g. meat, bone and blood meal, tallow and skin) and generate a large variety of solid and liquid waste (Cuetos et al., 2008). The latter represents 5-10% of the total animal weight depending on the degree of further processing of the slaughtered animals, with the majority of "waste" being cattle paunch, or undigested feed (Jensen, in submission). Cattle slaughterhouse waste (SHW), which includes multiple waste streams such as stomach and intestinal content, fat, manure, blood and rendering residues, has emerged as an industrial waste with strong potential to recover energy and nutrient resources through waste management. SHW is considered a good substrate for anaerobic digestion, however, the composition of SHW is highly variable with methane yields ranging between 230 and 700 L_{CH4} kg^{-1}VS (Edstrom et al., 2003; Cuetos et al., 2008; Hejnfelt and Angelidaki, 2009; Zhang and Banks, 2012b). Anaerobic treatment of SHW also includes risks associated with the high concentration of ammonia (NH_3) and/or long chain fatty acids (LCFA), potential inhibitors of the methanogenic activity (Cuetos et al., 2008). Ammonia inhibition is related with its capacity to diffuse into microbial cells and a subsequent proton imbalance and/or ion imbalance (Kayhanian, 1999), whereas the adsorption of LCFA onto the cell membrane, interfering with membrane functionality, is widely accepted as the mechanism of LCFA inhibition (Palatsi et al., 2009; Chen et al., 2008). Since ammonia is a by-product of protein acidification and LCFAs are an intermediate product from the degradation of fat, oil and grease, slaughterhouse wastewater and other high-value wastes are also high-risk, with inhibition being directly linked to the composition. Nevertheless, process instability and inhibition may be minimised through anaerobic co-digestion, which uses the degradation properties of a mixture of wastes to mitigate or dilute specific compounds (Mata-Alvarez et al., 2011).

Anaerobic co-digestion (AcoD) is a process where two or more substrates with complementary characteristics are mixed for combined treatment. AcoD has been reported as a feasible solution to overcome ammonia and LCFA inhibition and to improve the methane yield of SHW digestion. SHW have been successfully co-digested with biowaste (Zhang and Banks, 2012b), manure (Hejnfelt and Angelidaki, 2009) and mixture of biowaste and manure (Edstrom et al., 2003; Murto et al., 2004; Alvarez and

Liden, 2008; Cuetos et al., 2008). In AcoD, the improvement in methane production is mainly a result of the increase in organic loading rate (Astals et al., 2012); however, when possible, it is important to choose the best co-substrate and blend ration in order to: (i) favour positive interactions, i.e. synergisms, macro- and micro-nutrient equilibrium and moisture balance; (ii) dilute inhibitory or toxic compounds; (iii) optimise methane production and (iv) enhance digestate stability (Astals et al., 2011; Mata-Alvarez et al., 2011). Even though all these factors should be considered, the decisions on the ratio between wastes had been typically simplified to the optimisation of the carbon-to-nitrogen (C/N) ratio, where optimum reported values vary from 20 to 60 (Alvarez et al., 2010; Esposito et al., 2012; Wang et al., 2012). At the present time, there is limited knowledge about the influence of waste composition (carbohydrates, protein and lipids) on AcoD performance as well as on interactions between substrates that may enhance or attenuate inhibition thresholds, degradation rates, or biogas yields of the process. The degradation of carbohydrates, protein and lipids are characterised by different metabolic pathways, rates and methane yields (Angelidaki and Sanders, 2004); therefore knowledge about the influence of the substrate macro-composition would enhance the understanding and utility of potential and/or novel AcoD applications.

Reliable AcoD modelling is required to predict, in a clear and quantifiable manner, the effect of mixing two or more wastes in a digester and remove potentially negative impacts from mixing based on random or heuristic decisions (Astals et al., 2011; Mata-Alvarez et al. 2011). In addition, a better mechanistic understanding of how different feeds mix may reduce the time and costs associated with laboratory experiments as well as improve co-substrate selection and dose rates (Gali et al., 2009). Models are also useful to estimate important biochemical parameters such as biodegradability, hydrolysis rate and inhibition constant, which are critical in AD design, performance and troubleshooting (Batstone et al., 2009; Jensen et al., 2011). Recent nonlinear parameter estimation methods have provided an increase in level of resolution around prediction of the impacts of AcoD (Batstone 2003 and 2004).

The aim of the present study was to identify the interactions (synergisms and antagonisms) between carbohydrates, protein and lipids that take place during anaerobic co-digestion, focusing on process kinetics and the anaerobic biodegradability of the

substrates for a mechanistic model-based understanding of AcoD. This aims at identifying AcoD opportunities and, consequently, improving the anaerobic digestion of slaughterhouse and other similar wastes.

5.2. Materials and Methods

Biomethane potential test

Biomethane potential (BMP) tests were carried out according to Angelidaki et al. (2009) in 240 mL glass serum bottles at mesophilic temperature. All tests contained 120 mL inoculum, the amount of substrate that met an inoculum to substrate ratio (ISR) of 2 (VS-basis) and deionised water, added to make up the total test volume to 160 mL. Bottles were flushed with 100% N_2 gas for 3 min (1 L min-1), sealed with a rubber stopper retained with an aluminium crimp seal and stored in temperature-controlled incubators (37 ± 1°C). Tests were mixed by inverting once per day. Blanks containing inoculum and no substrate were used to correct for background methane potential in the inoculum. All tests and blanks were carried out in triplicate, and all error bars indicate 95% confidence in the average of the triplicate. Biogas volume was measured by manometer at the start of each sampling event. Accumulated volumetric gas production was calculated from the pressure increase in the headspace volume (80 mL) and expressed under standard conditions (0 °C, 1 atm). At each sample event, the biogas composition (CH_4, CO_2 and H_2) was determined using a PerkinElmer Autosystem 1022 Plus gas chromatograph equipped with a thermal conductivity detector (see Jensen et al., 2011 for GC configuration and procedure).

Two sets of BMP tests were done in order to assess the role of carbohydrates (Ch), protein (Pr) and lipids (Li) in AcoD. Each set of tests consisted of the mono-digestion of three substrates, representative of carbohydrates, protein and lipids, and the co-digestion of 7 mixtures, performed in VS-basis (Fig. 5.1). The first set of BMPs was done with pure substrates, i.e. cellulose, casein and olive oil, whereas in the second set of BMPs complex substrates from an slaughterhouse, i.e. paunch, blood and dissolved air flotation fat sludge (DAF), were used as sources of carbohydrate, protein and lipid, respectively.

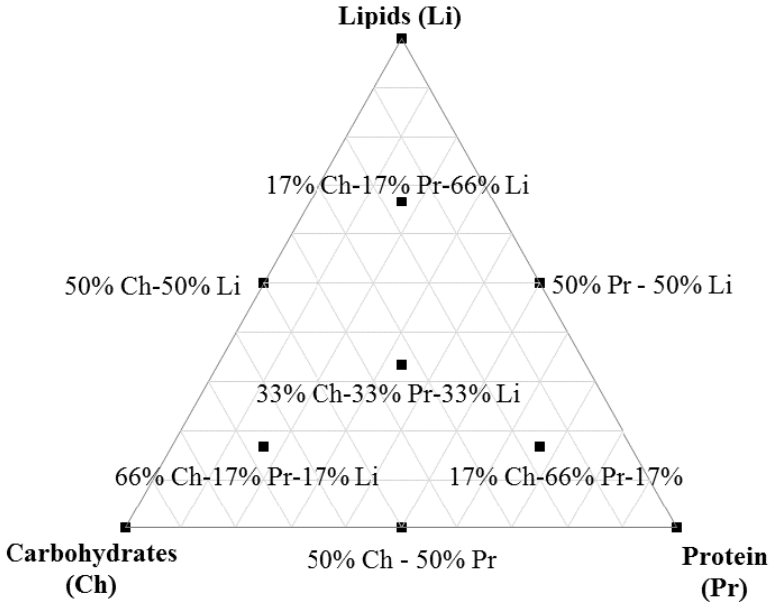


Fig. 5.1. Design of the co-digestion mixtures, organic mass basis (VS), between carbohydrates, protein and lipids

Model implementation and data analysis

Mathematical analysis of the BMPs was based on the IWA Anaerobic Digestion Model No. 1 (ADM1) (Batstone et al., 2002). As hydrolysis step is considered the rate-limiting step during the AD of complex substrates, the BMPs were modelled using a first order kinetic (eq. 5.1) (Jensen et al., 2011; Pratt et al., 2012).

$$r = \left(0_{t< t_{delay}} , \sum_i \left(f_i \cdot k_{hyd,i} \cdot S_i \cdot C_i \cdot I \right)_{t>t_{delay}} \right) \quad \text{(eq. 5.1)}$$

where r is the process rate (mL CH_4 L^{-1} day^{-1}), f_i is the substrate biodegradability (-), $k_{hyd,i}$ is the first order hydrolysis rate constant of the substrate (day^{-1}), S_i is the substrate concentration (g VS L^{-1}), C_i is the COD-to-VS ratio of the substrate, I is the inhibition factor and t_{delay} is the lag-phase, which is global across all substrates. The inhibition factor was included to model LCFA inhibition when lipids were either mono- or co-digested, where the thermodynamic inhibition function as in Pratt et al. (2012) was used instead of the conventional non-competitive inhibition function (eq. 5.2).

$$I = \left(\frac{K_{I,li}}{S_{li} + K_{I,li}} \right)^n \quad \text{(eq. 5.2)}$$

where I is the LCFA inhibition factor, which range from 0 (total inhibition) to 1 (no inhibition), S_{li} is the lipid concentration, $K_{I,li}$ is the inhibition constant (g VS L^{-1}) and n is the inhibition exponent. The exponent allows for an increase in inhibition progression rate compared with the standard non-competitive function.

The model was implemented in Aquasim 2.1d. Parameter estimation and uncertainty analysis were simultaneously estimated, with a 95% confidence limit, as for Batstone et al. (2003 and 2009). Parameters uncertainty was estimated based on a one-tailed t-test on parameter standard error around the optimum, and non-linear confidence regions were also tested to confirm the linear estimate was representative of true confidence. The objective function used was the sum of squared errors (χ^2), where average data from triplicate experiments were used.

Table 5.1. Characterisation of the pure substrates

	Units	Cellulose	Casein	Olive oil
TS	g kg^{-1}	918	946	1000
VS	g kg^{-1}	915	913	1000
COD_T	g O_2 kg^{-1}	976	1401	2890

Substrates and inoculum origin

Pure substrate included analytical grade cellulose and casein purchased from Sigma-Aldrich® and white-label refined olive oil, which contains mainly palmitic, oleic and linoleic acid (AOCS, 2013). Slaughterhouse wastes, i.e. paunch, blood and fat from a dissolved air flotation (DAF), were obtained from a slaughterhouse of Queensland (Australia). Table 5.1 shows a basic characterisation of the pure substrates, while Table 5.2 shows a complete physical-chemical characterisation of SHW. The CODt of cellulose and olive oil were calculated by multiplying the VS concentration by the theoretical oxygen demand of cellulose (1.07 g COD g^{-1} VS) and oleic acid (2.89 g COD g^{-1} VS), respectively, while the CODt of DAF sludge, which could not be analysed due to analytical interferences, was estimated by multiplying its VS concentration by 3.0 g COD g^{-1} VS. The inoculum was collected from an anaerobic digestion at a municipal WWTP in Queensland. The inoculum was treating mixed

primary sludge and waste activated sludge, the specific methanogenic activity of the inoculum at 37 ºC was 0.2 g COD CH_4 g^{-1} VS day^{-1}.

Table 5.2. Characterisation of the slaughterhouse wastes

	Units	Paunch	Blood	DAF
TS	g kg^{-1}	117	187	360
VS	g kg^{-1}	106	178	353
COD_T	g O_2 kg^{-1}	106	266	1053
COD_S	g O_2 kg^{-1}	2.5	253	3.7
VFA	g L^{-1}	0.64	1.86	0.52
- Acetic acid	g L^{-1}	0.36	1.47	0.22
- Propionic acid	g L^{-1}	0.18	0.19	0.27
- Butyric acid	g L^{-1}	0.08	0.15	0.01
- Valeric acid	g L^{-1}	0.03	0.05	0.02
Ethanol	g L^{-1}	0.02	0.14	0.06
Oil and grease	g kg^{-1}	4.5	1.5	265
Total proteins	g kg^{-1}	10.2	129.5	11.8
Soluble proteins	g kg^{-1}	1.7	128.2	0.4
Total carbohydrates	g kg^{-1}	55.5	3.7	0.6
Soluble carbohydrates	g kg^{-1}	1.6	0.1	0.4
TKN	g kg^{-1}	0.60	26.7	1.2
TKP	g kg^{-1}	0.21	0.20	0.29
Chloride	mg L^{-1}	147	2617	84
Ammonium	mg N L^{-1}	143	391	49
Nitrite	mg N L^{-1}	0.2	1.1	0.5
Nitrate	mg N L^{-1}	0.05	0.97	0.01
Phosphate	mg P L^{-1}	161	164	162
Sulphate	mg S L^{-1}	9.3	38	19
Aluminium	mg g^{-1} TS	0.86	n.d.	n.d.
Calcium	mg g^{-1} TS	4.09	n.d.	7.48
Iron	mg g^{-1} TS	0.84	0.25	0.29
Lead	mg g^{-1} TS	0.003	0.004	0.011
Magnesium	mg g^{-1} TS	0.46	n.d.	n.d.
Phosphor	mg g^{-1} TS	2.13	0.13	2.53
Potassium	mg g^{-1} TS	1.39	n.d.	0.19
Silicium	mg g^{-1} TS	0.24	0.001	0.20
Zinc	mg g^{-1} TS	0.02	n.d.	0.01

5.3. Results and discussion

5.3.1. Biomethane potential tests result

Synthetic substrates

Methane production of cellulose and casein followed first order process kinetics with B_0 values of 319 ± 6 and 431 ± 7 mL CH_4 g^{-1} VS, respectively; whereas olive oil, with a B_0 of 816 ± 33 mL CH_4 g^{-1} VS, showed a sigmoidal profile (Fig. 5.2). B_0 values and their uncertainty were outputs of the BMP modelling. Olive oil shape was probably due to LCFA inhibition of the methanogens, although the initial olive oil concentration (4.8 g L^{-1}) was far above the reported half maximal inhibitory concentration (IC_{50}) values for LCFA, which range from 50 to 1500 mg L^{-1} (Palatsi et al., 2009). In addition, the short lag phase (1.5 days) indicated that inhibition and adsorption was followed rapidly by conversion through methanogenesis, which is in contrast to the normal longer lag period (> 10 days) corresponding to a strong inhibition of the methanogens (Hwu et al., 1998; Salminen et al., 2000; Palatsi et al., 2009). The shorter lag period can likely be related to the relatively high inoculum-to-lipid ratio used in our tests (Hwu et al., 1998; Salminen et al., 2000).

To compare the response from pure substrates with those from co-digestion, we generate a simple prediction curve based on the combination of substrates over time, proportioned to the amount of substrate present. Fig. 5.2 shows the three pure substrates (top left), and predicted and actual curves for each substrates. These demonstrate a clear kinetic advantage caused by mixing substrates, but without any impact on methane yield (net B_0). Kinetic improvement where mixtures present high concentration of olive oil was clearly due to attenuation of inhibition. This could be a consequence of lower LCFA concentrations in the mixture and the synergy between substrates. Therefore, it can be concluded that substrate diversification improved the AD rate and reduced the inhibitory effect of LCFA. The present results are in agreement with Kuang et al. (2002) who concluded that the addition of glucose (carbohydrate) and cysteine (protein), either singly or in combination, decreased LCFA inhibition and improved the formation of granular biomass in high rate anaerobic reactors. Feeding glucose and/or cysteine to an LCFA inhibited digester also stimulates the degradation of LCFA and the growth of methanogenic archaea to enable a rapid recovery of digester performance (Kuang et al. 2006).

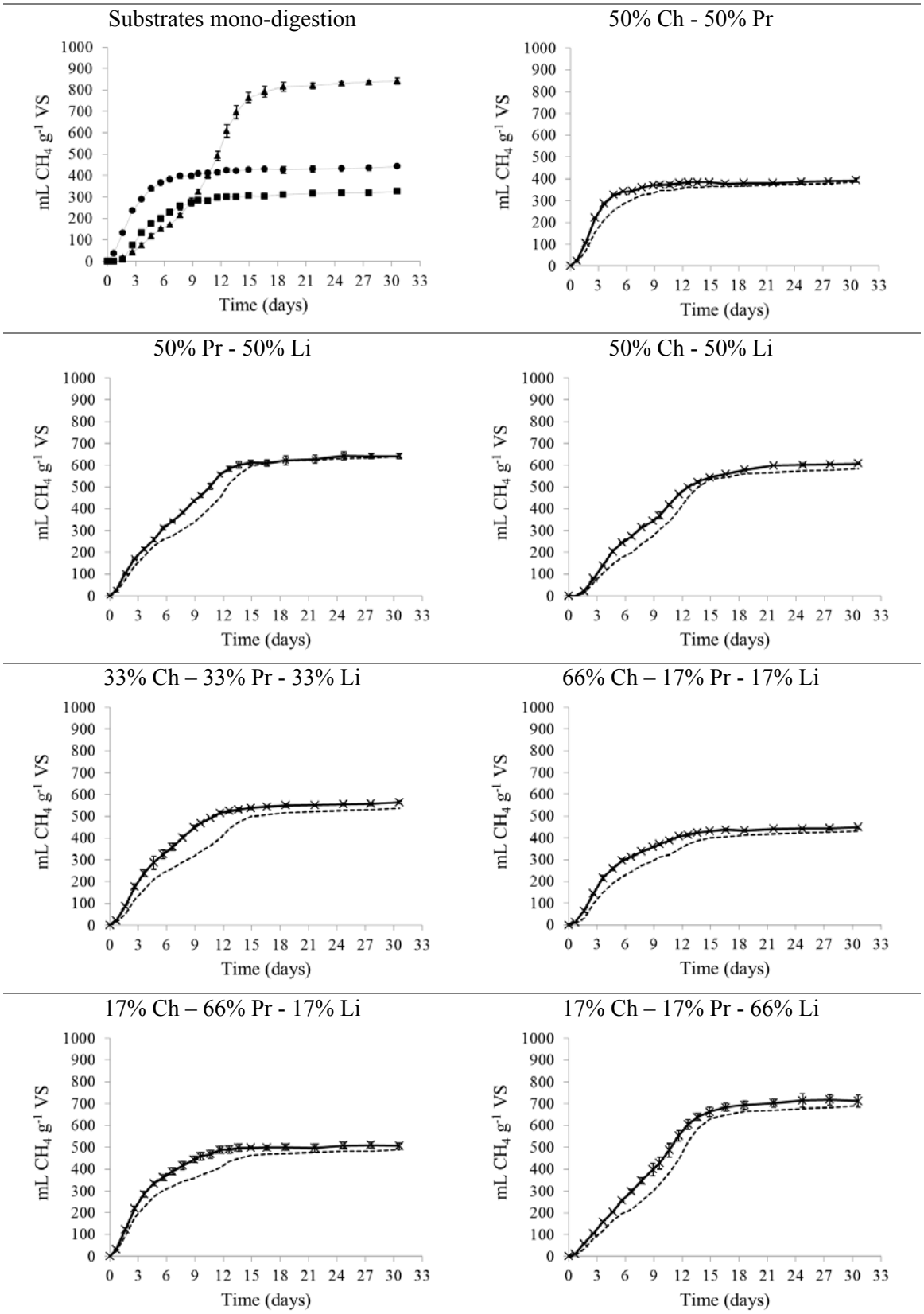


Fig. 5.2. Cumulative methane production in the course of time of synthetic substrates: mixture (×), theoretical profile of the mixture (dashed line), cellulose (■), casein (▲) and olive oil (●).

Cattle slaughterhouse wastes

As Table 5.2 shows, paunch, blood and DAF are high in carbohydrates, protein and lipids, respectively. When the SHW mono-digestion BMP results were compared with the results obtained from the pure substrates there was very strong overlap in methane profiles when comparing both the casein and blood tests, and the olive oil and DAF sludge tests. In contrast, paunch, due to its lignocellulosic composition, presented a flattened profile and reduced B_0 compared to cellulose. Paunch, blood and DAF presented B0 of 237 ± 14, 410 ± 8 and 824 ± 38 mL CH_4 g^{-1} VS, respectively. Again, B_0 values and their uncertainty were obtained through the BMP modelling. When the B_0 values where compared with the values reported by Hejnfelt and Angelidaki (2009) there was a good agreement in the B_0 of blood (450 mL CH_4 g^{-1} VS), whereas the B_0 reported for fat (560 mL CH_4 g^{-1} VS) was much lower than in the present study. Differences in the B_0 of fat be can be related with the fat origin and structure. The B_0 of paunch is in the range of those values reported for paunch and lignocellulosic agricultural wastes (Tong et al., 1990; Tritt et al., 1991). DAF sludge showed LCFA inhibition similar to the olive oil test.

All AcoD mixtures between SHW presented an improvement in the digestion kinetics when compared with the theoretical predictions (Fig. 5.3). The lipid-rich SHW mixtures (50%Ch - 50%Li; 50%Pr - 50%Li; 33%Ch - 33%Pr - 33%Li and 17%Ch - 17%Pr - 66%Li) showed a greater improvement in the process kinetics than that observed for pure substrates, whereas the other mixtures presented a similar trend. In the lipid-rich mixtures, the increase of the slope in the cumulative methane production, related with the greater LCFA methanisation period, was observed at day 4-5 instead of day 7. Therefore, AcoD mitigated LCFA inhibition in the SHW tests similar to the synthetic tests. Again, the reduction of LCFA inhibition could be related to lower LCFA concentration in the mixture and a synergy between substrates. However, the increased mitigation of LCFA inhibition in the SHW tests compared to the synthetic tests could be due to the adsorption of the LCFA on the surface of the paunch and/or blood, thus lowering the absorption of LCFA on the methanogen cell membrane. Consequently, the LCFA inhibition was further reduced and the methane production stimulated (Palatsi et al., 2009; Cuetos et al., 2010).

Two mixtures (50%Ch - 50%Li; 17%Ch - 17%Pr - 66%Li) resulted in a B_0 significantly higher than the theoretical prediction. The 15% difference between the theoretical B_0 and actual B_0 may be related with the capacity of the hydrolytic biomass present in the paunch to further hydrolyse the DAF sludge (slurry with small fat conglomerates). This conclusion is supported by a COD balance, as the paunch and blood COD were not enough to justify the difference of 80 and 95 mL CH_4 g^{-1} VS, respectively, between the theoretical and actual B_0. Paunch refers to the stomach contents of cattle and contains rumen micro-organisms consisting of bacteria, protozoa, and fungi, which are highly efficient at hydrolysis of lignocellulosic material. Nevertheless, paunch also contains, in a minor degree, lipolityc biomass which is able to breakdown lipids to fatty acids (Kim et al., 2009). For paunch lipolityc biomass, the degradability of unprotected lipids has been estimated to be about 90%, while the hydrolysis of structural plant lipids is thought to be lower due to the need to remove surrounding cellular matrices (Kim et al., 2009). In any case, the presence of lipid-degrader biomass in the paunch may have improved the degradation rate and extent of DAF in the aforementioned mixtures.

Small improvements in B_0 values were recorded in other AcoD mixtures, however, the difference between the theoretical and actual values were lower than 7%, and were considered not significant. The minor improvement in the process kinetics and B_0 recorded in the mixture between paunch and blood (50%Ch – 50%Pr) is in agreement with the result obtained by Elbeshbishy and Nakhla (2012) when co-digesting a 50% starch (carbohydrates) and 50% bovine serum albumin (protein) mixture (weight-basis). However, the same authors reported that the 80% starch and 20% bovine serum albumin mixture had a significant impact on the process kinetics and B_0 as both were much higher than the expected values (Elbeshbishy and Nakhla, 2012). Finally, it must be noted that the reported methane yields for mixed slaughterhouse are in the range of 400 - 600 mL CH_4 g^{-1} VS (Edstrom et al., 2003; Cuetos et al., 2008; Hejnfelt and Angelidaki, 2009; Zhang and Banks, 2012b). However, as shown by the results obtained in the present study, the methane potential and kinetic are greatly influenced by the SHW composition, with similar impacts and variability expected during full scale implementations.

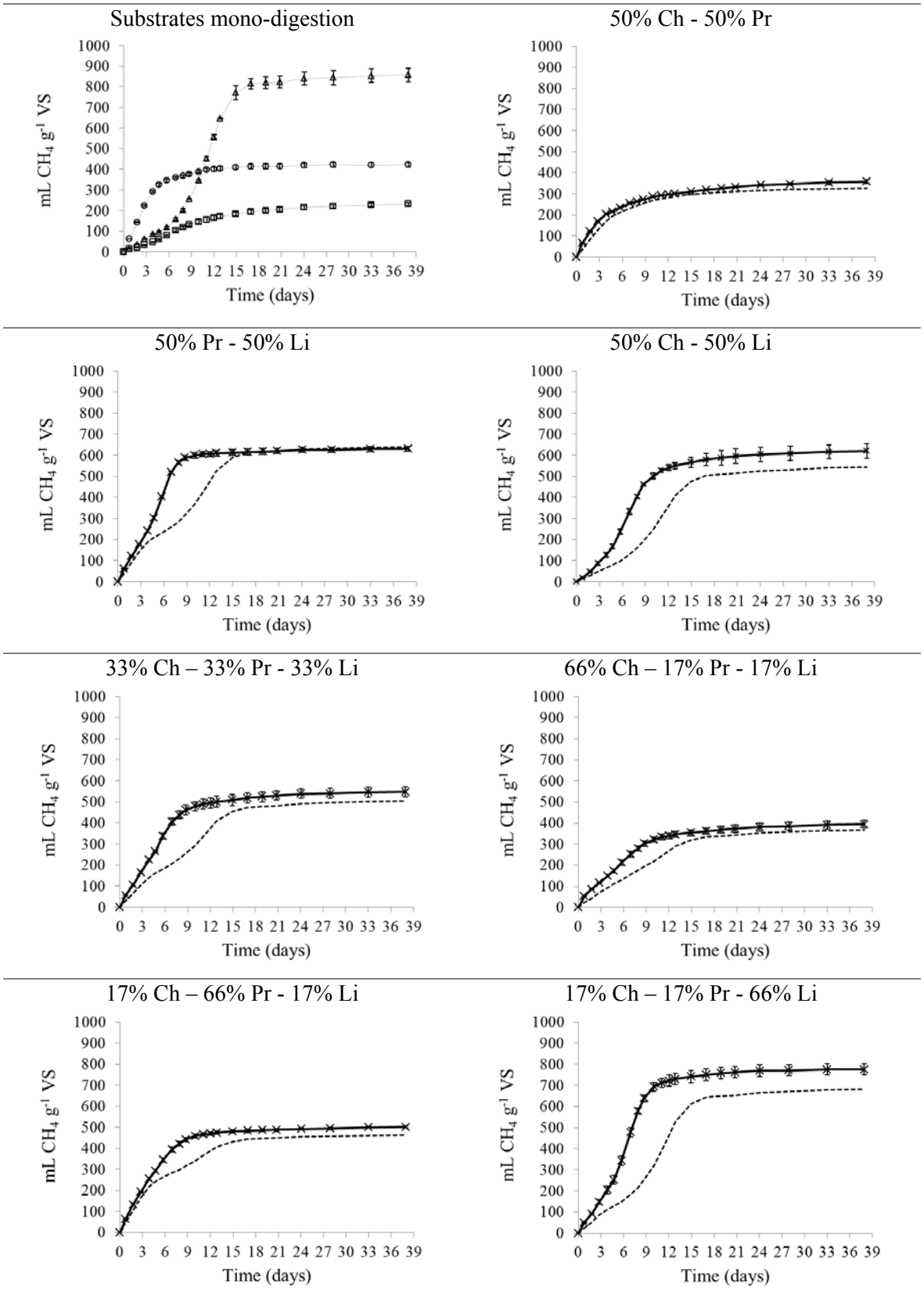


Fig. 5.3. Cumulative methane production in the course of time of each SHW mixture (×), theoretical profile (dashed line), paunch (□), blood (Δ) and DAF (○).

5.3.2. Model-based parameter estimation

The kinetic parameters estimated in the present work, either mono- or co-digestion, are substrate biodegradability (f), degradation kinetic (k_{hyd}) and LCFA inhibition, which quantifies the fraction of material that may be degraded under anaerobic conditions and the speed of degradation. The model parameters and the 95% confidence interval estimated for pure substrates and slaughterhouse wastes are provided in Table 5.3 and 5.4, respectively.

The high biodegradabilities, between 85% and 97%, obtained in all scenarios for cellulose, casein and olive oil are in agreement with the B_0 values obtained and confirmed the absence of any antagonism AcoD phenomena related with the organic matter intrinsic composition which could reduce substrate biodegradability. Blood and DAF also presented in all scenarios high biodegradabilities (> 85 %), whereas paunch, as lignocellulose material, presented lower values (~75 %). The high biodegradabilities of the SHW are in agreement with already reported values, which range from 70 to 90 % (Tritt et al., 1991; Hejnfelt and Angelidaki, 2009; Zhang and Banks, 2012b). In contrast, the hydrolysis rate of synthetic substrates and SHW presented a higher variability between substrates and scenarios. The hydrolysis rate and the biodegradability of blood and DAF are statistically similar to those modelled for casein and olive oil, respectively.

The interaction between all substrates presented different trends. These results suggest that the interactions between substrates do not only depend on the macro-composition but also on other physicochemical properties such as structure. The improvement of the process kinetic when the AcoD profiles were compared with the expected ones was reflected, either for pure substrate and SHW, by the increase of the hydrolysis rate of one or more compounds, when compared with the mono-digestion values, and by a reduction of the lipids IC_{50}. For synthetic substrates and SHW mixtures rich in lipids (50%Ch - 50%Li; 50%Pr - 50%Li; 33%Ch - 33%Pr - 33%Li and 17%Ch - 17%Pr - 66%Li), the increase of the LCFA methanisation and, therefore, the absence of the sigmoidal shape in the BMP profile were modelled by a significant reduction of the LCFA IC_{50} instead of increasing the $k_{hyd,li}$, which never exceeded the value modelled for olive oil and DAF. In this matter, although DAF presented higher IC_{50} than olive oil, the

reduction of the IC_{50} values presented a similar behaviour in both scenarios: (1) IC_{50} values, except for the mixtures with 17% lipids, decreased as the lipid initial concentration diminished and (2) the mixture 33%Ch - 33%Pr - 33%Li presented the lower IC_{50} value. Regarding pure substrates, the IC_{50} of the olive oil (1.3 g VS L^{-1}) was reduced to 0.9 g VS L^{-1} when co-digested with low quantities of cellulose and casein (17%Ch - 17%Pr - 66%Li) and to about 0.8 g VS L^{-1} when singly co-digested with cellulose or casein (50%Ch - 50%Li and 50%Pr - 50%Li). The IC_{50} value was even more reduced (~0.7 g VS L^{-1}) when the olive oil only represented the 17% of the VS content. Nevertheless, the lower IC_{50} value (0.6 g VS L^{-1}) was obtained for the 33%Ch - 33%Pr - 33%Li, phenomena that highlights the synergism between substrates. The present results are in agreement with Kuang et al. (2006) who reported that feeding a mixture of glucose and cysteine to an inhibited LCFA digester enabled a faster recovery of the digester performance than feeding glucose or cysteine by itself. The same authors also concluded that glucose was more effective than cysteine; however, the IC_{50} values obtained in the present study for cellulose and casein are statistically similar, therefore, both substrates are equally effective to reduce LCFA inhibition and stimulate methanisation.

The reduction of the LCFA inhibition for the SHW mixtures was in absolute values, except for the mixture 17%Ch - 17%Pr - 66%Li, more significant than for pure substrate mixtures, since the IC_{50} values were reduced from 1.7 g VS L^{-1} to 0.9 – 1.0 g VS L^{-1}. These facts confirmed the existence of an extra mechanism, not present in pure substrates AcoD, that reduced the methanogens LCFA inhibition; probably the absorption of LCFA on the surface of the paunch and/or blood. Finally, regarding the two SHW mixtures that produced more methane than expected (50%Ch - 50%Li; 17%Ch - 17%Pr - 66%Li), it is important to highlight that the model estimated a paunch and DAF biodegradability of 85 and 99 %, respectively, much higher than when mono-digested, however, there was not an improvement of the hydrolysis rates. These results suggest that the absorption of DAF onto the paunch not only improved DAF biodegradability but also paunch.

Table 5.3. Model parameters for pure substrates mono- and co-digestion

Parameter	Description	Units	Cellulose (Ch)	Casein (Pr)	Olive oil (Li)	50%Ch 50%Pr	50%Pr 50%Li	50%Ch 50%Li	33%Ch 33%Pr 33%Li	66%Ch 17%Pr 17%Li	17%Ch 66%Pr 17%Li	17%Ch 17%Pr 66%Li
f_{ch}	biodegradability of Ch	-	0.93 ± 0.02	-	-	0.90 ± 0.10	-	0.98 ± 0.02	0.97 ± 0.01	0.95 ± 0.05	0.95 ± 0.05	0.96 ± 0.04
f_{pr}	biodegradability of Pr	-	-	0.87 ± 0.01	-	0.91 ± 0.09	0.81 ± 0.02	-	0.97 ± 0.01	0.79 ± 0.04	0.95 ± 0.03	0.97 ± 0.03
f_{li}	biodegradability of Li	-	-	-	0.88 ± 0.03	-	0.93 ± 0.01	0.93 ± 0.01	0.91 ± 0.01	0.86 ± 0.05	0.90 ± 0.04	0.94 ± 0.03
$k_{hyd,ch}$	hydrolysis constant of Ch	day^{-1}	0.26 ± 0.02	-	-	0.33 ± 0.12	-	0.27 ± 0.03	0.34 ± 0.01	0.32 ± 0.07	0.20 ± 0.07	0.12 ± 0.05
$k_{hyd,pr}$	hydrolysis constant of Pr	day^{-1}	-	0.35 ± 0.03	-	0.75 ± 0.31	0.40 ± 0.05	-	0.36 ± 0.02	0.19 ± 0.05	0.26 ± 0.06	0.55 ± 0.10
$k_{hyd,li}$	hydrolysis constant of Li	day^{-1}	-	-	2.33 ± 0.52	-	0.79 ± 0.04	0.81 ± 0.16	0.89 ± 0.07	2.09 ± 0.39	2.71 ± 0.29	1.16 ± 0.28
$K_{I,li}$	inhibitor constant	g VS L^{-1}	-		5.78 ± 0.90	-	0.58 ± 0.14	0.73 ± 0.17	0.07 ± 0.03	0.46 ± 0.37	1.37 ± 0.45	1.44 ± 0.34
n	inhibitor exponent	-	-		3.50 ± 0.44	-	0.79 ± 0.12	0.94 ± 0.12	0.34 ± 0.05	0.66 ± 0.30	1.77 ± 0.39	1.47 ± 0.24
t_{dealy}	lag period	day	1.56 ± 0.19	0.45 ± 0.13	2.02 ± 0.39	1.00 ± 0.20	0.08 ± 0.05	0.68 ± 0.11	0.38 ± 0.04	1.01 ± 0.21	0.35 ± 0.23	0.43 ± 0.17
IC_{50}	50% lipids inhibitory concentration	g VS L^{-1}	-	-	1.27 ± 0.05	-	0.82 ± 0.02	0.79 ± 0.03	0.58 ± 0.07	0.70 ± 0.05	0.65 ± 0.04	0.85 ± 0.04

Table 5.4. Model parameters for slaughterhouse waste mono- and co-digestion

Parameter	Description	Units	Cellulose (Ch)	Casein (Pr)	Olive oil (Li)	50%Ch 50%Pr	50%Pr 50%Li	50%Ch 50%Li	33%Ch 33%Pr 33%Li	66%Ch 17%Pr 17%Li	17%Ch 66%Pr 17%Li	17%Ch 17%Pr 66%Li
f_{ch}	biodegradability of Ch	-	0.74 ± 0.04	-	-	0.80 ± 0.17	-	0.87 ± 0.07	0.71 ± 0.07	0.76 ± 0.10	0.86 ± 0.04	0.64 ± 0.11
f_{pr}	biodegradability of Pr	-	-	0.87 ± 0.01	-	0.86 ± 0.14	0.87 ± 0.04	-	0.98 ± 0.02	0.93 ± 0.05	0.92 ± 0.02	0.97 ± 0.03
f_{li}	biodegradability of Li	-	-	-	0.85 ± 0.04	-	0.85 ± 0.03	0.98 ± 0.02	0.99 ± 0.01	0.95 ± 0.04	0.99 ± 0.01	0.97 ± 0.03
$k_{hyd,ch}$	hydrolysis constant of Ch	day^{-1}	0.11 ± 0.02	-	-	0.11 ± 0.05	-	0.14 ± 0.04	0.07 ± 0.04	0.15 ± 0.07	0.09 ± 0.04	0.15 ± 0.09
$k_{hyd,pr}$	hydrolysis constant of Pr	day^{-1}	-	0.31 ± 0.03	-	0.47 ± 0.18	0.55 ± 0.50	-	0.76 ± 0.13	0.50 ± 0.17	0.59 ± 0.07	0.62 ± 0.18
$k_{hyd,li}$	hydrolysis constant of Li	day^{-1}	-	-	2.65 ± 0.34	-	2.20 ± 0.45	2.47 ± 0.48	1.10 ± 0.32	0.77 ± 0.44	0.65 ± 0.21	2.02 ± 0.53
$K_{I,li}$	inhibitor constant	g VS L^{-1}	-		18.7 ± 0.7	-	2.82 ± 0.50	3.75 ± 0.31	0.88 ± 0.39	0.82 ± 0.54	0.70 ± 0.25	2.87 ± 0.64
n	inhibitor exponent	-	-		7.52 ± 0.46	-	2.30 ± 0.33	2.90 ± 0.38	0.99 ± 0.25	0.96 ± 0.49	0.79 ± 0.19	1.96 ± 0.37
t_{dealy}	lag period	day	1.47 ± 0.66	0.24 ± 0.20	0..31 ± 0.30	0.44 ± 0.12	0.11 ± 0.11	0.44 ± 0.29	0.15 ± 0.15	0.19 ± 0.19	0.09 ± 0.09	1.07 ± 032
IC_{50}	50% lipids inhibitory concentration	g VS L^{-1}	-	-	1.74 ± 0.05	-	0.99 ± 0.03	1.01 ± 0.02	0.86 ± 0.09	0.91 ± 0.08	0.97 ± 0.04	1.22 ± 0.02

5.4. Conclusions

The work demonstrates in a clear and quantifiable manner the synergism mechanisms that occur during AcoD and discards that AcoD can generate any antagonisms effect because of the substrate intrinsic composition. Other conclusions are summarised as follows:

- Substrate diversification improved process kinetics. The synergisms of mixing substrates lead to an improvement in AD kinetics for all mixtures. However, as a general trend, the ultimate methane production was not affected.

- Mixing waste is a feasible option to reduce the impact of inhibitory compounds. The introduction of a carbohydrates and/or protein source to lipids reduced the LCFA inhibition, present in lipid AD.

- Paunch and DAF resulted, when compared with the theoretical one, in a higher methane yield. Results suggest that the biomass present in the paunch may contribute to improved hydrolysis of the partially biodegradable fat conglomerates present in the DAF.

6. Co-digestion of pig manure and glycerol: experimental and modelling study

Abstract

It is a fact that the rapid increase of biodiesel production over the last years has resulted in the generation of large and constant amounts of glycerol, which is causing an oversupply problem. Since glycerol is a biodegradable organic compound exempt of nitrogen, it can be applied as a co-substrate in the anaerobic digestion process of pig manure (PM). In order to analyse the feasibility of a mixture of pig manure and glycerol in anaerobic processes and to define the effect originated by the nitrogen limitation when large amounts of glycerol are added, several biodegradabilty batch tests were performed with different mixtures. These were named as: 100% PM, 80% PM, 60% PM, 40% PM and 20% PM, in pig manure wet weight-basis. Furthermore, a modified model based on anaerobic digestion model no.1 (ADM1) was used to simulate the methane production profiles for the mixtures tested. Specifically, both experimental and model results show the power of the co-digestion technology. In particular, the mixture of 80% PM produced the highest methane production with 215 mL CH_4 g^{-1} COD, almost 125% more methane than when pig manure was mono-digested. In contrast, the one with 20% PM was clearly inhibited by the volatile fatty acid due to the low nitrogen concentration of the mixture. In addition, the specific methane production predicted by the model was in good agreement with the experimental results, although in some samples the shape of the profiles did not match perfectly. Moreover, the modified ADM1 appears to be a useful tool to predict the methane production and the limitations related to the lack/excess of nitrogen during the co-digestion process of pig manure and glycerol.

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- Astals S, Ariso M, Galí A, Mata-Alvarez J (2011). Co-digestion of pig manure and glycerine: Experimental and modelling study. J Environ Manage 92:1091-1096

6.1. Introduction

Traditionally, animal feed was grown in farms and residues from animals were returned to the land as fertilizers. As time passed by, in the mid-twentieth century, farmers specialized in the cattle field. This was a consequence of the increasing market demands, the development of genetic material and farming equipment and the availability of cheaper feed. All these changes lead to the increase of the animal farm size and the beginning of the intensive livestock farming (IPPC, 2003). In contemporary society, in most countries, the pig production is concentrated in certain regions, where generally there is not enough land available to utilize all the manure production as a fertiliser. From an environmental point of view, in these regions the impact of the intensive pig farming is one of the most important issues society should be concerned about as a result of the increasing contamination of the soil, the water and the air (Danés et al., 1996). These potential negative contributions to the environment from the pig manure (PM) make its correct management and treatment necessary.

The biodiesel production in the European Union has increased from 500,000 tonnes in 1998 to 9,000,000 tonnes in 2009 (EBB, 2010). The main by-product of the biodiesel production is crude glycerol, which is about 10% of the weight of the initial raw matter (Dasari et al., 2005). However, since the crude glycerol is a mixture of glycerol itself, with alcohols, water, salts, heavy metals, free fatty acids, unreacted mono-, di- and triglycerides, methyl esters, among others it has few direct uses and it possesses a very low value (Frangui and Milford, 1999; Pagliaro and Rossi, 2008). As a consequence, biodiesel producers refine the crude glycerol through filtration, chemical additions, and fractional vacuum distillation to yield various commercial grades before the by-product is moved to different markets in other industries. In other cases, if it is to be used in the alimentary, the cosmetic or the drug industry further treatments like bleaching, deodoring, and ion exchange are needed to remove its trace properties (Pachauri and He, 2006; Pagliaro and Rossi, 2008). The main drawback of the crude glycerol purification for the aforementioned purposes is its high cost and that make it out of the range of the economic feasibility of the small and medium size plants (Pachauri and He, 2006). At the present time, the outlook of the glycerol is uncertain since the existing glycerol market cannot absorb the large rise of this product brought from the biodiesel plants completely. The main evidence of this situation is the fall of the price of the

glycerol which has made this by-product become a waste (Johnson and Taconi, 2007). Within this scenario, it is important to consider that a low-grade glycerol utilization should be developed in order to make the cost of the biodiesel production sustainable in the long term.

The advantages of the anaerobic co-digestion have been widely described in Section 1.4. However, some drawbacks exist as well: (i) the high cost of waste transfer from the co-substrate generation point to the anaerobic plant, (ii) the risk of spreading poisonous substances originated from the industrial or municipal waste and (iii) the harmonisation of different policies of the waste generators. What is more, co-digestion will change the digestion behaviour and the quality of the digestate; furthermore, the addition of unknown co-substrate should be prevented. In order to better the results of the co-digestion and to detect the amounts of inhibitory or toxic compounds, which can lead to a process breakdown or decrease the methane production, it is necessary to carry out several laboratory experiments such as the biodegradability test and/or the lab-scale digester (Braun et al., 2002). As detailed in Section 1.4.4, the achievement of a successful combination of different types of waste requires careful management since random or heuristic decisions on the ratio between waste streams or feedstock to full-scale plants often have negative effects on the digester medium, and a significant reduction of the methane production (Zaher et al., 2009). One of the consequences of this need is the rise on the search for an accurate modelling of the anaerobic degradation of wastes (Angelidaki et al., 1997).

In the present study the feasibility of co-digestion of pig manure and glycerol based on both experimental and modelling results has been evaluated. To achieve this objective, several anaerobic biodegradability tests have been performed with different percentages of glycerol. Finally, the experimental data have been compared to the results of the modified version of ADM1, in order to check if the model can predict the co-digestion process and the nitrogen limitation.

6.2. Materials and Methods

Biomethane potential test

The main objectives of this study were the quantification of the methane potential for each substrate (mL CH_4 g^{-1} COD_{added}) and the analysis of the change of the profile when there is a nitrogen limitation. These tests were performed using reactors of a total volume of 250 mL, which were filled with substrate and an inoculum at a 0.75 of $COD_{substrate}$/ $VS_{inoculum}$ ratio. Therefore, equal amounts of COD for each sample were added into the digesters in order to compare the obtained results while the effective reactor volume was set up to 230 mL with deionised water. The methane production during a running test was measured by using a displacement liquid device equipped with a biogas wash vessel to remove the CO_2 from the biogas (Benabdallah et al., 2007).

Structure of the model

In particular Galí et al. (2009) developed a model for agro-wastes in MATLAB/SIMULINK, with the code written in C language, were differential equations were used instead of algebraic equations (see more details in Section 3.3). The data from the practical information (substrate characterization, stoichiometry and kinetics) is taken from a Microsoft Excel file which, afterwards, is reported to Matlab. Simulink acts as a flow sheet diagram software where the different units (reactors) are connected with the influent flow-rates. Fig. 3.2 shows the model scheme when it is operated by one or two indistinct stirred reactors. To build up the model, an extended characterization of the substrate must be done, which allows calculating the percentage in which COD is structured, while previous batch test have been done to determine the disintegration constant. Finally, introducing the initial amount of substrate and the retention time, all the variables are defined and the model is ready to be run (Galí et al., 2009).

Mixed sewage sludges and inoculum origin

The pig manure and the inoculum were brought to the laboratory from an industrial plant, which treats the manure anaerobically, located in Lleida (Spain). Analytical grade glycerol was purchased from Panreac Quimica, S.A.

6.3. Results and discussion

As detailed above, some substrates can present some limitations and appear to be low efficient when they are degraded anaerobically. The main constraint of the pig manure is the imbalance of its nutrient content -low carbon-to-nitrogen (C/N) ratio- which decreases the microorganism activity. In this study, synthetic glycerol was used to avoid interferences from the minority compounds which could be present in industrial glycerol, and to analyse the viability of the co-digestion between substrates.

Table 6.1. Characteristics of PM, GLY, DPM and each mixture

Parameter	Units	PM	GLY	80% PM	60% PM	40% PM	20% PM	DPM
Density	kg L^{-1}	1.0	1.3	1.1	1.1	1.2	1.2	1.0
TS	g L^{-1}	50.4	850.5	180	380	617	800	16.0
VS	g L^{-1}	35.4	850.3	155	372	610	797	9.8
CODt	g O_2 L^{-1}	69.7	2,256	308	883	1,432	2,086	13.3
NTK	g N L^{-1}	5.3	0.00	4.6*	3.6*	2.5*	1.3*	1.7
TAN	g N L^{-1}	4.4	0.00	3.3	2.9	2.0	1.0	1.4
C/N	g C g^{-1}N	16.4	-	23.4	202	434	1,131	12.4
pH	-	7.5	7.0	7.4	7.4	7.4	7.3	8.2
VFA	mg L^{-1}	5.7	0.00	4.8	3.8	2.3	1.4	0.2
Partial Alk.	g $CaCO_3$ L^{-1}	8.2	0.1	6.5	5.2	3.5	1.7	5.0
Total Alk.	g $CaCO_3$ L^{-1}	16.7	0.1	14.1	11.0	7.8	4.1	6.3

* Estimated through mass balance

6.3.1. Substrate and inoculum characterisation

The characteristics of the pig manure (PM), the glycerol (GLY) and the inoculum (digested pig manure, DPM) used in this study and the tested mixtures are shown in Table 6.1. Five samples with different concentration levels in wet weight-basis of PM were tested (100% PM, 80 % PM, 60 % PM, 40 % PM and 20 % PM) with the aim at scanning all the possible mixtures between these two substrates and at creating different scenarios. As a co-substrate, glycerol can give a solution to some issues when it is digested anaerobically with pig manure. Particularly, it can balance the C/N ratio of the mixture and it can dilute the ammonium nitrogen concentration in the digester medium. Nevertheless, the low alkalinity of the glycerol reduces the alkalinity in the mixture-feed and, as consequence, in the digester as well. It is important to take into consideration the fact that the alkalinity, which is the medium-buffering capacity,

should be high enough to avoid the destabilization of the system originated by the possible accumulation of volatile fatty acids.

6.3.2. Biomethane potential test

The ultimate methane production (B_0) of the pig manure, the mixtures and the blank were determined through biodegradability tests for quadruplicate, so twenty four batch assays were carried out. Fig. 6.1 shows the average value of B_0 profiles, after withdrawing the methane production from the blank (3.4 mL CH_4 g^{-1} COD).

As represented in Fig. 6.1, the methane production of each sample was similar during the first 16 days. After this first period, the mixtures of 80% PM, 60% PM and, in minor degree, of 40% PM continued generating methane during the following 10 days. This may be a result of the adaption of the microorganisms.

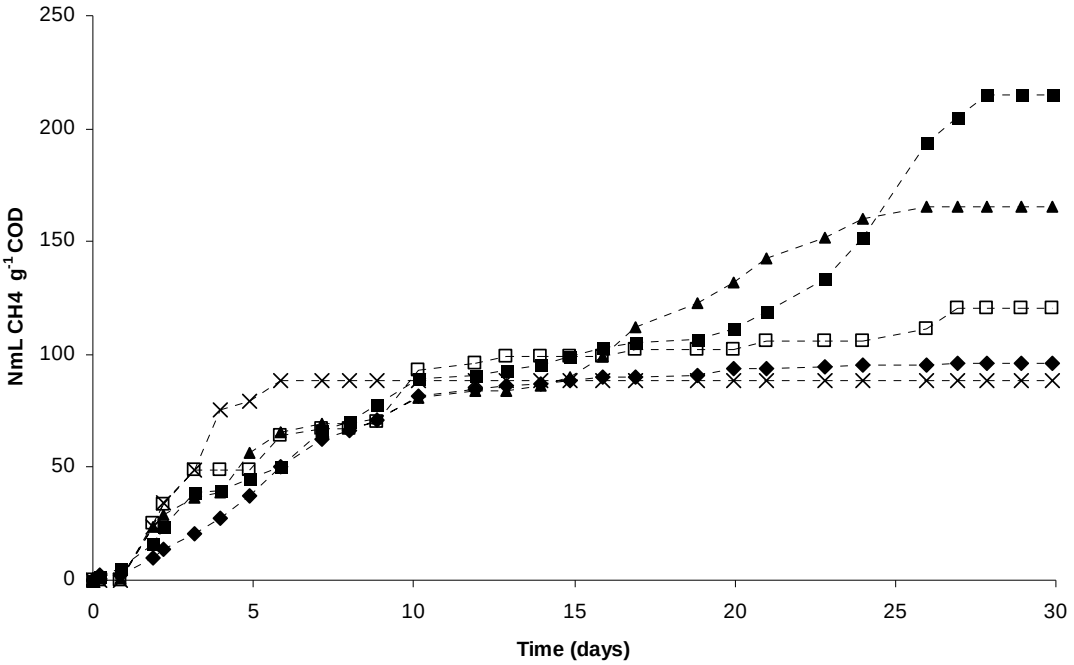

Fig. 6.1. Cumulative methane production for pig manure (♦) and each mixture: 80% PM (■), 60% PM (▲), 40% PM(□) and 20% PM(×)

The mixture of 80% PM produced the highest methane production with 215 mL CH_4 g^{-1} COD, which is about 125% more methane than when PM was mono-digested (96 mL CH_4 g^{-1} COD). In contrast, 20% PM had the lowest B_0 with 88 mL CH_4 g^{-1} COD. As reported by several authors (Parkin and Owen, 1986; Kayhanian and Hardy, 1994), the

optimum C/N ratio is placed between 20 and 40. The fact that the mixture of 80% PM has a C/N ratio of 23.4, which is within the optimum range, could be the reason why the highest methane production from that mixture was obtained. Table 6.2 shows the final B_0 of the biodegradability tests and the COD, the TS and the VS removal of each tested sample. As can be seen, all the mixture-samples present higher TS and VS removal percentages than PM, something that highlights the synergism established when glycerol is added to the reactor. From an environmental point of view, a higher methane production implies a reduction of greenhouse gases (GHG) emissions through the higher production of electricity from a renewable source instead of from fossil fuel. Therefore, a higher solid removal leads to a more stabilized digestate that will reduce GHG emissions during the storage and after its application in the field (Clemens et al., 2006).

Table 6.2. Ultimate methane production and matter removal of each tested sample

	Units	**PM**	**80% PM**	**60% PM**	**40% PM**	**20% PM**
$B_{0,\,COD}$	mL CH_4 g^{-1} COD_{fed}	95.7	214.8	165.2	120.6	88.2
$B_{0,\,TS}$	mL CH_4 g^{-1} TS_{fed}	130.8	200.4	125.5	78.5	60.3
$B_{0,\,VS}$	mL CH_4 g^{-1} VS_{fed}	187.9	249.6	134.1	92.9	73.3
$COD_{removal}$	%	27.2	61.4	47.2	34.5	25.2
$TS_{removal}$	%	21.1	78.1	91.1	81.2	51.6
$VS_{removal}$	%	30.0	90.9	93.0	83.1	51.9
$B_{0,\,COD}$	mL CH_4 g^{-1} COD_{fed}	57.5	61.2	56.9	54.4	59.9
$COD_{removal}$	%	62.0	66.0	61.3	58.7	64.7

Another remarkable result was that all the mixtures produced more methane than pig manure except for the mixture of 20% PM, which was clearly inhibited by its high level of volatile fatty acid produced by the nitrogen limitation (Benabdallah et al., 2009). Consequently, intermediate compounds were accumulated as they could not be degraded and converted to methane. The effluent of the 20% PM showed significant amounts of acetic, propionic and butyric acid, a phenomenon that illustrated the poor digestion conditions. This is demonstrated through the propionic/acetic ratio present at

the end of the test. Its value, which was 2.3 exceeded 1.4, a critical threshold limit to breakdown the anaerobic digestion process (Hill et al. 1987).

6.3.3. Modelling the co-digestion process

The ADM1 modified model designed by Galí et al. (2009) was used to validate the tested mixture of pig manure and glycerol, as well as to cover the nitrogen limitation when large amounts of glycerol were added. The model was uploaded with the initial parameters of the tested samples (Table 6.1) and then it was run for a simulation time of 30 days.

As the amount of pig manure decreased, the more quantity of glycerol the mixture had, and the less quantity of nitrogen had. This was a result of the negligible nitrogen content of the glycerol. This is important because the microorganisms that carry out the anaerobic digestion process need several nutrients for their growth, and one of the most important elements is nitrogen. Moreover, if there was a deficit of it, the process would reduce its kinetic. It should also be considered the effect of the increase in bacterial mass needed for the higher rate turn over (Angelidaki et al., 1997).

When the co-digestion experiments were reproduced by the modified ADM1 model, the results were satisfactory, matching the experimental results, particularly the ones concerning the final methane production data (see Table 6.2 and Fig. 6.2). It can also be observed that the shape of the predicted profiles of 100% PM, 60% PM and of 40% PM were nearly the same as the experimental ones. However, in the case of the 80% PM, the model did not predict any limitation which, consequently, displayed an ideal profile. Although this happened, in the experimental values there was an intermediate step indicating that there was a limitation and/or adaptation. Moreover, the model made a good prediction of the increase of the methane production when the glycerol was added to the pig manure. These last results are coherent because glycerol has a high rate of organic carbon that increases the biodegradability of the final sample. With reference to the mixture of 20% PM, even though the model indicated that the methane production would show a gradual increase, the experimental results indicated that there was a resulting step that occurred rapidly in the process and from which it was inhibited never

to recover again. It is important to indicate that both the model and the experimental data reached the same final B_0 result. This similarity could be explained by the nitrogen limitation which causes a stop at the first stage of the process while the model represents a soft inhibition.

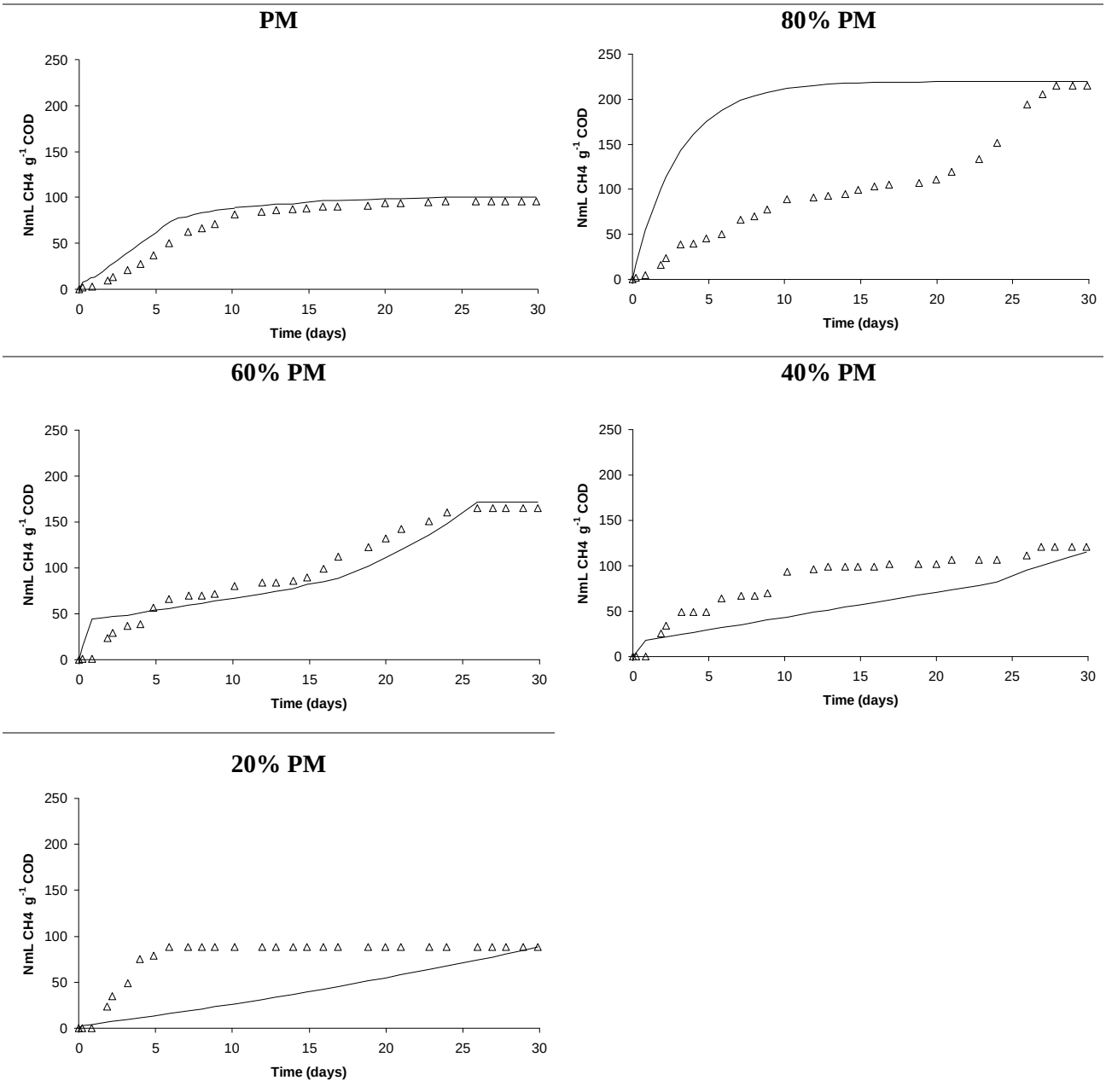

Fig. 6.2. Co-substrate biodegradability profiles: experimental (Δ) and simulation (-)

6.4. Conclusions

In this work, the anaerobic mesophilic co-digestion of pig manure with glycerol was tested in order to enhance the methane production obtained when pig manure is digested and, therefore, to improve the environmental and economic benefits of the process. The main conclusions extracted from the study are summarised as follows:

- In biodegradability batch tests of pig manure with glycerol, the co-digestion improved the methane production. Specifically, the mixture of 80% PM had the highest B_0 with 215 mL CH_4 g^{-1} COD. This mixture produced about 125% more methane than when PM was mono-digested.

- The lower production obtained with the 20% PM mixture showed the effect of a nutrient limitation, which highlighted the problem of performing mixtures in full-plants without developing previous studies.

- The modified version of the ADM1 model developed by Galí et al. (2009) predicted correctly the co-substrate degradation of pig manure and glycerol, specially, considering the final biogas production.

7. Co-digestion of sewage sludge and glycerol: synergism and inhibition mechanisms

Abstract

Crude glycerol, by-product of the biodiesel production, has stood out as an ideal co-substrate for anaerobic digestion. However, the presence of some inhibitory compounds and the risk of overloading limit crude glycerol dose. Three biomethane potential tests sets were done in order to analyse synergism and inhibitory mechanisms when sewage sludge and crude glycerol are co-digested. Moreover, nonlinear parameter estimation was used in order to estimate biodegradability, kinetic and inhibition parameters and to better support the conclusions. The crude glycerol used in this study presented a high specific methane potential (550 ± 24 mL CH_4 g^{-1} VS) and biodegradability (99 ± 1%). The obtained specific methane potential is higher than the theoretical one (426 mL CH_4 g^{-1} VS), probably due to the presence of unreacted lipids. Model derived results indicated, as all scenarios could be modelled with a single set of coefficients, that there was not synergism between glycerol and sewage sludge. Therefore, the higher methane production obtained in the co-digestion assays was due to crude glycerol addition. The half maximal inhibitory constant for glycerol was 1.03 g L^{-1}. Nevertheless, no sever inhibition was observed until the glycerol concentration in the digester was above 3.5 g L^{-1}. Finally, after a detailed study of the anaerobic digestion intermediates, it was concluded that propionate is the main inhibitory response when glycerol is used as co-substrate.

- Astals S, Batstone DJ. Mata-Alvarez J, Jensen P. Co-digestion of sewage sludge and glycerol: synergism and inhibition mechanisms. Article in preparation

7.1. Introduction

Crude glycerol (GLY), by-product of the biodiesel production, is a mixture of glycerol itself, alcohol, water, salts, heavy metals, fatty acids, unreacted mono-, di- and triglycerides and methyl esters. Although its quality and composition depends mainly on the raw matter origin, the chemical process used to obtain the biodiesel and the glycerol refining treatment (Pagliaro and Rossi, 2008; Robra et al., 2010).

As shown by the increasing number of papers, GLY has stood out as ideal co-substrate for anaerobic digestion (Astals et al., 2012a). This is because of its high: theoretical methane production (426 mL CH_4 g^{-1}), biodegradability (~100%) and purity. Anaerobic co-digestion (AcoD) of sewage sludge (SS) and GLY is of interest (Mata-Alvarez et al., 2011) since: (i) the elevated content of water in SS acts as solvent for glycerol; (ii) the alkalinity of SS gives a buffering capacity for the temporary accumulation of volatile fatty acids; (iii) the wide range of macro- and micro-nutrients present in the SS are essential for bacterial growth, and (iv) glycerol supplies rapidly biodegradable matter and, therefore, higher biogas yields to the system. Nevertheless, the presence of some inhibitory compounds like salts (from the catalyst and/or acidification) and methanol should be considered as they can limit GLY dose (Siles et al., 2010; Robra et al., 2010; Castrillon et al., 2012). Despite these facts, the highest risk of process inhibition when using GLY as co-substrate is overloading and the resulting digester acidification (Fountoulakis et al., 2010; Astals et al., 2011; Astals et al., 2012a, Nuchadang et al., 2012). Some studies have reported the GLY limiting concentration at mesophilic conditions. For instance, Amon et al. (2006) reported a 6% w/w of GLY when treating a mixture of PM, maize silage and rapeseed meal; Robra et al. (2010) suggested that the GLY dose should not exceed 10 % w/w in a system fed with cattle slurry; and Astals et al. (2012a) found a limit of 4% w/w of GLY when mixed with PM.

Reliable AcoD modelling is required to predict, in a clear and quantifiable manner, the effect of mixing two or more wastes in a digester and remove potentially negative impacts from mixing based on random or heuristic decisions (Astals et al., 2011; Mata-Alvarez et al. 2011). Moreover, the development and use of models may reduce the time and costs associated with laboratory experiments as well as improve co-substrate selection and dose rates (Gali et al., 2009). Models are also useful to estimate important

biochemical parameters such as biodegradability, kinetic parameters and inhibition constants, which are critical in AD design, performance and troubleshooting (Batstone et al., 2009; Jensen et al., 2011). In this field, nonlinear parameter estimation methods should allow improve parameter estimation and uncertainty to modelling and assessment of AcoD (Batstone 2003 and 2004).

7.2. Materials and Methods

Biomethane potential test

Biomethane potential (BMP) tests were carried out in 160 mL glass serum bottles at mesophilic temperature. All tests contained 70 mL inoculum, different quantities of substrate and deionised water, added to make up the total test volume to 85 mL. Bottles were flushed with 99.99% N_2 gas for 3 min (1 L min^{-1}), sealed with a rubber stopper retained with an aluminium crimp seal and stored in temperature controlled incubators (37 ± 1°C). Tests were mixed by inverting once per day. Blanks containing inoculum and no substrate were used to correct for background methane potential in the inoculum. All tests and blanks were carried out in triplicate, and all error bars indicate 95% confidence in the average of the triplicate. Biogas volume was measured by manometer at the start of each sampling event. Accumulated volumetric gas production was calculated from the pressure increase in the headspace volume and expressed under standard conditions (0 °C, 1 atm). At each sample event, the biogas composition (CH_4, CO_2 and H_2) was determined using a PerkinElmer Autosystem 1022 Plus gas chromatograph equipped with a thermal conductivity detector (see Jensen et al., 2011 for GC configuration and procedure).

Three sets of BMP were carried out in order to analyse different aspects about sewage sludge and glycerol anaerobic co-digestion. The first BMP set was done in order to identify synergism between substrates and the methane potential of the mixtures. The second BMP set was carried out in order to analyse the effect of the GLY concentration in the digester medium. The third BMP set was done to identify the inhibitory mechanism when GLY overloading takes place. Details of each BMP set up are provided below.

In the first BMP set, five mixtures between SS and GLY, i.e. 0.25, 0.5, 1, 2 and 4% of GLY wet-basis (w/w) concentration, were tested. All tests had an inoculum to substrate ratio (ISR) of 2 (VS-basis). Therefore, as the GLY concentration increased the amount of SS was reduced. Additionally, two digesters only fed with SS and GLY were used as reference; both of them performed at an ISR of 2. In the second BMP set, all digesters were fed with an identical mass of inoculum (70 g) and sewage sludge (16 g), which met an inoculum to SS ratio (ISR) of 2, and the amount of GLY required to achieve 0.25, 0.5, 1 and 2% w/w concentration in the digester medium. Consequently, the ISR was increased as the GLY dose rose. Again, two digesters only fed with SS and GLY, at an ISR of 2, were used as reference. In the third BMP set, only GLY, at an ISR of 2, was tested. At each sample event, sample from the digester medium was removed and the glycerol, acetate, propionate, butyrate, valerate, ethanol, propanol, butanol and 1,3-pronediol concentrations analysed.

Model implementation and data analysis

Mathematical analysis of the BMPs was based on the IWA Anaerobic Digestion Model No. 1 (ADM1) (Batstone et al., 2002). Sewage sludge degradation was modelled using a first order kinetics, since hydrolysis step is considered the rate-limiting step during SS degradation (eq. 7.1). In contrast, glycerol, as soluble compound, was modelled by Monod kinetics with an inhibition function (eq. 7.2). A non-competitive inhibition function, as in Pratt et al. (2012), was included to model digester overloading when glycerol was either mono- or co-digested. Co-digestion scenarios were modelled by combining both equations and the inhibition function affecting both rate processes (eq. 7.3).

$$r_{ss} = f_{ss} \cdot k_{hyd,ss} \cdot S_{ss} \cdot C_{ss} \qquad \text{(eq. 7.1)}$$

$$r_{gly} = f_{gly} \cdot k_{m,gly} \cdot \left(\frac{S_{gly}}{S_{gly} + K_s} \right) \cdot \left(\frac{K_I}{K_I + S_{gly}} \right)^n \cdot C_{gly} \qquad \text{(eq. 7.2)}$$

$$r_{AcoD} = f_{ss} \cdot k_{hyd,ss} \cdot S_{ss} \cdot C_{ss} \cdot \left(\frac{K_I}{K_I + S_{gly}} \right)^n + f_{gly} \cdot k_{m,gly} \cdot \left(\frac{S_{gly}}{S_{gly} + K_s} \right) \cdot \left(\frac{K_I}{K_I + S_{gly}} \right)^n \cdot C_{gly} \qquad \text{(eq. 7.3)}$$

where r_i is the process rate (mL CH_4 L^{-1} day^{-1}), f_i is the substrate biodegradability (-), S_i is the substrate concentration (g VS L^{-1}), C_i is the COD-to-VS ratio of the substrate, $k_{hyd,ss}$ is the first order hydrolysis rate constant of the SS (day^{-1}), $k_{m,gly}$ is the maximum uptake rate of GLY (g VS L^{-1} day^{-1}), K_s is the half-saturation constant of GLY (g VS day^{-1}), K_I is the inhibition coefficient (g VS L^{-1}), and n is the inhibition exponent. The exponent allows for an increase in inhibition progression rate compared with the standard non-competitive function.

The model was implemented in Aquasim 2.1d. Parameter estimation and uncertainty analysis were simultaneously solved, with a 95% confidence limit, as for Batstone et al. (2003 and 2009). Parameters uncertainty was estimated based on a two-tailed t-test on parameter standard error around the optimum, and non-linear confidence regions were also tested to confirm the linear estimate was representative of true confidence. The objective function used was the sum of squared errors (χ^2), where average data from triplicate experiments were used.

Table 7.1. Physico-chemical characterization of the sewage sludge and the glycerol

	Units	SS	GLY
TS	g kg^{-1}	38	829
VS	g kg^{-1}	31	746
CODt	g O_2 kg^{-1}	56	1056
Glycerol content	g kg^{-1}	-	723
Methanol content	g kg^{-1}	-	1.1

Wastes and inoculum origin

The crude glycerol was acquired from a biodiesel plant located in Victoria (Australia). The mixed sewage sludge was obtained from a municipal wastewater treatment plant (WWTP) of the Melbourne metropolitan area (Australia). Table 7.1 shows a basic physico-chemical characterisation of the SS and the GLY used in the present study. The inoculum was collected from an anaerobic digestion at a municipal WWTP in Queensland (Australia). The inoculum was treating mixed primary sludge and waste activated sludge, the specific methanogenic activity of the inoculum at 37 ºC was 0.2 g COD CH_4 g^{-1} VS day^{-1}.

7.3. Results and discussion

7.3.1. First BMP set: identifying substrate synergism

Fig. 7.1 shows the average cumulative biogas production in the course of time of the sewage sludge, the glycerol and each mixture. As can be seen, all mixture as well as SS presented a first-order profile. However, the reduction of the methane production rate recorded for the 2 and 4% w/w mixture as well as in the glycerol profile indicated that some inhibition took place during the digestion process.

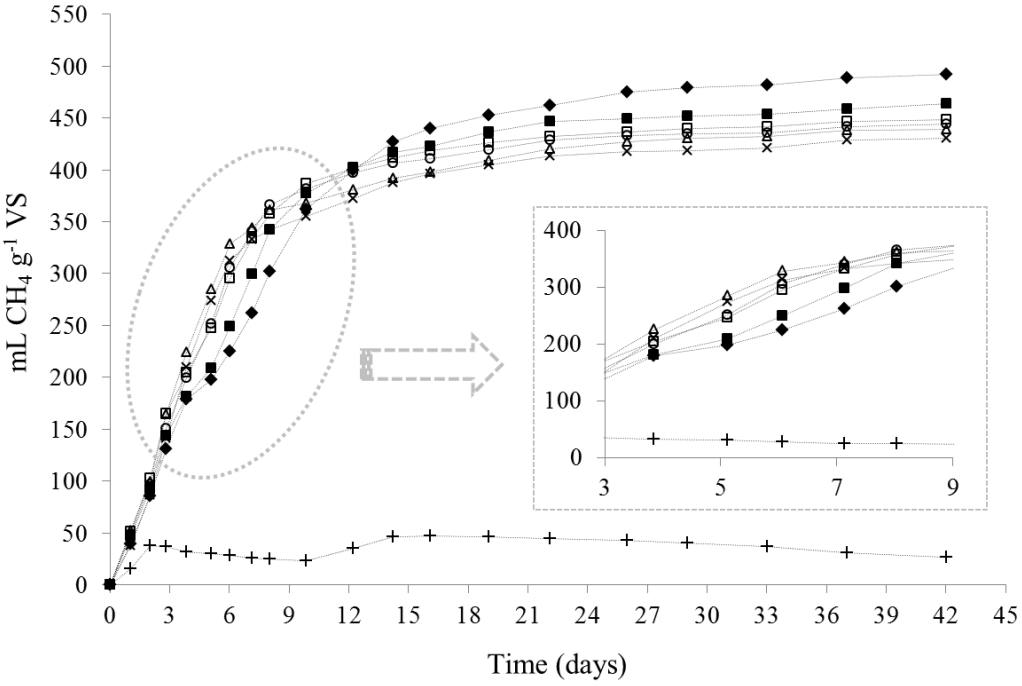

Fig. 7.1. Cumulative methane production, at a constant ISR, for sewage sludge (×), glycerol (+) and each mixture: 0.25% PM (Δ), 0.5% (○), 1% (□), 2% (■) and 4% (♦).

Since the reduction in the methane production rate occurred in those tests with a higher GLY dose, it was clear that the inhibition was related to the GLY concentration in the digester medium. Moreover, considering that the reduction in the microbiological activity was recorded at day 4 (for 2 and 4% w/w mixtures), it was hypothesised that the inhibition phenomena was related to the accumulation of volatile fatty acids, nitrogen limitation and/or low pH values (Fountoulakis et al., 2010; Astals et al., 2011) instead of the higher concentration of an inhibitory compound present in the glycerol. In principle, the presence of an inhibitory compound, such as salts or methanol, would have inhibited the assay from the beginning of the assay rather than on the fourth day.

Even more, the latter phenomena are normally related with digester long term operation instead of discontinuous assays.

As the methane potential of the GLY could not be directly determined due to inhibition, a mass balance was carried out taking into account the specific methane potential (B_0) (437 ± 22 mL CH_4 g^{-1} VS) and VS initial composition of the SS and co-digestion BMP tests. The obtained B_0 for GLY was 550 ± 24 mL CH_4 g^{-1} VS, which is higher than the theoretical B_0 for glycerol (426 mL CH_4 g^{-1} VS). The difference between B_0 could be related with the presence of unreacted lipids in the crude glycerol. Moreover, as the obtained B_0 for GLY was not correlated (P=0.6943) to the GLY concentration it was concluded that, under the assay conditions, there was not synergism between substrates. Consequently, it could be concluded that the higher methane potential obtained in the co-digestion assays was related to the higher B_0 and biodegradability of the GLY. The previous conclusion was confirmed with the model outputs, as all BMP were modelled with a single set of coefficients and parameters. Table 7.2 shows the model derived outputs and their uncertainty, while Fig. 7.2 shows the experimental versus the modelling BMP profile. Finally, with the obtained K_I and n, the half maximal inhibitory concentration (IC_{50}) of the glycerol was calculated to be 1.03 g VS L^{-1}; conditions reached by the 1% w/w mixture (1.2 g VS L^{-1}) and exceeded by the 2% (2.0 g VS L^{-1}) and 4% (3.2 g VS L^{-1}) mixture. Nevertheless, any sever effect was observed in any of the performed assays.

Table 7.2. Estimated model parameters and uncertainty for SS and GLY under mono- and co-digestion conditions

	Units	Value
$\mathbf{B_{0,ss}}$	mL CH_4 g^{-1} VS	432 ± 6
$\mathbf{B_{0,gly}}$	mL CH_4 g^{-1} VS	492 ± 5
$\mathbf{f_{ss}}$	-	0.69 ± 0.01
$\mathbf{f_{gly}}$	-	0.99 ± 0.01
$\mathbf{k_{hyd,ss}}$	day^{-1}	0.19 ± 0.02
$\mathbf{k_{m,gly}}$	g VS L^{-1} day^{-1}	10.14 ± 2.27
$\mathbf{K_s}$	g VS L^{-1}	7.52 ± 0.43
$\mathbf{K_I}$	g VS L^{-1}	1.29 ± 0.01
n	-	1.18 ± 0.22

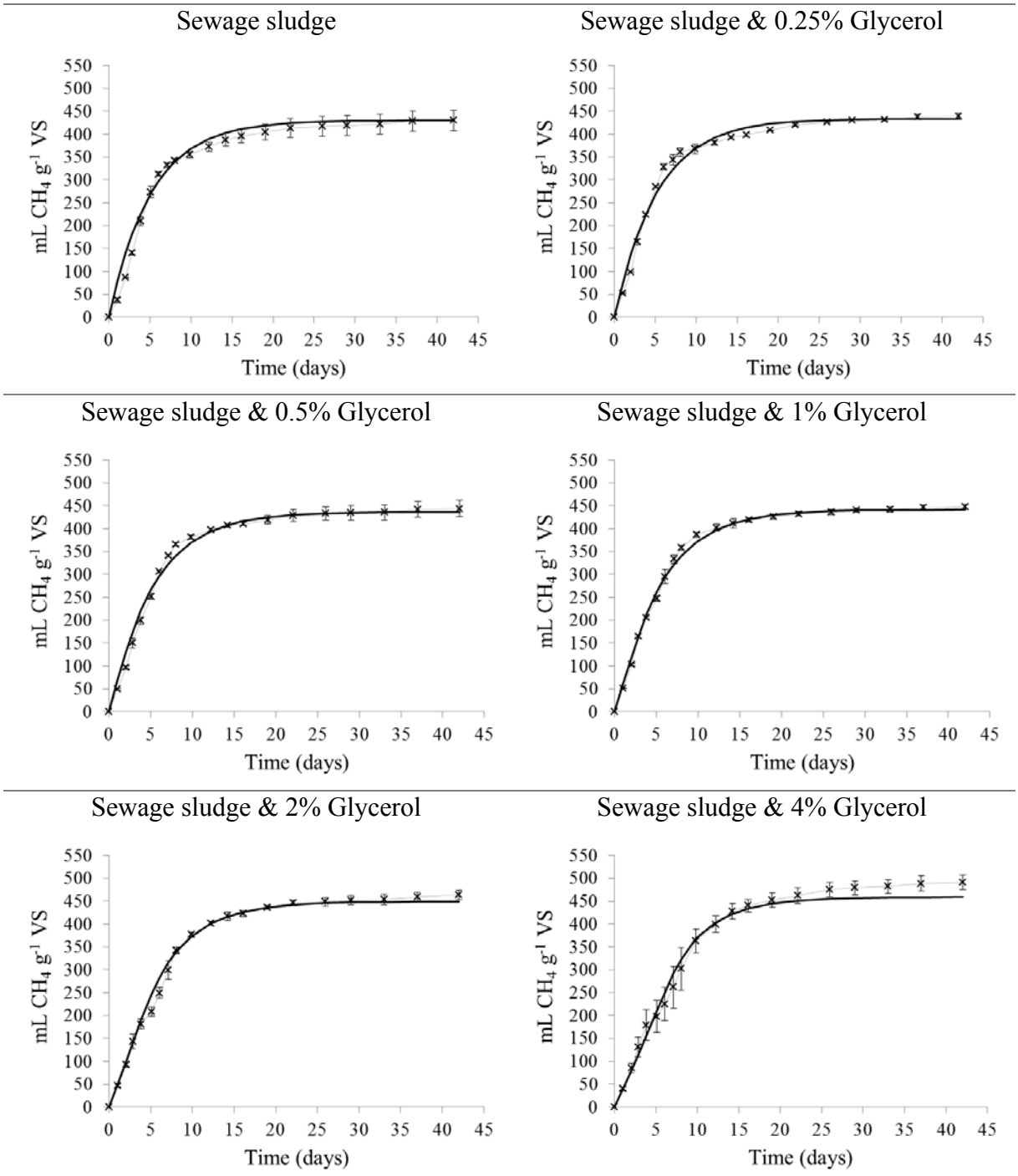

Fig. 7.2. Experimental and modelled cumulative methane productions in the course of time the sewage sludge and each mixture: Experimental data (×) and modelled profile (solid line).

7.3.2. Second BMP set: effect of the glycerol concentration in the digester medium

The second set of BMP experiments was done to determine the effect of the GLY concentration in a sewage sludge digester. Fig. 7.3 shows the profile, in addition to the SS and GLY, when the GLY concentration in the digester was 0.25, 0.5 and 1%

mixtures. The 2% GLY concentration is not shown because it produced less methane than the blank digester.

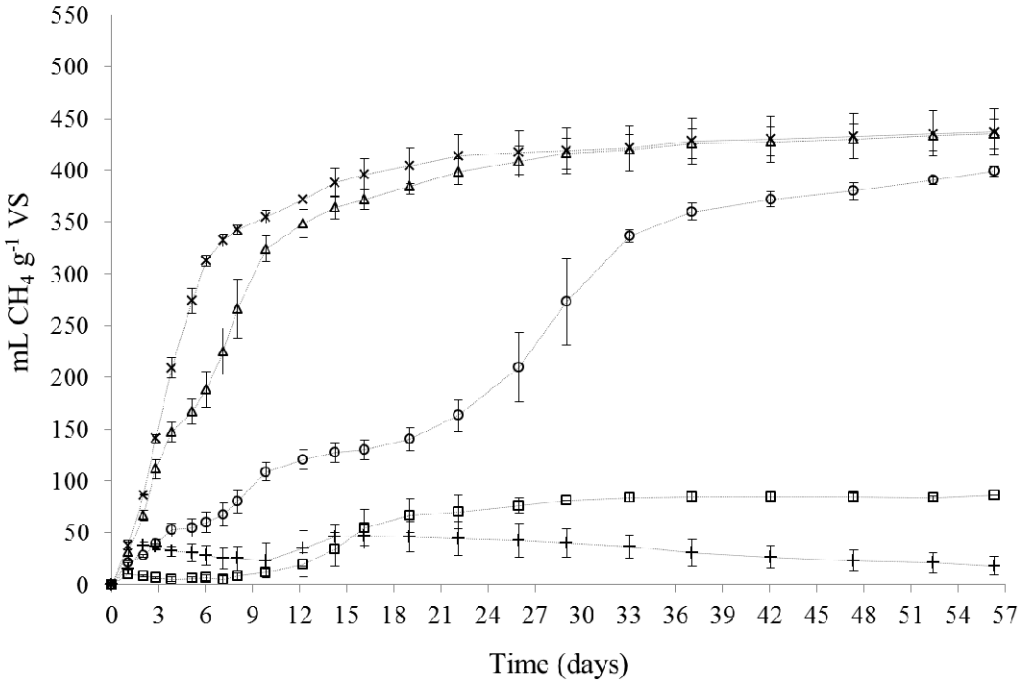

Fig. 7.3. Cumulative methane production, at a variable ISR, for sewage sludge (×), glycerol (+) and each mixture: 0.25% (Δ), 0.5% (○), 1% (□).

As shown by the low methane productions, all co-digestion tests having a GLY concentration higher than 0.5% in the digester medium (i.e. 1%, 2% and the crude glycerol) presented a strong inhibition of the digestion process, probably due to system overloading (ISR < 1) (Raposo et al., 2012). In contrast, the mixture with a concentration of 0.25% presented, at day 4, a reduction of the methane production rate like the showed in the previous section. The softer inhibition of the 0.25% mixture was related with the lower glycerol concentration (1.9 g VS L^{-1}) and an appropriated ISR value (~1.5). The 0.5% (3.7 g VS L^{-1}) test showed at the begging of the assay a sever inhibition of the methane production, indicating that there as an inhibition, followed by an increase of the methane production rate. A similar BMP profile was reported in chapter 6 (Fig 6.2) when co-digesting pig manure and glycerol. The lower specific methane production of the 0.5% mixture, when compared with the SS and the 0.25% mixture, reflected the disruption of the AD process caused by the inhibition.

7.3.3. Third BMP set: identifying inhibitory mechanism of glycerol

In the third BMP experiments set only glycerol was analysed. However, not only the methane production in the course of time was followed (Fig 7.4A) but also the concentration of glycerol, acetate, propionate, butyrate, valerate, ethanol, propanol, butanol and 1,3-propanediol were analysed at each sample event (Fig 7.4B). Butyrate and valerate are not shown in Fig. 4B since their concentrations were very low in comparison to glycerol, acetate and propionate. In contrast, ethanol, propanol, butanol and 1,3-propanediol concentrations were not detected (< 5 mg L^{-1}).

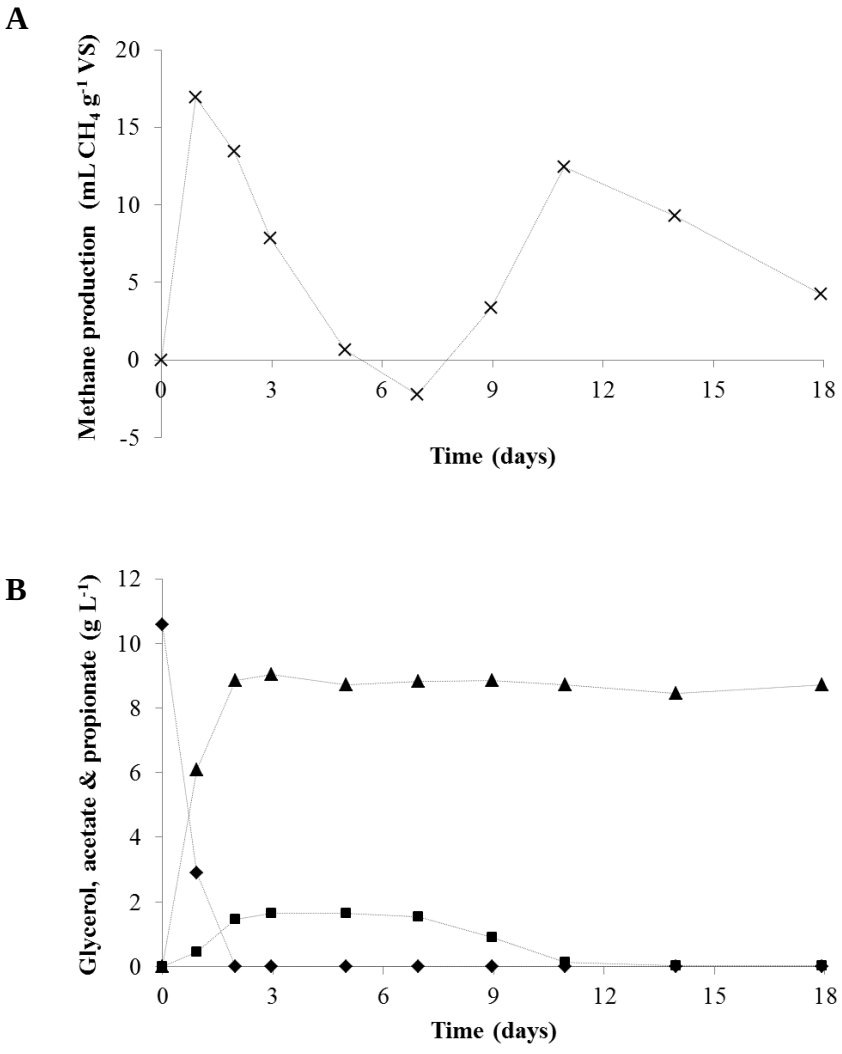

Fig. 7.4. (A) Cumulative methane production of the glycerol BMP test; (B) evolution of the concentration of: glycerol (♦), acetate (■) and propionate (▲).

As can be seen in Fig 7.4B, glycerol uptake was very fast since its 10.6 g L^{-1} were degraded in less than 2 days. During the first couple of day glycerol was converted to propionate, then, in a minor degree, to acetate and subsequently to methane. However, when the propionate concentration reached 8.8 g L^{-1} the acetate production as well as the methane production were stopped, result of the major disruption of the AD process. However, after a lack period of 5 days, acetate was converted to methane (Fig 7.4). The aforementioned propionate concentration was far above the reported limit values, which range between 1.5 and 7.4 g L^{-1} (Ahring et al., 1995; Nielsen et al., 2007; Wang et al., 2009). It should be noted that no trace of H_2 was detected in the gas pahse during the 18 days of the assays indicating the lower production of Acetyl-CoA precursor of acetate and butyrate. Regarding the other VFA, butyrate concentration gradually increased until day 11, when a concentration of 0.21 g L^{-1} was reached. Then, the concentration decreased to a 0.10 g L^{-1} (day 18). In contast. valerate concentration increased progressively during the whole study from 0.0 to 0.36 g L^{-1} (day 18). Taking into account those facts, it was concluded that glycerol was converted first to lactate (Fig 7.5) and then, through the acrylate pathway, to propionate (MetaCyc, 2013). Consequently, and taking into account that the final pH of the GLY digester was 6.6, it is clear that the AcoD process was disrupted by the high concentration of propionate instead low pH values.

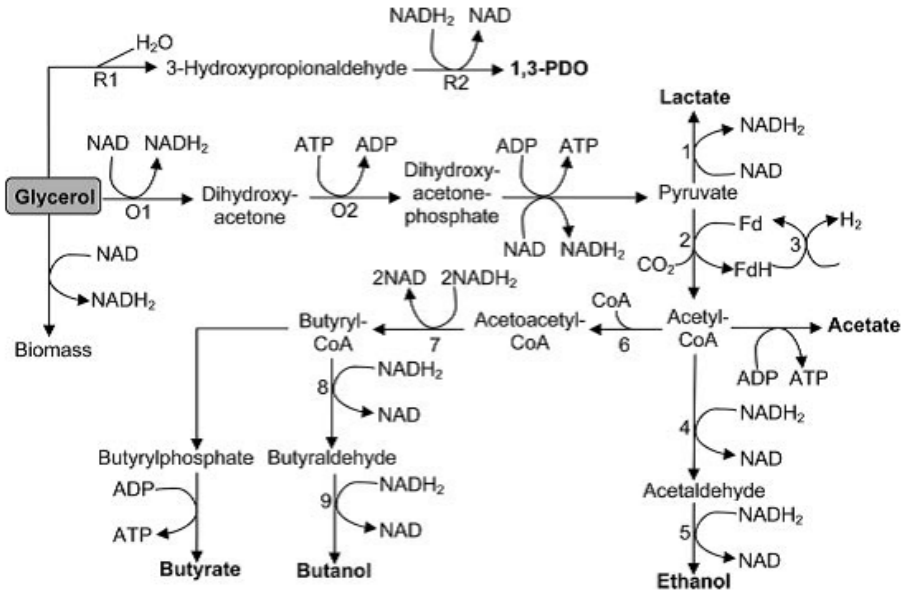

Fig. 7.5. Metabolic pathways for glycerol in clostridia (Johnson and Taconi, 2007)

7.4. Conclusions

In the present study, anaerobic co-digestion of sewage sludge and glycerol was tested in order to determine the synergism and inhibition phenomena between both substrates. Therefore, GLY dose could be optimised and process inhibition avoided. The main conclusions extracted from the study are summarised as follows:

- Crude glycerol is an ideal cosubstrate due to its high specific methane potential (550 ± 24 mL CH_4 g^{-1} VS) and biodegradability (f_{gly} = 99 ± 1%).

- Model derived results indicated that, under the assay conditions, there was not synergism between substrates. Consequently, the higher methane production recorded in the co-digestion assays was due to glycerol addition.

- The half maximal inhibitory concentration (IC_{50}) of glycerol was calculated to be 1.03 g VS L^{-1}. A reduction in the methane production rate occurred in those co-digestion tests with a glycerol concentration above 1 g VS L^{-1}, whereas sever inhibition of the digestion process was recorded when the glycerol concentration in the digester medium was higher than higher than 3.5 g L^{-1}.

- Propionate accumulation is suggested as the main inhibitory response when crude glycerol is used as co-substrate.

8. Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: biogas and digestate

Abstract

Crude glycerol derived from biodiesel production is characterised by its high concentration of organic carbon and its solubility in water; properties that make it a suitable co-substrate to improve the efficiency of a manure digester. An increase of about 400% in biogas production was obtained under mesophilic conditions when pig manure was co-digested with a 4% of glycerol, on a wet-basis, compared to mono-digestion. The increase in biogas production was mainly a consequence of the increase in organic loading rate. However, the differences could also be related to the synergy between both substrates and the carbon-to-nitrogen ratio. Moreover, the analysis of the macro-compounds, protein, lipids, carbohydrates and fibers, showed lower removal efficiencies in the co-digester as the microorganisms obtained nutrients from the soluble carbohydrates provided by the glycerol. The digestate stability, evaluated through a respirometric assay, showed that co-substrate addition does not exert a negative impact in the digestate quality.

- Astals S, Nolla-Ardèvol V, Mata-Alvarez J (2011). Anaerobic co-digestion between pig manure and crude glycerol at mesophilic and thermophilic conditions. International Symposium on Anaerobic Digestion of Solid Waste and Energy Crops. Vienna (Austria). 28th August – 1st September of 2011
- Astals S, Nolla-Ardèvol V, Mata-Alvarez J (2012). Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: Biogas and digestate. Bioresour Technol 110:63-70

8.1. Introduction

Since 1992, biodiesel has been produced at industrial scale in Europe through transesterification of vegetable oil, animal fat and/or used kitchen oil with alcohol (EBB, 2010). At present, more than 9,000,000 tonnes of biodiesel per year are produced in about 120 plants, which have a combined production capacity of over 20,000,000 tonnes of biodiesel per year (EBB, 2010). The main by-product of biodiesel production is crude glycerol, which is about 10% of the weight of the initial raw matter. Specifically, crude glycerol is a mixture of glycerol, alcohol, water, salts, heavy metals, free fatty acids, unreacted mono-, di- and tri-glycerides and methyl esters in varying amounts depending on the quality of the raw matter and the chemical process used to obtain the biodiesel (Pagliaro and Rossi, 2008; Robra et al., 2010).

At the present time, in some regions, the glycerol has to be disposed of as waste since (i) the existing glycerol market cannot absorb the large rise in by-product production (Johnson and Taconi, 2007); (ii) treatment and refinement of crude glycerol is too expensive for small and medium plants (Pachauri and He, 2006); and (iii) crude glycerol does not have a lot of direct uses due its impurities (Pagliaro and Rossi, 2008). In other regions, crude glycerol can be sold for 80 to 300 € per tonne depending on the regional market availability and the glycerol purity (Johnson and Taconi, 2007). Within this scenario, many research efforts to develop economical utilisations of crude glycerol have been made in order to make the cost of the biodiesel production sustainable in the long term. Among them, the valorisation of this residue as a co-substrate in anaerobic digestion (AD) plants is a promising solution, since a renewable source of energy is obtained from the treatment. Several successful studies, in batch and/or continuous experiments, have been published with reference to the benefits of the addition of glycerol to enhance the AD of agro-wastes (Amon et al., 2006; Anna et al., 2009), cattle manure (Chen et al., 2008; Mladenovska et al., 2003; Robra et al., 2010), fruit and vegetable wastes (Ma et al., 2008), organic fraction of municipal solid waste (Fountoulakis and Manios, 2009), pig manure (Álvarez et al., 2010; Amon et al., 2006; Astals et al., 2011; Galí et al., 2009), sewage sludge (Fountoulakis et al., 2010), mixture of pig manure and OFMSW (Schievano et al., 2009), mixture of olive mill and slaughterhouse wastewaters (Fountoulakis and Manios, 2009) and mixture of manure and organic industrial wastes (Holm-Nielsen et al., 2008).

Anaerobic co-digestion (AcoD) consists of the anaerobic digestion of a mixture of two or more substrates with complementary characteristics. As a result, biogas and organic matter removal yields are enhanced (Mata-Alvarez et al., 2011). Mixing animal manure and glycerol is of interest (Mata-Alvarez et al., 2000; 2011) since (i) the elevated content of water in manure acts as solvent for glycerol; (ii) the high alkalinity of manure gives a buffering capacity for the temporary accumulation of volatile fatty acids; (iii) the wide range of macro- and micro-nutrients present in the manure are essential for bacterial growth; and (iv) glycerol supplies rapidly biodegradable matter. Even though the AcoD of animal manure has been widely investigated, most of the studies have focused on process performance and biogas yield whereas little attention has been paid to digestate quality. However, both the biogas and the digestate have to be managed in an appropriate way in order to make AD plants feasible. Utilisation of the digestate as organic fertiliser or soil conditioner seems to be the best option for its recycling, since it contains considerable amounts of residual organic carbon (Alburquerque et al., 2011; Salminen and Rintala, 2002). However, digestate properties are conditioned by the raw materials used as substrate and the development of the anaerobic process in the digester. Furthermore, the introduction of a co-substrate can lead, in some cases, to the production of unstable digestates witch may exert negative impacts on organic matter mineralisation and nutrient turn-over in the plant-soil system (Alburquerque et al., 2011).

8.2. Materials and Methods

Laboratory scale continuous digesters

The CSTR were performed at mesophilic conditions (37 ºC) as described in section 3.2.2. Moreover, it should be noted that two different batches of pig manure were used as feed supply: batch 1 was used from day 1 to 99 and batch two from day 100 to 196; the glycerol was the same throughout the experiment.

Wastes and inoculum origin

Fresh pig manure (PM) and digested pig manure, used as inoculum, were obtained from a centralised plant, which treats the manure anaerobically, located in Lleida (Spain). After collection, pig manure was stored at 4 ºC until its utilisation. The crude glycerol (GLY) was obtained from an industrial plant in Huesca (Spain) which mainly produces

biodiesel through the transesterification of vegetable oils like sunflower, soybean and/or rape. The glycerol was stored at 4 ºC.

8.3. Results and discussion

8.3.1. Start-up of mesophilic anaerobic co-digestion

As can be seen in Table 8.1, the acclimatisation of the anaerobic microorganisms to crude glycerol (GLY), carried out in the co-digestion digester (D2), was divided into four different periods. The percentage of GLY, on a wet-basis (w/w), was increased as the co-digester showed signs of adaptation to the new influent, i.e. stabilisation of the daily biogas production and the intermediate-to-partial alkalinity ratio (IA/PA ratio), since the addition of low quantities of GLY meant a significant increase in solid and organic matter content of the feed supply.

Table 8.1. Operational mode of the star-up of mesophilic anaerobic co-digestion

	Units	**I**	**II**	**III**	**IV**	**V**
Proportion PM-GLY (w/w)	-	100/0	99/1	97/3	95/5	96/4
Proportion PM-GLY (TS/TS)	-	100/0	70/30	43/57	31/69	36/64
Proportion PM-GLY (VS/VS)	-	100/0	58/42	31/69	21/79	25/75
Operation time	days	1 - 11	12 - 21	22 - 35	36 - 48	49 - 60

The stability of the process was evaluated by the IA/PA ratio instead of the volatile fatty acids-to-total alkalinity ratio (Ferrer et al., 2010). However, both ratios are based on the same concept: if the acid concentration, estimated by the IA, exceeds the buffer capacity provided by the HCO_3^- species, determined by the PA, the digester will sour inhibiting the microorganism's activity and, specially, affecting methanogens. Therefore, to consider the process stable, the IA/PA ratio has to be kept below 0.4. Other authors have evaluated the digester stability with the intermediate-to-total alkalinity ratio (IA/TA ratio) (Fernández et al., 2001); however, the IA/TA ratio is less sensitive than the IA/PA ratio and is not adequate for systems with high alkalinity.

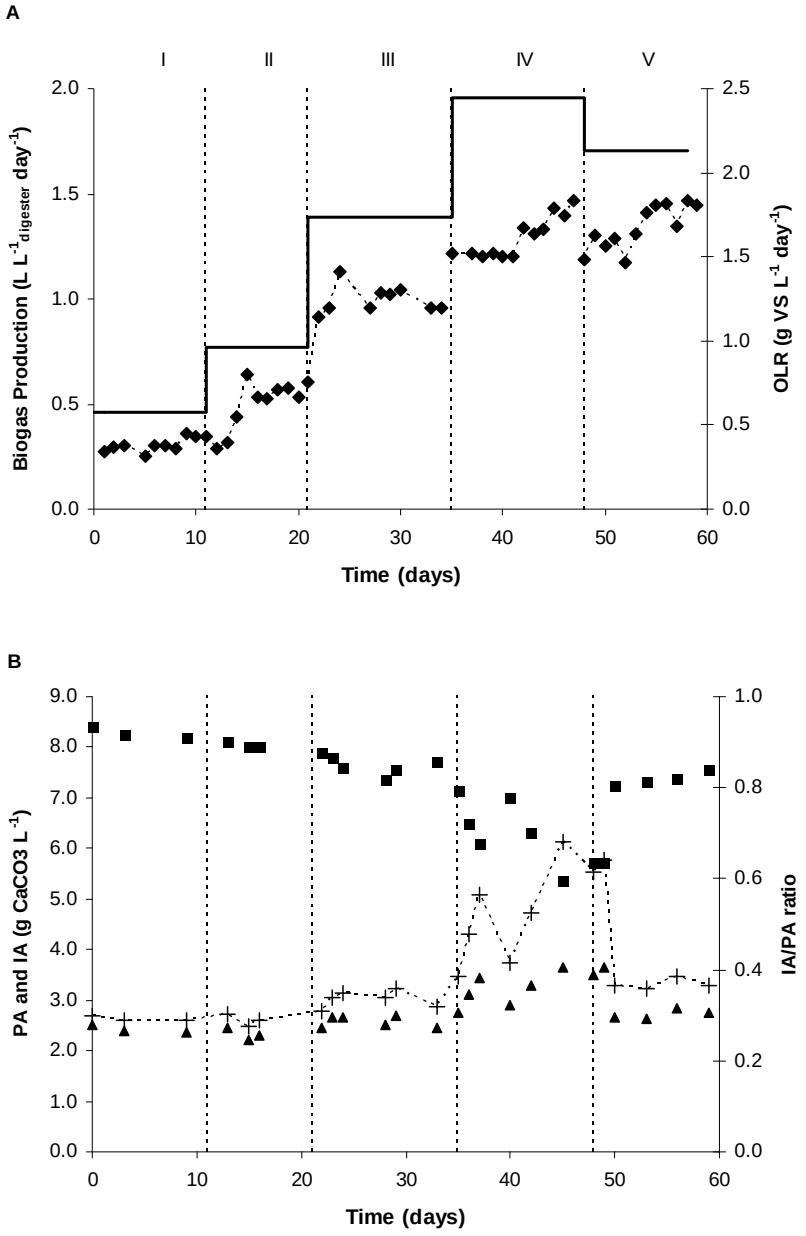

Fig. 8.1. Start-up of mesophilic anaerobic co-digestion of pig manure and glycerol: (A) Biogas production (♦) and organic loading rate (─, secondary axis); (B) partial alkalinity (■), intermediate alkalinity (▲) and IA/PA ratio (+, secondary axis)

At the beginning of the experiment (period I), both digesters, D1 and D2, were only fed with pig manure (PM) until day 11 when both systems showed similar operational parameters (i.e. biogas production, pH and alkalinity). Then in period II, a 1% of GLY (w/w), was added to the feed supply of D2 while the reference digester (D1) was kept fed with PM. As expected the addition of GLY had an important effect in the organic loading rate (OLR) and in the biogas production (Fig. 8.1A). In contrast, the IA and the

PA in D2 showed similar values to the ones obtained in period I (Fig. 8.1B), whereas only a small reduction of the pH from 8.1 to 7.9 could be noticed (data not shown). In period III, the increase in GLY in the influent, from 1% to 3% w/w, had a clear effect on the IA/PA ratio, which rose from 0.29 to 0.34. This effect was even clearer in period IV, when the IA/PA ratio achieved values over 0.60, which exceeded the critical value (0.4) to assure a stable AD process. At the beginning of each period, the increase in the VFA concentration, and therefore in the IA, due to the increase of the GLY content in the feed supply was a result of the VFA turn-over until the anaerobic microorganisms adjust to the new influent (Angelidaki et al., 1997). However, in period IV, where the content of GLY was augmented to 5% w/w, the system did not show signs of adaptation.

Process instability, which was leading the digester to failure, was a consequence of several factors: (i) the negligible alkalinity of the GLY reduced the alkalinity of the mixture and, as a consequence, in the digester; (ii) GLY represented a source of rapidly biodegradable organic matter, which generated large amounts of VFA; and (iii) the high OLR as a result of the addition of GLY (as can be seen in Table 8.1, in period IV about 80% of the organic matter in the influent was provided by the GLY). After 12 days in period IV (day 49), and to avoid process failure, the percentage of GLY was reduced from 5 to 4% w/w (period V). The reduction of the GLY content had a satisfactory effect on process stability, because after two days (day 51), the IA/PA ratio decreased to values lower than 0.4. It should be pointed out that during the whole start-up process the pH values were stable (between 7.9 and 7.6). However, it is probable that, if the alkalinity values had been lower, the pH would have dropped more as a result of VFA accumulation. Finally, it should be noted that biogas production in period IV and in period V was nearly the same (Fig. 8.1A), a clear sign of organic overloading in period IV. Therefore, a 4% w/w of GLY in the feed supply was considered to be the limiting concentration to maintain a stable AD process. Moreover, this value is similar to the limiting concentrations of GLY obtained by other authors that have carried out experiments with manure. For instance, Amon et al. (2006) reported a 6% w/w of GLY with a mixture of pig manure, maize silage and rapeseed meal; and Robra et al. (2010) proposed a 5% w/w of GLY in a system fed with cattle slurry.

8.3.2. Mesophilic anaerobic co-digestion: first period

The characteristics of the influent of the first stage period (period VI) are reported in Table 8.2. When the PM (influent of D1) and the mixture (influent of D2) are compared, the addition of crude glycerol had an important effect on parameters related to the matter content (TS, VS, COD). The addition of GLY resulted in a 120% increase in TS while the VS and the COD increased by around 190%. Moreover, due to its solubility in water, the main impact of GLY was on parameters related to soluble organic matter. The addition of GLY led to an increase in the VS/TS ratio from 0.6 to 0.8, and the VSS/TSS ratio maintained similar values in both influents (~0.7). In contrast, a decrease in the VSS/VS ratio (from 0.7 to 0.3) and an increase in the CODs/CODt ratio (from 0.5 to 0.8) were observed. The GLY used in this study was neutral (pH 6.5) and with a negligible concentration of nitrogen compounds and alkalinity. In fact, when both influents were compared, the pH was the same while a slight reduction (around 4%) was observed for the nitrogen compounds and alkalinity. The daily biogas production, at standard temperature and pressure (STP) conditions, of both digesters is presented in Fig. 8.2.

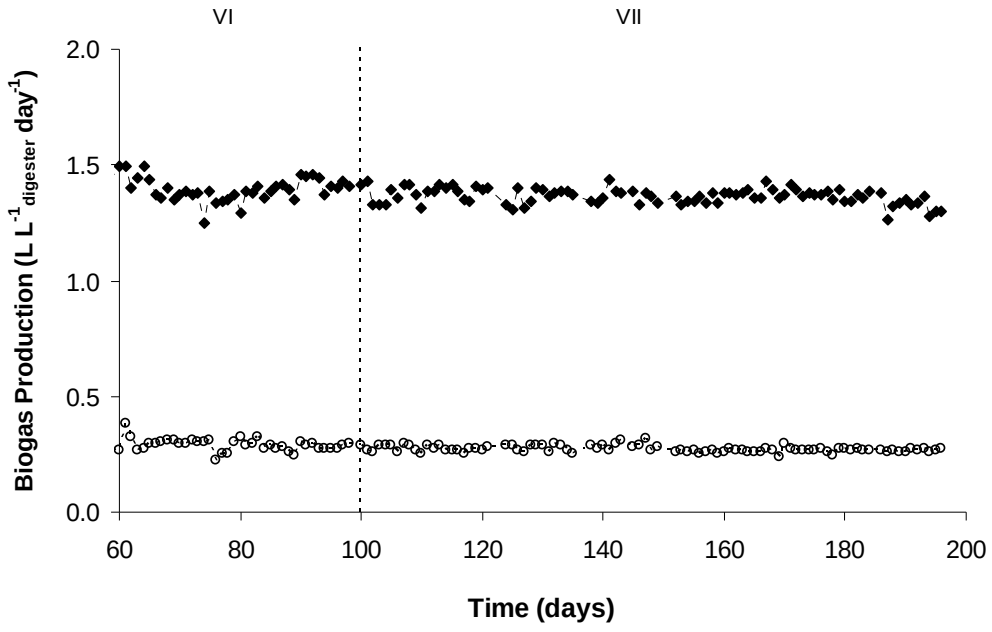

Fig. 8.2. Daily biogas production in the reference (o) and in the co-digestion (♦) digester

Table 8.2. Characterisation of influents and effluents of reference and co-digestion digesters

		Period VI				Period VII			
		D1		D2		D1		D2	
	Units	influent	effluent	influent	effluent	influent	effluent	influent	effluent
PM : GLY	% (w/w)	100 / 0		96 / 4		100 / 0		96 / 4	
OLR	gSV L_R^{-1} day^{-1}	0.6 ± 0.1		1.9 ± 0.1		0.5 ± 0.01		1.7 ± 0.1	
Influent and effluent composition									
TS	g L^{-1}	21.5	16.9	47.2	19.3	18.8	14.7	44.3	17.2
VS	g L^{-1}	12.9	8.3	37.8	9.8	10.5	6.2	34.9	7.8
TSS	g L^{-1}	12.8	10.5	12.6	11.9	8.3	7.8	8.4	9.4
VSS	g L^{-1}	9.5	6.7	9.6	8.2	6.0	4.6	6.0	6.3
CODt	g O_2 L^{-1}	24.7	12.6	71.3	15.1	21.0	9.3	66.9	11.0
CODs	g O_2 L^{-1}	12.8	3.7	58.3	4.3	13.2	1.9	56.2	2.1
pH	-	7.7	8.1	7.8	8.0	7.7	7.9	7.6	7.8
Partial Alk.	g CaCO3 L^{-1}	4.7	9.1	4.4	8.7	4.1	8.6	3.9	8.4
Total Alk.	g CaCO3 L^{-1}	9.1	11.0	8.9	10.7	8.8	10.4	8.5	10.2
VFA	g L^{-1}	5.1	0.08	5.2	0.07	7.4	0.16	7.6	0.17
- Acetic acid	g L^{-1}	4.2	0.06	4.1	0.05	5.9	0.08	6.0	0.11
- Propionic acid	g L^{-1}	0.1	0.04	0.2	0.02	0.3	0.05	0.3	0.04
- Butyric acid	g L^{-1}	0.5	0.02	0.5	0.04	0.7	0.03	0.7	0.02
- Valeric acid	g L^{-1}	0.3	n.d.*	0.3	n.d.	0.3	n.d.	0.6	n.d.
$N\text{-}NH_4^+$	g L^{-1}	1.2	1.4	1.2	1.3	1.0	1.2	0.9	1.01
$N\text{-}NH_3$	g L^{-1}	0.07	0.18	0.08	0.13	0.05	0.12	0.04	0.08
NTK	g L^{-1}	1.6	1.6	1.5**	1.9	1.5	1.5	1.4**	1.4
Removal efficiency									
$TS_{removal}$	%	21.4		59.1		21.8		61.2	
$VS_{removal}$	%	35.7		74.1		41.0		77.7	
$COD_{removal}$	%	49.0		78.8		55.7		84.9	
Biogas characteristics									
Production	L_{biogas} day^{-1}	1.16		5.58		1.06		5.44	
SBP-V_R	L_{biogas} L_R^{-1} day^{-1}	0.29		1.40		0.27		1.36	
SBP-SV_{added}	L_{biogas} g VS_{added}^{-1}	0.45		0.74		0.50		0.78	

* n.d. non detected (< 0.01 g L^{-1})

** Estimated through mass balance

In period VI, biogas production from D1 was approximately 1.2 L day^{-1} while D2 produced approximately 5.6 L day^{-1}, which represents an increase in biogas production of 380%. It has to be highlighted that an increase of 380% represents the highest biogas increase among the studies that have used GLY as co-substrate, where the average increase vary from 100% to 200% (Amon et al., 2006; Fountoulakis and Manios, 2009;

Fountoulakis et al., 2010; Ma et al., 2008). It is clear that the difference in biogas yield is mainly a consequence of the increase in OLR, which increased by 190% (from 0.64 g VS L_R^{-1} day^{-1} to 1.88 g VS L_R^{-1} day^{-1}). Nevertheless, the difference observed between both specific biogas productions, 0.45 L_{biogas} g^{-1} VS in D1 and 0.74 L_{biogas} g_{-1} VS in D2, emphasises the high biodegradability of glycerol and the synergy between both substrates in the co-digester medium. The difference can also be related to the carbon-to-nitrogen (C/N) ratio and the free ammonia nitrogen present in the digester.

It is well known that one of the main issues for the co-digestion process lies in balancing the C/N ratio. In fact, ideal co-substrates for manures, substrates with high nitrogen contents and high alkalinity, are wastes which have a high C/N ratio, like crude glycerol (Mata-Alvarez et al., 2011). Moreover, it has been shown that optimum values for C/N ratio are within the range of 20 to 70. The co-digestion digester had a C/N ratio of 48, calculated as COD/TKN (Álvarez et al., 2010), which is within the optimum range. In contrast, the reference digester had a low C/N ratio (C/N = 15). Total ammonia nitrogen (TAN) inhibition is especially distinct when digesting manures (Hansen et al., 1998) and a wide range of inhibiting TAN concentrations have been reported. The differences can be attributed to the characteristics of the substrates and inoculums, environmental conditions (temperature and pH) and adaptation periods (Chen et al., 2008). Since NH_3 has been reported to be the main cause of inhibition, especially affecting methanogens, it has to be pointed out that the NH_3 concentration depends basically on three parameters: TAN concentration, temperature and pH (eq. 8.1) (Chen et al., 2008; Kayhanian, 1999).

$$N\text{-}NH_3 = \frac{TAN \cdot 10^{pH}}{e^{\left(\frac{6344}{273.15+T}\right)} + 10^{pH}} \qquad \text{(eq. 8.1)}$$

In period VI, the TAN concentration in D1 and D2 were similar; however, the NH_3 concentration was notably different in both digesters (0.18 g L^{-1} in D1 and 0.13 g L^{-1} in D2). The influence of NH_3 on the anaerobic process can be described by the inhibition equation reported in the ADM1. In this model, free ammonia inhibition is a non-competitive inhibition affecting the acetate uptake rate (eq. 8.2), where S_{NH3} is the free

ammonia concentration and $K_{I,NH3}$ is the inhibition constant (0. 26 g L^{-1} - Angelidaki et al., 1999). Values from eq. 8.2 range from 0 (total inhibition) to 1 (no inhibition).

$$I_{NH_3 X_{ac}} = \frac{1}{1 + \frac{S_{NH_3}}{K_{I,NH_3}}} \qquad \text{(eq. 8.2)}$$

Therefore, D1 ($I_{NH3,Xac}$ = 0.59) was slightly more inhibited by free ammonia than D2 ($I_{NH3,Xac}$ = 0.66). This fact can be explained by the dilution effect in the TAN concentration made by the addition of GLY and the slight decrease in pH in D2 (Table 8.2). However, this inhibition did not lead to an increase in VFA (below 0.2 g L^{-1} in both digesters) or process instability, since the interact ion between NH_3, VFA and pH led the AD to an "inhibited steady state", which is a condition where the process is running stable but with lower methane yields (Angelidaki and Ahring, 1994).

In addition to the increase in biogas production, a higher VS and CODt removal yield was obtained during co-digestion when compared to mono-digestion. In absolute numbers, D1 degraded 36 and 49% of the available VS and CODt, respectively, while D2 eliminated 74% of the VS and the 79% of the CODt. Although both digestates exhibited similar compositions in terms of solids and COD, the digestate from D1 exhibited, for all these parameters, lower values than the digestate from D2. This phenomenon can be explained as a conjunction of two factors. First, the addition of GLY represented an important supply of organic carbon resulting in an increase in biomass (Ma et al. 2010), so all the extra organic matter could be degraded and overloading inhibition was avoided. Through a mass balance, where the average anaerobic biomass yield is 0.1 g $COD_{biomass}$ g^{-1} $COD_{eliminated}$ and the biomass growth rate is 0.8 g $VS_{biomass}$ g^{-1} $COD_{eliminated}$, an increase of 0.9 g $VS_{biomass}$ day^{-1} (0.2 g VS L^{-1}) was obtained. However, this biomass growth was not enough to explain the difference in VS between both digestates, because the difference was 1.5 g VS L^{-1}. Second, the biomass of D2 did not hydrolyse all the particulate matter supplied by the PM as it used GLY as a major source of carbon, while the biomass of D1 had to obtain nutrients from the particulate matter as it was the only source of nutrients. In fact, the biogas potential of the mixture supplied to D2 was 6.1 L_{biogas} day^{-1}, where 4.9 L_{biogas} day^{-1} came from the GLY (the theoretical biogas production per gram of glycerol is 0.73 L_{biogas} g^{-1} GLY) and

1.2 L_{biogas} day^{-1} came from the PM. However, taking into account that the biogas production of D2 was 5.6 L_{biogas} day^{-1} it can be understood that some degradable compounds remained in the D2 effluent since there is a difference of about 0.5 L_{biogas} day^{-1} between the potential and the obtained biogas production.

8.3.3. Mesophilic anaerobic co-digestion: second period

Periods VI and period VII were run with the same operational conditions; consequently similar results were obtained in both periods in terms of solid removal efficiencies, biogas yield (Table 8.2) and biogas production (Fig. 8.2). Actually, the second stage (period VII) was carried out to study the differences between both processes and therefore, not only standard parameters were monitored but also protein, lipids, carbohydrates and fibers.

Table 8.3. Characterisation of protein, lipids, carbohydrates and fibers in influents and effluents of reference and co-digestion digesters in period VII

		D1		**D2**	
	Units	**influent**	**effluent**	**influent**	**effluent**
PM : GLY	% (w/w)	100 / 0		96 / 4	
Influent and effluent composition					
Protein	g L^{-1}	3.5	1.6	3.4*	2.5
Lipid	g L^{-1}	1.4	0.4	2.2	1.4
Carbohydrates	g L^{-1}	5.5	4.2	29.3	3.9
Fiber	g L^{-1}	2.3	1.6	2.2*	2.0
Removal efficiency					
$Protein_{removal}$	%	55.5		25.2	
$Lipid_{removal}$	%	69.9		34.9	
$Carbohydrates_{removal}$	%	25.4		86.7	
$Fiber_{removal}$	%	30.3		11.0	

* Estimated through mass balance

It has been reported that AD of pig manure is limited by its low hydrolysis rate (Bonmatí et al., 2001). This fact is even more significant in very degraded pig manures, like the one used in this study, which was characterised by an NH_4^+/NTK ratio of about 0.7. As can be observed in Table 8.3, pig manure had large amounts of protein and

carbohydrates, being fibers the main fraction of the carbohydrates, while the lipids content was small. In contrast, the 4% w/w mixture was very rich in carbohydrates, fibers were less than the 10% of the fraction, and the amounts of protein and lipids were small. The 4% w/w mixture contained more lipids than PM, probably due to unreacted glycerides supplied by the GLY. The microorganisms from D1 degraded more protein, lipids and fibers than the microorganisms from D2 (Table 8.3). These results suggest that microorganisms in D1 had to hydrolyse large quantities of particulate matter to obtain nutrients. In contrast, bacteria in D2 had plenty of nutrients because of the large amounts of carbohydrates provided by the GLY and therefore did not need to hydrolyse large amounts of particulate matter.

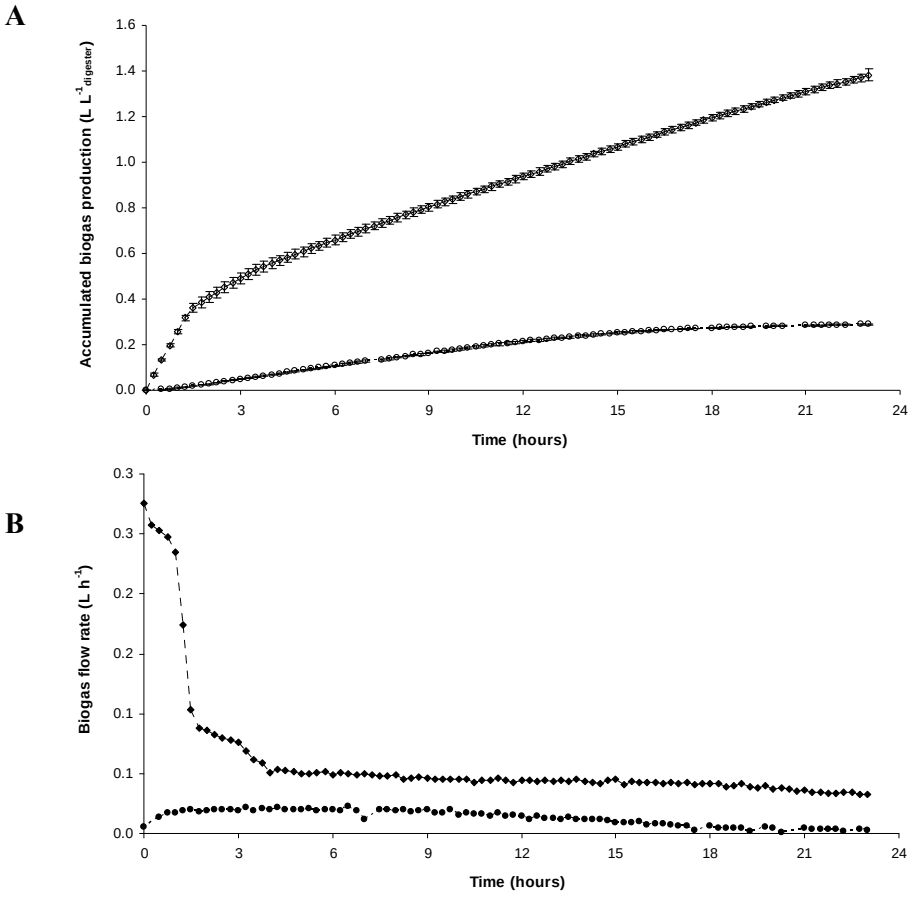

Fig. 8.3. (A) Accumulated biogas production in a day in the reference (o) and in the co-digestion (◊) digester. (B) Biogas flow rate in the reference (●) and in the co-digestion (♦) digester organic loading rate

Fig. 8.3 shows that the biomass of D2 produced 25% of the daily biogas production in the first two hours, whereas during the same period, biogas production from D1 was very low (10% of the daily biogas production); fact that highlighted the difference between both feed supplies in terms of easy biodegradable organic matter. After this initial period, the disparity between the degradation rates makes it clear that the PM digester was limited by its hydrolysis rate (0.08 L_{biogas} h^{-1}), while the co-digestion digester transformed fluently soluble carbohydrates into biogas (0.2 L_{biogas} h^{-1}). Biogas production of D1 showed a plateau after 18 h (95% of the daily biogas production was already produced) as a consequence of organic matter exhaustion. In contrast, biogas production of D2 did not show a plateau, as the biomass of D2 needed more time to consume all the available organic matter, which could be obtained with large HRT.

8.3.4. Digestate stability for agricultural use

The stabilisation of organic waste is related to the mineralisation of part of its organic compounds. Many parameters have been used as indicators of the mineralisation of organic streams (Al Momami et al., 2004; Tambone et al., 2009): (i) COD/DOC ratio, where lower ratios imply a higher degree of mineralisation; (ii) the average oxidation state (AOS – eq. 8.3), which ranges from +4 for CO_2, the most oxidised state of C, and -4 for CH_4, the most reduced state of C; or (iii) the COD/TKN ratio, which decreased due to COD degradation.

$$AOS = \frac{4(DOC - COD)}{COD} \qquad \text{(eq. 8.3)}$$

However, for semi-solid wastes, a respiration index, like BOD_{5d}, seems more adequate (Alburquerque et al., 2011; Ponsà et al., 2008). As shown in Table 8.4, the BOD_{5d} of the non-digested and digested samples highlight the waste stabilisation during the AD process. Actually, the BOD_{5d} was reduced by about 80% in D1 and more than 90% in D2; values that are similar to the data reported by other authors, who determined the stabilisation, by aerobic respirometrics techniques, of some organic waste before and after the AD process (Tambone et al., 2009). Moreover, when the BOD_{5d} of the PM (9.7 g O_2 L^{-1} – 7.7 mg O_2 VS^{-1} h^{-1}) was compared with the stability limit value proposed by Ponsà et al. (2008) and Alburquerque et al. (2011), (2 mg O_2 VS^{-1} h^{-1} and 6g O_2 L^{-1},

respectively) for a safety agricultural use, the need for a stabilisation treatment was accentuated. Since the PM exceeded the limit values, it should not be directly applied to soil as fertiliser or conditioner. Furthermore, the introduction of GLY as a co-substrate increased the BOD_{5d} from 9.7 to 32.3 g O_2 L^{-1} as a consequence of the presence of easily biodegradable compounds (Barrena et al., 2006). In contrast, digestate stability of both digesters, in terms of BOD_{5d}, was nearly the same: 1.8 g O_2 L^{-1} (2.4 mg O_2 VS^{-1} h^{-1}) for D1 and 2.0 g O_2 L^{-1} (2.1 mg O_2 VS^{-1} h^{-1}) for D2. These values are a little higher than those proposed by Ponsà et al. (2008) but lower than the more restrictive limit (<2.5 g O_2 L^{-1}) proposed by Alburquerque et al. (2011). Additionally, the latter authors also suggested DOC (<1.5 g C L^{-1}) and the DOC/TKN ratio (< 1.5 g C g N^{-1}) of digestates as stability indicators for its agricultural use. These parameters are of importance since a high percentage of TKN as NH_4^+ (80% and 70% for D1 and D2 respectively) improve the N-fertiliser potential of the digestate and low carbon doses favour carbon mineralisation and rapid ammonium nitrification in the soil-plant system (Riffaldi et al., 1996).

Table 8.4. Digestate quality parameters

		D1		**D2**	
	Units	**influent**	**effluent**	**influent**	**effluent**
PM : GLY	% (w/w)	100 / 0		96 / 4	
Influent and effluent characteristics					
BOD	g O_2 L^{-1}	9.7	1.8	32.3	2.0
DOC	g C L^{-1}	4.6	0.8	17.5	1.0
Conductivity	mS cm^{-1}	17.2	17.0	16.6	17.3
Fluoride	g L^{-1}	0.6	0.6	n.d.	n.d.
Chloride	g L^{-1}	1.2	1.2	1.2	1.2
Phosphate	g L^{-1}	n.d.	n.d.	n.d.	n.d.
Sulphate	g L^{-1}	0.3	0.3	n.d.	n.d.
Sodium	g L^{-1}	0.7	0.7	0.7	0.7
Potassium	g L^{-1}	2.2	2.8	2.2	2.8
Calcium	g L^{-1}	0.5	0.5	0.2	0.2
Magnesium	g L^{-1}	0.2	0.2	0.1	0.1
COD/DOC ratio	mol O_2 mol C^{-1}	1.08	0.89	1.20	0.75
AOS	-	-0.30	0.44	-0.82	0.85
DOC/TN	g C g N^{-1}	3.1	0.5	12.5	0.7

* n.d. non detected (< 0.1 g L^{-1})

8.4. Conclusions

Anaerobic co-digestion between crude glycerol and pig manure at mesophilic conditions was carried out in a continuous digester while an identical digester, only supplied with pig manure, was used as a reference. From this study the following conclusions can be drawn:

- Co-digestion between pig manure and glycerol was satisfactory to improve the biogas production since the addition of glycerol increased the digester organic loading rate, balanced the carbon-to-nitrogen ratio and decreased the free ammonia concentration in the digester medium.

- The microbial community biomass of the co-digestion digester did not hydrolyse all the particulate matter supplied by the pig manure since it used glycerol as a major source of nutrient, while the microbial community biomass of the reference digester had to obtain nutrients from the particulate matter as it was the only source of nutrients.

- The disparity between the organic compounds removal (proteins, lipids, carbohydrates and fibers) and the biogas flow rates made clear that the anaerobic digestion of pig manure anaerobic digestion is limited by the disintegration-hydrolysis step while the co-digestion digester transformed fluently soluble carbohydrates into biogas.

- The respirometric values of both feed supply were largely reduced as a consequence of the anaerobic treatment. Moreover, the values of both digestates were near the most restrictive limit values proposed for a safety agricultural.

9. Thermophilic co-digestion of pig manure and crude glycerol: process performance and digestate stability

Abstract

Anaerobic co-digestion has been widely used to enhance biogas production of digesters and, therefore, to improve the anaerobic plants economic feasibility. In the present study, glycerol, a by-product of the biodiesel industry, was used as a co-substrate for pig manure. The results showed that the thermophilic anaerobic co-digestion of pig manure supplemented with 3% of glycerol, on wet-basis, was satisfactory. The specific biogas production of the co-digester was 180% higher than the one obtained by the reference digester, which was only fed with pig manure. The improvement was related with the doubling of the organic loading rate, the high biodegradability of the crude glycerol, the slight reduction of the free ammonia concentration and the optimisation of the carbon-to-nitrogen ratio. Moreover, the analysis of the organic matter (protein, lipids, carbohydrates and fibers) and the biogas flow rates showed that the microorganisms in the co-digester obtained large amounts of nutrients from the glycerol, whereas the microorganisms of the reference digester mainly produced biogas from the particulate matter. However, the digestate obtained from the co-digester cannot be directly applied as soil fertiliser or conditioner due to the presence of high levels of biodegradable matter, which may exert negative impacts on the plant-soil system. Therefore, a longer hydraulic retention time, a reduction of the glycerol concentration and/or a post-treatment is required if the digestate is to be used as soil fertiliser or conditioner. In contrast, pig manure digestate can be directly applied on land.

- Astals S, Nolla-Ardèvol V, Mata-Alvarez J (2011). Anaerobic co-digestion between pig manure and crude glycerol at mesophilic and thermophilic conditions. International Symposium on Anaerobic Digestion of Solid Waste and Energy Crops. Vienna (Austria). 28th August – 1st September of 2011
- Astals S, Nolla-Ardèvol V, Mata-Alvarez J. Thermophilic co-digestion of pig manure and crude glycerol: process performance and digestate stability. Submitted to Journal of Biotechnology

9.1. Introduction

As shown by the dramatic rise of papers published, anaerobic co-digestion (AcoD) is, at the present time, the most relevant topic within anaerobic digestion research (Mata-Alvarez et al., 2011). AcoD has been used to enhance digesters biogas production and therefore make farm-scale plants economically feasible since the real driving force behind manure-based AD has been the income that electricity sales represent (Pavan et al., 2007). Even though, the improvement of the biogas yield is mainly consequence of the increase in the organic loading rate (ORL), when possible it is important to choose the best co-substrate and blend ration in order to: (i) favour positive interactions, i.e. positive synergisms, macro- and micro- nutrient equilibrium and moisture balance; (ii) dilute inhibitory and/or toxic compounds, (iii) optimize methane production and (iv) enhance digestate stability (Mata-Alvarez et al., 2000 and 2011; Astals et al., 2011; Alburquerque et al., 2012a). A substrate for co-digestion can be crude glycerol, a by-product of the biodiesel industry, which is currently produced in high quantities due to the increase of biodiesel production (Johnson and Taconi, 2007). The mixture between manure and glycerol is of interest since (i) the elevated content of water in manure acts as solvent for glycerol; (ii) the high alkalinity of manure gives a buffering capacity for temporary accumulation of volatile fatty acids; (iii) the wide range of macro- and micro-nutrients present in the manure are essential for bacterial growth and (iv) glycerol supplies easily biodegradable matter (Mata-Alvarez et al., 2011; Astals et al., 2012a). The positive interaction between manure and glycerol has widely been reported at mesophilic conditions (Mladenovska et al., 2003; Amon et al., 2006; Chen et al., 2008; Galí et al., 2009; Álvarez et al., 2010; Robra et al., 2010; Astals et al., 2011 and 2012; Nuchdang and Phalakornkule, 2012; Regueiro et al., 2012; Castrillon et al., 2013), whereas only Holm-Nielsen et al. (2008) have studied it at thermophilic conditions, even though their research was not devoted to AcoD purposes.

In this context, the higher installation of centralized AD plants that treat manures from a large number of farms and the increasing use of co-substrates have raised the need for effective sanitation procedures during the operation of AD plants. It should be noted that digestate sanitation and stability are of the utmost importance since the use of it as soil organic fertiliser or conditioner represents the most appropriated, economical and environmentally, disposal solution (Salminen and Rintala, 2002; Holm-Nielsen et al.,

2009). From a hygienisation point of view, thermophilic digestates fulfil the American and the European legislation for land application, while a post-treatment is required for mesophilic digestates prior their utilisation on land (Carrington et al., 1991; Guzman et al., 2007). Regarding digestate stability, the introduction of a co-substrate can lead to the production of unstable digestates which may exert negative impacts on organic matter and nutrient turnover in the plant-soil system (Alburquerque et al., 2012b). To be specific, digestate stability depends on the co-substrate properties and dose and on the development of the anaerobic process (Astals et al., 2012a). However, at the present time, the prevalence of efficiency criteria for biogas production over digestate stability can lead to short residence time of the material in the digester and as a result the digestate produced might not be completely exhausted in terms of easily biodegradable organic matter (Alburquerque et al., 2012a).

The main objective of this paper is to investigate the thermophilic anaerobic co-digestion between pig manure and crude glycerol in terms of process performance and digestate stability, when the co-digester is operated at crude glycerol limiting concentration. Moreover, the performance of the co-digestion at thermophilic and mesophilic conditions are compared.

9.2. Materials and Methods

Laboratory scale continuous digesters

The CSTR were performed at thermophilic conditions (55 ºC) as described in section 3.2.2.

Wastes and inoculum origin

Pig manure was obtained from a centralised plant, which treats manure anaerobically, located in Lleida (Spain). Crude glycerol was obtained from an industrial biodiesel plant in Huesca (Spain) which mainly produces biodiesel through the transesterification of vegetable oils like sunflower, soybean and/or rape. The GLY used in this study was neutral (pH 6.3) and presented high concentration of organic matter (CODt = 1.3 kg O_2 kg^{-1} and CODs = 1.2 kg O_2 kg^{-1}). After collection, PM and GLY were stored at 4 ºC until their utilisation.

9.3. Results and discussion

9.3.1. Thermophilic anaerobic co-digestion performance

As detailed in chapter 7, before the present study both digesters were operated at mesophilic conditions for more than 100 days with the reference digester (D1) treating pig manure and the co-digestion digester (D2) treating manure plus 4% (w/w) glycerol. The start-up of both thermophilic anaerobic digesters was carried out by increasing the temperature from mesophilic (35 ºC) to thermophilic conditions (55 ºC) in a single step, and with one day without feeding. As shown in Fig. 9.1, D1 showed a reduction in the methane production, which could be related with the lower PM biodegradability (the PM used at thermophilic conditions was different than the used at mesophilic condition), but no important signs of process instability. In contrast, the reduction of the biogas yields as well as the increase of the intermediate-to-partial alkalinity ratio (data not shown) indicated that D2 was not adapting to the new operational conditions. To avoid digester acidification the percentage of GLY was decreased from 4% to 3% w/w.

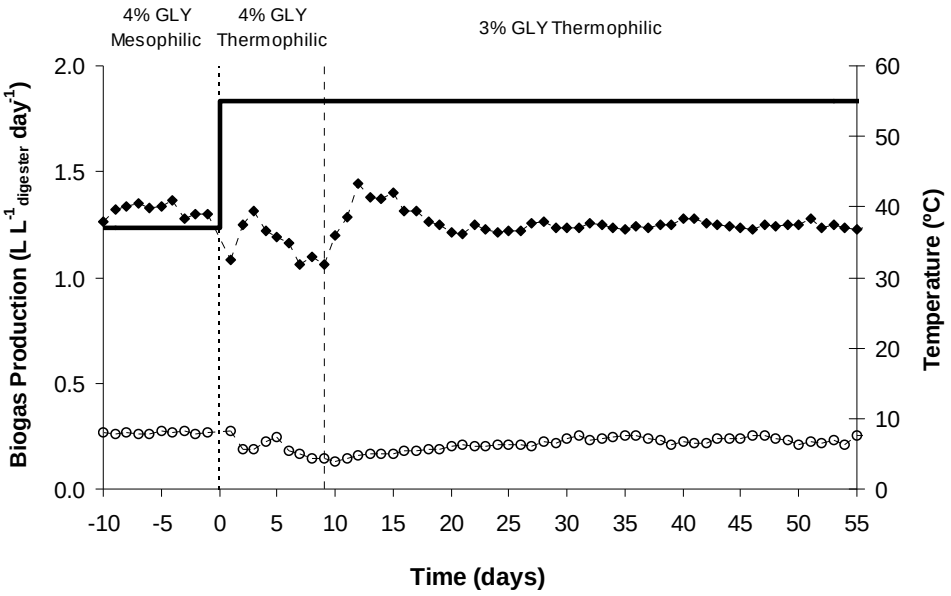

Fig. 9.1. Evolution of the daily biogas production in the reference (○) and in the co-digestion (♦) digester and temperature profile (─, secondary axis)

The reduction of the GLY percentage had a satisfactory effect on the digester stability. In fact, after some days, pH, total and partial alkalinity and biogas production rates were constant and the steady state conditions were considered to be achieved (Fig. 9.1). At steady state conditions, the influent and the effluent of both digesters were characterised

during a period equivalent to two HRT (Table 9.1). During this period, not only the standard analyses were monitored but also protein, lipids, carbohydrates and fibers.

Table 9.1. Characterisation of influents and effluents of reference and co-digestion digesters

		D1		D2	
	Units	**influent**	**effluent**	**influent**	**Effluent**
PM : GLY	% (w/w)	100 / 0		97 / 3	
OLR	g VS L_R^{-1} day^{-1}	1.4 ± 0.1		2.6 ± 0.1	
Influent and effluent composition					
TS	g L^{-1}	30.7	23.6	49.6	25.7
VS	g L^{-1}	21.2	15.9	39.6	17.3
TSS	g L^{-1}	26.5	18.1	25.0	19.8
VSS	g L^{-1}	20.4	13.6	19.5	14.8
CODt	g O_2 L^{-1}	33.0	24.4	72.7	30.7
CODs	g O_2 L^{-1}	5.5	5.0	40.6	9.1
pH	-	7.7	8.1	7.7	8.0
Partial Alk.	g $CaCO_3$ L^{-1}	7.4	7.7	7.2	7.0
Total Alk.	g $CaCO_3$ L^{-1}	10.6	10.6	10.3	10.4
VFA	g L^{-1}	2.0	1.6	2.0	3.0
- Acetic acid	g L^{-1}	1.0	0.8	1.0	1.3
- Propionic acid	g L^{-1}	0.7	0.6	0.6	1.1
- Butyric acid	g L^{-1}	0.1	0.1	0.1	0.2
- Valeric acid	g L^{-1}	0.2	0.1	0.2	0.4
TAN	g N L^{-1}	2.7	2.9	2.6	2.8
N-NH_3	g N L^{-1}	0.46	1.03	0.42	0.91
NTK	g N L^{-1}	2.8	2.9	2.7*	2.8
Protein	g L^{-1}	0.6	0.1	0.6*	0.4
Lipids	g L^{-1}	0.9	0.5	1.5	0.8
Carbohydrates	g L^{-1}	19.9	15.4	28.0	16.1
Fibers	g L^{-1}	9.9	6.3	9.6*	7.2
Removal efficiency					
$TS_{removal}$	%	23.3		48.3	
$VS_{removal}$	%	24.8		52.5	
$COD_{removal}$	%	26.1		57.7	
$Protein_{removal}$	%	85.3		57.4	
$Lipid_{removal}$	%	42.6		40.7	
$Carbohydrates_{remov}$	%	22.6		42.9	
$Fiber_{removal}$	%	35.5		24.7	
Biogas characteristics					
Biogas production	L_{biogas} day^{-1}	0.91		4.96	
SBP-V_R	L_{biogas} L_R^{-1} day^{-1}	0.23		1.24	
SBP-VS_{fed}	L_{biogas} g VS_{fed}^{-1}	0.17		0.47	

As shown in Fig. 9.2A, the accumulated biogas production between feedings in D2 showed a sigmoidal shape instead of the exponential shape obtained when treating the same substrates at mesophilic conditions (Fig 9.3). This profile could be explained through the inhibition of the acetate uptake rate by NH_3, which is more accentuated at thermophilic than at mesophilic conditions (Angelidaki and Ahring, 1994). However, free ammonia inhibition did not lead to process instability, since the interaction between NH_3, VFA and pH led the AD to an "inhibited steady state", a condition where the process is running stable but with lower methane yields (Hansen et al., 1998). In contrast, the accumulated biogas profile of D1 seemed to be more limited by the organic matter exhaustion and solubilisation rate, giving biogas flow rates below 0.02 L_{biogas} $L_{digester}$ h^{-1} during the whole period (Fig 9.2B). The characterisation of D1 influent showed that carbohydrates were the main fraction of the organic matter (94%), being fibers about 50% of them, whereas the protein and lipids content was very low. Moreover, the analysis of the particulate compounds present in the digestate of D1 showed that even though protein and lipids showed higher removal efficiencies they only represent about 15% of the organic matter removed, whereas fibers, typically characterised by a low biodegradability, represent about 70% of the organic matter removed (Table 9.1). In contrast, the relatively high biogas flow rate of the AcoD, above 0.06 L_{biogas} $L_{digester}$ h^{-1} during the first 10 hours, showed that D2 transformed soluble carbohydrates, provided by GLY, fluently into biogas. The difference between both feed supplies in terms of easy biodegradable organic matter was also shown by the protein, lipids and fibers removal efficiencies, which showed higher values for D1 (Table 9.1). These results suggest that microorganisms in D1 had to hydrolyse large quantities of particulate organic matter to obtain food, whereas bacteria in D2 did not need to since they had enough easy available substrate supplied by the addition of glycerol. In D2, the absence of a plateau 24h after feeding in the accumulated biogas production (Fig 9.2A) and the difference between VS concentration of both effluents (Table 9.1) indicated that some biodegradable organic matter was still present in the co-digester effluent and therefore more time, a larger HRT, should be required to convert all the biodegradable matter into biogas.

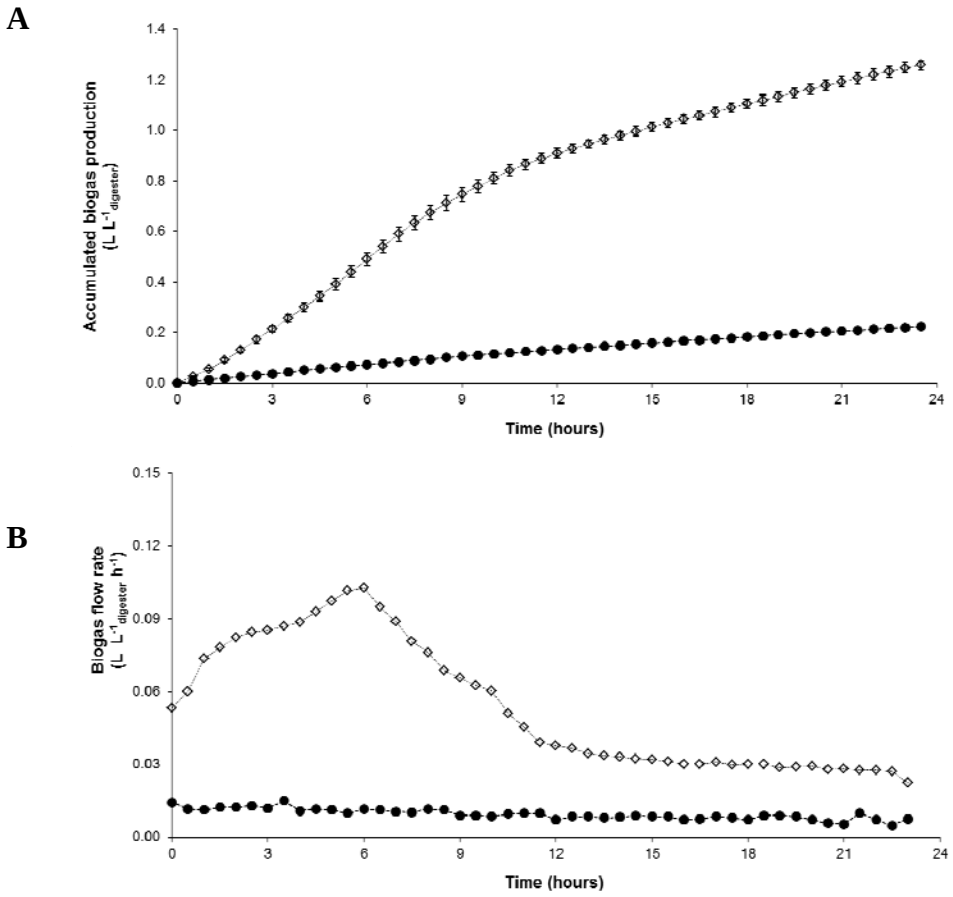

Fig. 9.2. (A) Accumulated biogas production between feedings in the reference digester (●) and in the co-digestion digester (◊). (B) Biogas flow rate in the reference digester (●) and in the co-digestion digester (◊)

9.3.2. Digestate quality for agricultural use

Mono-digestion and co-digestion effluents were characterised by high conductivity values, around 20 mS cm^{-1}, due to the high ion concentration, mainly chloride, sodium and potassium (Table 9.2). Nevertheless, nowadays, there is not a conductivity threshold value for agricultural use of digestates. Consequently, special care must be taken since high doses or continued applications of digestate can lead to an excessive salt accumulation in soil, which might inhibit plant growth (Alburquerque et al., 2012a,b). Regarding the nitrogen compounds, both effluents presented almost 100% of it as TAN (inorganic form) (Table 9.1). It is well known that the higher concentration of TAN improves the fertiliser potential of the digestate when compared with the feed supply. However, nitrogen dynamics in the soil are conditioned by the storage and

spreading procedures since malpractices, which do not favour TAN nitrification, can lead to nitrogen volatilisation and/or leaching (Alburquerque et al., 2012b). Another factor that has a great influence in the nitrogen turnover is the concentration of easily biodegradable organic matter in the digestate since an excess of it can cause N-immobilisation and/or oxygen exhaustion because of an excessive increase in soil microbial activity (Alburquerque et al., 2011; Bernal et al., 2009).

In order to assess the concentration of biodegradable organic matter in the digestate several parameters and threshold values have been reported. However, for semi-solid wastes, respiration indexes, like BOD_{5d}, and analytical parameters related to soluble organic matter, such as CODs or DOC, seems more adequate (Alburquerque et al., 2012; Astals et al., 2012a). The addition of 3% of GLY (w/w) to PM produced a notable increase of the aforementioned parameters in the influent with especial remark to the tripling of the BOD_{5d} (from 8.7 to 31.0 g O_2 L^{-1}) (Table 9.2). As shown by the reduction of the BOD_{5d} values in both digestates, a 40% in D1 and a 75% in D2, AD is a feasible technology to diminish the presence of easy biodegradable organic matter. Nevertheless, if the threshold values suggested by Ponsa et al. (2008) and Alburquerque et al. (2011) (2 mg O_2 g^{-1} VS h^{-1} and 6 g O_2 L^{-1}, respectively) for a safety agricultural use of digestate are taken into account, D1 effluent could be included in the less restrictive quality criteria and used as soil fertiliser, whereas D2 effluent cannot be directly applied as fertiliser due to its respirometric instability. Moreover, the latter authors also suggested the DOC (<1.5 g C L^{-1}) and the DOC/TKN ratio (< 1.5 g C g^{-1} N) of digestates as stability indicator. As can be seen in Table 9.2, D1 effluent only complies with the DOC/TKN limit value whereas D2 did not reach any of the aforementioned values. Assessing the stability of both digestates through the combination of the aforementioned parameters, it is clear that the effluent from the co-digester could not be directly used as fertiliser or soil conditioner, therefore, a longer HRT, a reduction of the GLY concentration and/or a post-treatment is required in order to avoid detrimental effect on the plant-soil system and on the environment. In contrast, the effluent from the pig manure digester could be directly applied even though a final refining would be recommended (Bustamante et al., 2012).

Table 9.2. Digestate quality parameters

		D1		D2	
	Units	**influent**	**effluent**	**influent**	**Effluent**
PM : GLY	% (w/w)	100 / 0		97 / 3	
Influent and effluent characteristics					
BOD_{5d}	g O_2 L^{-1}	8.7	5.1	31.0	8.3
DOC	g C L^{-1}	4.0	3.8	15.0	5.7
DOC/TKN	g C g^{-1} N	1.4	1.3	5.6	2.0
Conductivity	mS cm^{-1}	20.4	19.2	19.5	18.8
Fluoride	g L^{-1}	0.2	0.2	0.2	0.2
Chloride	g L^{-1}	1.1	1.1	1.1	1.1
Phosphate	g L^{-1}	0.2	n.d.*	0.2	n.d.
Sulphate	g L^{-1}	0.1	n.d.	0.1	n.d.
Sodium	g L^{-1}	0.5	0.5	0.5	0.5
Potassium	g L^{-1}	1.7	1.7	1.7	1.7
Calcium	g L^{-1}	0.2	n.d.	0.2	n.d.
Magnesium	g L^{-1}	n.d.*	n.d.	n.d.	n.d.

* n.d. non detected (< 0.1 g L^{-1})

9.3.3. Comparison between mesophilic and thermophilic anaerobic co-digestion of pig manure and glycerol

Even though the digester configuration (different HRT and OLR) and the PM used at thermophilic conditions (TAcoD) were different than the used mesophilic conditions (MAcoD) (Chapter 8), the results of the present study are compared with the ones reported for mesophilic conditions in order to evaluate the influence of temperature on process performance and digestate quality.

As shown in Fig. 9.1, the GLY limiting concentration was higher at MAcoD (4% w/w) than at TAcoD (3% w/w); nonetheless, the amount of COD supplied by GLY was very similar in both cases, 2.4 g O_2 L_R^{-1} day^{-1} (MAcoD) and 2.7 g O_2 L_R^{-1} day^{-1} (TAcoD). The addition of GLY to PM not only tripled (MAcoD) and doubled (TAcoD) the OLR, when compared with the PM digester, but also led, as no antagonism effect took place, to an increase of the SBP-VS_{fed} of about 180% in both scenarios. In both cases, the improvement of the SBP-VS_{fed} was related with the high biodegradability of the

glycerol and the synergy between substrates, i.e. optimisation of the C/N ratio and reduction of the free ammonia nitrogen concentration.

Regarding the accumulated biogas production profile between feedings, neither MAcoD nor TAcoD showed a plateau 24h after feeding (Fig. 8.3A and 9.2A). These results, together with the difference of the organic matter concentration between the mono- and co-digestion digestates (VS and CODt) indicated that AcoD microorganisms needed more time to degrade all the biodegradable matter. Moreover, the comparison of the influent and effluent organic matter (protein, lipids, carbohydrates and fibers) showed that both, MAcoD and TAcoD, were able to remove all the GLY and, therefore, the difference in the VS between mono- and co-digestion should be related with biodegradable compounds present in the PM. Additionally, the fact that the difference between mono- and co-digestion SBP-VS_{fed} was the same in both scenarios (0.3 L_{biogas} g^{-1} VS_{fed}), highlighted that the difference between MAcoD and TAcoD SBP-VS_{fed} was mainly related with PM biodegradability (biogas yield and organic matter removal) instead of co-digestion performance. The higher concentration of biodegradable organic matter in the AcoD digestates was also reflected in the stability indicators (BOD_{5d}, DOC, DOC/TKN), however, the effluent of MAcoD complied with the three stability limits suggested by Alburquerque et al. (2011), whereas the TAcoD digestate did not reach any of them. As both AcoD removed all GLY, the difference between MAcoD and TAcoD stability values could be related with the accumulation of AD intermediate products and the presence of partly-biodegradable PM compounds. However, since both PM seemed already highly degraded, it is likely that the difference was mainly related with the concentration of intermediate products and especially VFA. It is well known that thermophilic digesters are characterised by higher VFA concentration than mesophilic ones. In fact, MAcoD showed VFA concentrations below 0.2 g L^{-1}, whereas the VFA concentration of the TAcoD was much higher, around 3.0 g L^{-1}.

Finally, when comparing the hygienisation of both systems and considering the low hygienisation efficiency of the mesophilic AD, it is likely that the MAcoD digestate did not fulfil the two requirements of the 3rd draft of the European Union for the unrestricted use of sludge in agriculture: E.coli concentration below 500 CFU per gram and 6 log_{10} reduction of E. coli (Environment DG, EU, 2000; Astals et al., 2012b).

Consequently, a post-treatment, such as composting or pasteurisation, is required prior to land application. In contrast, the effluent of the TAcoD is expected to achieve the requirements of the European hygienisation legislation (Astals et al., 2012b). Nevertheless, a longer HRT, a reduction of the GLY concentration and/or a post-treatment is required to improve the digestate stability and reach the values suggested by Alburquerque et al. (2011).

Co-digestion between PM and GLY was feasible either at mesophilic or thermophilic conditions, however, the choice of the operational temperature and glycerol dose should be made in terms of process energetic efficiency and digestate quality. Due to the lower NH_3 and VFA concentrations, MAcoD is to be more stable and less inhibited than TAcoD; consequently higher biogas yields are expected for MAcoD. In contrast, TAcoD produce a digestate that fulfil the hygienisation legislation for unrestricted agricultural use, whereas a post-treatment is normally required from MAcoD digestates to reach the levels of the sanitation legislation. A mentioned before, the lower respirometric stability of the TAcoD digestate could be solved with a longer HRT and/or reduction of the glycerol proportion in the feedstock, nevertheless, these options reduce the biogas yield of the TAcoD and, therefore, the plant economic feasibility.

9.4. Conclusions

The thermophilic (55 ºC) anaerobic co-digestion of pig manure supplemented with 3% of crude glycerol, on wet-basis, was very satisfactory in terms of biogas yield. From this study the following conclusions can be drawn:

- The addition of glycerol resulted in a higher specific biogas production (0.47 L_{biogas} g^{-1} VS_{fed}) than the mono-digestion of pig manure (0.17 L_{biogas} g^{-1} VS_{fed}); improvement related to the doubling of the organic loading rate, the high biodegradability of the crude glycerol, the slight reduction of the ammonia concentration and the optimisation of the carbon-to-nitrogen ratio.

- The organic matter characterisation and the evaluation of the biogas flow rates showed that the microbial community of the co-digester did not hydrolyse all the particulate matter supplied by the pig manure since it used glycerol as a major source of carbon, while the microbial community of the reference

digester had to obtain food from the particulate matter as it was the only source of it.

- The presence of relatively high amounts of biodegradable matter made the digestate obtained from the co-digester unsuitable to be directly applied as soil fertiliser or conditioner since it may exert negative impacts on the plant-soil system. A longer hydraulic retention time, a reduction of the glycerol concentration and/or a post-treatment is required to improve digestate stability. Pig manure digestate can be directly applied as soil fertiliser or conditioner.

- The comparison between mesophilic and thermophilic pig manure and glycerol co-digestion indicated that lower biogas yields and digestate stability are expected at thermophilic conditions because of the higher ammonia and volatile fatty acids concentration. However, the thermophilic digestate was likely to fulfil the requirements of the European hygienisation legislation for unrestricted agricultural use.

10. Conclusions and recommendations

10.1. Conclusions

In this study, anaerobic mono- and co-digestion has been evaluated and modelled for several waste streams and conditions. The main conclusions extracted from this work are compiled in this section:

Chapter 4: Anaerobic digestion of sewage sludge: a biodegradability and modelling study

- The ultimate methane potential of the sewage sludges ranged from 188 to 214 mL CH4 g^{-1} COD_{fed}, whereas the COD removals varied between 58 and 65%.

- The apparent first order solubilisation rate of the sewage sludges showed two homogeneous groups: (i) the lowest rate group from 0.23 to 0.35 day^{-1} and (ii) the highest rate group from 0.27 to 0.43 day^{-1}.

- No statistically significant relationship between the ultimate methane potential or the disintegration constant and the sewage sludge characterisation was found. Therefore, an empirical relationship based on sludge characterisation to estimate both values could not be established.

- A 5 – 65% solubilisation rate underestimation was found when the conventional first order rates, obtained from experimental data fitting, were compared with the best fit results of the model. The k_{dis} underestimation was related to soluble compounds accumulation, mainly long chain fatty acids and acetate.

- The comparison between the simulation and the experimental results showed the consistency of the developed methodology, which is mainly based on the composite concentration and its stoichiometric coefficients.

Chapter 5: Identification of synergistic impacts during anaerobic co-digestion of organic wastes

- Substrate diversification improved process kinetics. The synergisms of mixing substrates lead to an improvement in AD kinetics for all mixtures. However, as a general trend, the ultimate methane production was not affected.

- Mixing waste is a feasible option to reduce the impact of inhibitory compounds. The introduction of a carbohydrates and/or protein source to lipids reduced the LCFA inhibition, present in lipid AD.

- Paunch and DAF resulted, when compared with the theoretical one, in a higher methane yield. Results suggest that the biomass present in the paunch may contribute to improved hydrolysis of the partially biodegradable fat conglomerates present in the DAF.

Chapter 6: Co-digestion of pig manure and glycerol: experimental and modelling study

- In biodegradability batch tests of pig manure with glycerol, the co-digestion improved the methane production. Specifically, the mixture of 80% PM had the highest B_0 with 215 mL CH_4 g^{-1} COD. This mixture produced about 125% more methane than when PM was mono-digested.

- The lower production obtained with the 20% PM mixture showed the effect of a nutrient limitation, which highlighted the problem of performing mixtures in full-plants without developing previous studies.

- The modified version of the ADM1 model developed by Galí et al. (2009) predicted correctly the co-substrate degradation of pig manure and glycerol, specially, considering the final biogas production.

Chapter 7: Co-digestion of sewage sludge and glycerol: synergism and inhibition mechanisms

- Crude glycerol is an ideal cosubstrate due to its high specific methane potential (550 ± 24 mL CH_4 g^{-1} VS) and biodegradability (f_{gly} = 99 ± 1%).

- Model derived results indicated that, under the assay conditions, there was not synergism between substrates. Consequently, the higher methane production recorded in the co-digestion assays was due to glycerol addition.

- The half maximal inhibitory concentration (IC_{50}) of glycerol was calculated to be 1.03 g VS L^{-1}. A reduction in the methane production rate occurred in those co-digestion tests with a glycerol concentration above 1 g VS L^{-1}, whereas sever inhibition of the digestion process was recorded when the glycerol concentration in the digester medium was higher than higher than 3.5 g L^{-1}.

- Propionate accumulation is suggested as the main inhibitory mechanism when crude glycerol is used as co-substrate.

Chapter 8: Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: biogas and digestate

- Co-digestion between pig manure and glycerol was satisfactory to improve the biogas production since the addition of glycerol increased the digester organic loading rate, balanced the carbon-to-nitrogen ratio and decreased the free ammonia concentration in the digester medium.

- The microbial community biomass of the co-digestion digester did not hydrolyse all the particulate matter supplied by the pig manure since it used glycerol as a major source of nutrient, while the microbial community biomass of the reference digester had to obtain nutrients from the particulate matter as it was the only source of nutrients.

- The disparity between the organic compounds removal (proteins, lipids, carbohydrates and fibers) and the biogas flow rates made clear that the

anaerobic digestion of pig manure anaerobic digestion is limited by the disintegration-hydrolysis step while the co-digestion digester transformed fluently soluble carbohydrates into biogas.

- The respirometric values of both feed supply were largely reduced as a consequence of the anaerobic treatment. Moreover, the values of both digestates were near the most restrictive limit values proposed for a safety agricultural

Chapter 9: Thermophilic co-digestion of pig manure and crude glycerol: process performance and digestate stability

- The addition of glycerol resulted in a higher specific biogas production (0.47 L_{biogas} g^{-1} VS_{fed}) than the mono-digestion of pig manure (0.17 L_{biogas} g^{-1} VS_{fed}); improvement related to the doubling of the organic loading rate, the high biodegradability of the crude glycerol, the slight reduction of the ammonia concentration and the optimisation of the carbon-to-nitrogen ratio.

- The organic matter characterisation and the evaluation of the biogas flow rates showed that the microbial community of the co-digester did not hydrolyse all the particulate matter supplied by the pig manure since it used glycerol as a major source of carbon, while the microbial community of the reference digester had to obtain food from the particulate matter as it was the only source of it.

- The presence of relatively high amounts of biodegradable matter made the digestate obtained from the co-digester unsuitable to be directly applied as soil fertiliser or conditioner since it may exert negative impacts on the plant-soil system. A longer hydraulic retention time, a reduction of the glycerol concentration and/or a post-treatment is required to improve digestate stability. Pig manure digestate can be directly applied as soil fertiliser or conditioner.

- The comparison between mesophilic and thermophilic pig manure and glycerol co-digestion indicated that lower biogas yields and digestate stability are

expected at thermophilic conditions because of the higher ammonia and volatile fatty acids concentration. However, the thermophilic digestate was likely to fulfil the requirements of the European hygienisation legislation for unrestricted agricultural use.

10.2. Recommendations

For further investigation, the following recommendations are proposed:

Sewage sludge modelling

- Detect and explore a set of factors that affect sewage sludge anaerobic degradability and kinetics.

- Analyse more sewage sludges and/or more frequently in order to establish, if possible, default stoichiometric and parameters model values for ADM1 based on simple laboratory analysis.

- Extend the Anaerobic Digestion Model No.1 in order to incorporate other state variable, such as particle size, sulphur, ions and inhibitory compounds.

Anaerobic co-digestion

- Analyse the potential of anaerobic co-digestion to mitigate other well-known inhibitory mechanisms, like ammonia, salinity, sulphydric acid, etc.

- Further improve co-digestion models, based on the Anaerobic Digestion Model No.1. Improve and incorporate activation and inhibition functions in order to reproduce synergism and antagonism phenomena, respectively.

- Evaluate the influence of the co-substrate ratio on process performance and digestate stability. For example, highly and partly biodegradable wastes could be evaluated and compared.

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Abbreviations

AcoD	Anaerobic Co-digestion
AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model No.1
Alk	Alkalinity
AWMC	Advanced Water Management Centre
BMP	Biomethane Potential Test
BOD_{5d}	5-day Biochemical Oxygen Demand
BRN	Biological Nutrient Removal
B_0	Ultimate Methane Potential
CAS	Conventional Activated Sludge
Ch	Carbohydrates
C_i	COD-to-VS ratio of the substrate
C/N	Carbon-to-Nitrogen ratio
COD	Chemical Oxygen Demand
CODp	Particulate Chemical Oxygen Demand
CODs	Soluble Chemical Oxygen Demand
COD_{Saa}	COD of the soluble amino acids
COD_{Sfa}	COD of the soluble fatty acids
COD_{Ssu}	COD of the soluble sugars
COD_{Si}	COD of the soluble inerts
CODt	Total Chemical Oxygen Demand
COD_{TAc}	COD sum of acetate and acetic acid
COD_{TBu}	COD sum of butyrate and butyric acid
COD_{TPro}	COD sum of propionate and propionic acid
COD_{TVa}	COD sum of valerate and valeric acid
COD_{XC1}	COD of the sewage sludge composite
COD_{Xch}	COD of the particulate carbohydrates
COD_{Xi}	COD of the particulate inerts
COD_{Xli}	COD of the particulate lipids
COD_{Xpr}	COD of the particulate protein
COD_{VFA}	COD of the volatile fatty acids
CHP	Combined Heat and Power unit
CI	Confidence Interval
CSTR	Continuous Stirred Tank Reactor
DAF	Fat from a dissolved air flotation
DOC	Dissolved Organic Carbon
DPM	Digested Pig Manure
D1	Reference digester

D2	Co-digestion digester
EU	European Union
f_i	Substrate biodegradability
$f_{Si,XC1}$	Soluble inert from waste composite
$f_{Si,XC2}$	Soluble inert from dead biomass
$f_{Xch,XC1}$	Particulate carbohydrates from waste composite
$f_{Xch,XC2}$	Particulate carbohydrates from dead biomass
$f_{Xi,XC1}$	Particulate inerts from waste composite
$f_{Xi,XC2}$	Particulate inerts from dead biomass
$f_{Xpr,XC1}$	Particulate protein from waste composite
$f_{Xpr,XC2}$	Particulate protein from dead biomass
$f_{Xli,XC1}$	Particulate lipids from waste composite
$f_{Xli,XC2}$	Particulate lipids from dead biomass
G_{ch4}	Methane gas concentration
GC	Gas Chromatograph
G_{co2}	Carbone dioxide gas concentration
GHG	Greenhouse Gases
G_{h2}	Hydrogen gas concentration
G_{h2s}	Hydrogen sulphide concentration
GLY	Glycerol or crude glycerol
HRT	Hydraulic Retention Time
I	Fatty acids or glycerol inhibition factor
IA	Intermediate Alkalinity
IC	Inorganic Carbon
IC_{50}	Half maximal inhibitory concentration
IE	Inhabitant Equivalent
IFAS	Integrated Fixed Film Activated Sludge
ISR	Inoculum- to-Substrate Ratio
IWA	International Water Association
$k_{dis,XC1}$	Disintegration constant of waste composite
$k_{dis,XC2}$	Disintegration constant of dead biomass
$k_{hyd,i}$	First order hydrolysis rate constant of the substrate
K_I	Inhibition coefficient
$k_{m,gly}$	Maximum uptake rate of glycerol
K_s	Half-saturation constant
LCFA	Long Chain Fatty Acid
Li	Lipids
MAD	Mesophilic Anaerobic Digestion
MAcoD	Mesophilic Anaerobic Co-digestion
MBR	Membrane Bioreactor
MSW	Municipal Solid Waste
n	Inhibition exponent
n.d.	Non-detected or below detection limit

OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
OMSW	Olive Mill Solid Waste
OMW	Olive Mill Waste
PA	Partial Alkalinity
PM	Pig Manure
Pr	Protein
PS	Primary Sludge
r_i	Process rate
S_{aa}	Soluble amino acids concentration
S_{ac-}	Soluble acetate concentration
SBP-V_R	Specific biogas production per volume of digester
SBP-VS_{fed}	Specific biogas production per mass of volatile solid fed
S_{bu-}	Soluble butyrate concentration
S_{ch4}	Soluble methane concentration
S_{co2}	Soluble bicarbonate concentration
S_{fa}	Soluble large chain fatty acids concentration
S_{h+}	Soluble proton concentration
S_{hac}	Soluble acetic acid concentration
S_{hbu}	Soluble butyric acid concentration
S_{hco3-}	Soluble carbon dioxide concentration
S_{hpo42-}	Soluble dihydrogen phosphate concentration
S_{hpro}	Soluble propionate concentration
S_{hs-}	Soluble hydrogen sulphide
S_{hva}	Soluble valeric acid concentration
SHW	Slaughterhouse Waste
S_{h2}	Soluble hydrogen concentration
S_{h2po4-}	Soluble dihydrogen phosphate concentration
S_{h2s}	Soluble sulphide acid
S_i	Soluble inert concentration (in Chapter 4)
S_i	Substrate concentration (in Chapter 5 and 7)
S_{nh3}	Soluble free ammonia concentration
S_{nh4+}	Soluble ammonium concentration
S_{oh-}	Soluble hydroxyl concentration
S_{pro-}	Soluble propionate concentration
SRT	Solid Retention Time
SS	Sewage Sludge
S_{su}	Soluble sugar concentration
S_{va-}	Soluble valerate concentration
TA	Total Alkalinity
TAN	Total Ammonia Nitrogen
TAD	Thermophilic Anaerobic Digestion
TAcoD	Thermophilic Anaerobic Co-digestion

t_{delay}	Lag-phase
TKN	Total Kjeldahl Nitrogen
TKP	Total Kjeldahl Phosphorous
TS	Total Solids
TSS	Total Suspended Solids
UB	University of Barcelona
VFA	Volatile Fatty Acid
VS	Volatile Solids
VSS	Volatile Suspended Solids
WAS	Waste Activated Sludge
WW	Waste Water
w/w	Mixture on a wet-basis
WWTP	Wastewater Treatment Plant
X_{aa}	Amino acids degraders concentration
X_{ac}	Acetate degraders concentration
X_{ch}	Particulate carbohydrate concentration
X_{c1}	Composite concentration from waste
X_{c2}	Composite from dead biomass concentration
X_{c4}	Valerate and butyrate degraders concentration
X_{fa}	LCFA degraders concentration
X_{pr}	Particulate protein concentration
X_{h2}	Hydrogen degradation concentration
X_{i}	Particulate inert concentration
X_{li}	Particulate lipids concentration
X_{pro}	Propionate degraders concentration
X_{su}	Sugars degraders concentration
$\beta_{N,Xpr}$	Mass conversion parameters for protein
γ_{li}	Theoretical oxygen demand of lipids

Resumen en castellano

La codigestión anaeróbica es actualmente, como muestra el incremento de publicaciones, el tema más relevante en el campo de la digestión anaeróbica. La codigestión anaeróbica consiste en digerir dos o más sustratos de origen diferente, con el objetivo de compensar las carencias que los sustratos presentan cuando son digeridos individualmente. A pesar que la codigestión anaeróbica ha sido previamente estudiada, la mayoría de trabajos se han focalizado en la optimización del ratio carbono-nitrógeno o el porcentaje de cosustrato más que en analizar la influencia de la composición de la materia orgánica a digerir. Asimismo, existe poco conocimiento sobre los mecanismos sinérgicos que tienen lugar en un codigestor, aunque tales puede ser muy interesante para profundizar el conocimiento de la codigestión anaeróbica y, consecuentemente, hacer una mejor selección de los cosustratos y optimizar su dosis en el influente del digestor. Otro aspecto relevante en lo que se refiere a la viabilidad de la planta de codigestión es analizar cómo afecta la adición del cosustrato en el comportamiento del digestor y en la estabilidad del digerido.

En esta tesis, la mono- y codigestión de residuos urbanos, agropecuarios e industriales ha sido estudiada con el objetivo de profundizar en el conocimiento de esta opción tecnológica, la cual permite mejorar la viabilidad económica de las plantas y, consecuentemente, fomentar su implantación. Dentro del campo de la codigestión anaeróbica, el presente trabajo hace énfasis en lo que refiere a las interacciones entre sustratos (sinergias y antagonismos), el efecto de la codigestión sobre la calidad del digerido y su modelización. La modelización de la codigestión se ha utilizado para estimar parámetros bioquímicos necesarios para el diseño y operación de estos digestores o para predecir el comportamiento del digestor.

En los diferentes estudios de codigestión realizados: codigestión de residuos de matadero, codigestión de lodos de depuradora y glicerina, y codigestión de purín de cerdo y glicerina se ha podido quantificar que la codigestión de residuos es una opción tecnológica que permite incrementar la producción, mejorar la cinética de degradación y mitigar la inhibición de los microorganismos. Aunque el grado de mejora y los mecanismos que tienen lugar dependen de las propiedades susbtrato y cosustrato.

Introducción

Los cambios socioeconómicos de las últimas décadas, las altas concentraciones de población en núcleos urbanos, el desarrollo de la industria agroalimentaria y la intensificación de las explotaciones ganaderas, han propiciado la producción de grandes cantidades de residuos orgánicos que causan graves problemas medioambientales. Actualmente existen diversas tecnologías para tratar estos residuos, con o sin recuperación energética, entre las que destaca la digestión anaeróbica (DA). La DA tiene como principales ventajas: (1) la estabilización de la materia orgánica, (2) ser una fuente de energía renovable gracias al metano producido durante el proceso y (3) la reducción de la emisión de gases de efecto invernadero (Bonmatí y col., 2001). En la actualidad, la DA es un tecnología consolidada aunque con una implantación relativamente baja. Esta baja implementación es consecuencia, entre otros factores, de los bajos rendimientos en producción de metano que presentan algunos sustratos y la dificultad y desuniformidad de la burocracia necesaria para desarrollar el proyecto. Por otro lado, el constante incremento del precio del petróleo, la necesidad de ganaderos/industrias de diversificar sus ingresos, reducir la dependencia energética y la implantación de nuevas directivas ambientales han hecho crecer como nunca antes el interés por esta tecnología en toda Europa. Aunque la actual crisis económica ha hecho disminuir notablemente la cantidad de proyectos.

Des del punto de vista técnico, una de las mejores y simples opciones para mejorar el rendimiento de la DA, y consecuentemente su viabilidad económica, es la codigestión de residuo. La codigestión anaeróbica (CoDA) consiste en digerir una mezcla de dos o más sustratos de origen diferente para aprovechar la sinergia de las mezclas y compensar las carencias que los sustratos presentan cuando son digeridos individualmente (Mata-Álvarez y col., 2000 y 2011). La principales ventajas de la CoDA son: (1) incrementar la producción de metano; (2) aumentar la carga de materia orgánica biodegradable en el influente; (3) optimizar la humedad de la corriente de entrada; (4) diluir compuestos inhibitorios y/o tóxicos presentes en algunos residuos; (5) reducir la emisión de gases de efecto invernadero a la atmósfera, (6) mejorar la calidad del efluente; y (7) ahorrar costes de inversión y de operación al compartir una misma instalación (Alatriste-Mondragón y col., 2006; Mata-Álvarez y col., 2000 y 2011). Sin embargo, algunos inconvenientes deben también ser considerados: (1) el coste de

transporte del cosustrato hasta la planta de DA; (2) el riesgo de extender sustancias contaminantes; (3) la realización de mezcla ad vultum tuum o basadas en heurísticos que puedan afectar negativamente al digestor anaeróbico; y (4) la coordinación y armonización de las diferentes políticas de los generadores de residuos.

La CoAD es un tema que se ha puesto de actualidad en los últimos años pese a conocerse desde los años 1970 (Mata-Álvarez y col., 2011). La Figura 1 muestra la interrelación entre los sustratos con referencia a su ratio carbono-nitrógeno (C/N) (parámetro importante en la codigestión) y al porcentaje de artículos que trataron estos sustratos. En la figura, se observa que un alto porcentaje de los artículos sobre codigestión trabajaron con lodos de depuradora (27%) y estiércol (25%), consecuencia de la necesidad de aumentar las ganancias que representa la venta incentivada de la electricidad producida y reducir las emisiones de gases de efecto invernadero de estas instalaciones. Los cosustratos ideales para los lodos de depuradora y el estiércol, sustratos caracterizados por un alto contenido en nitrógeno y una elevada alcalinidad, son los residuos industriales y agrarios, que contienen menor alcalinidad y un ratio C/N mayor.

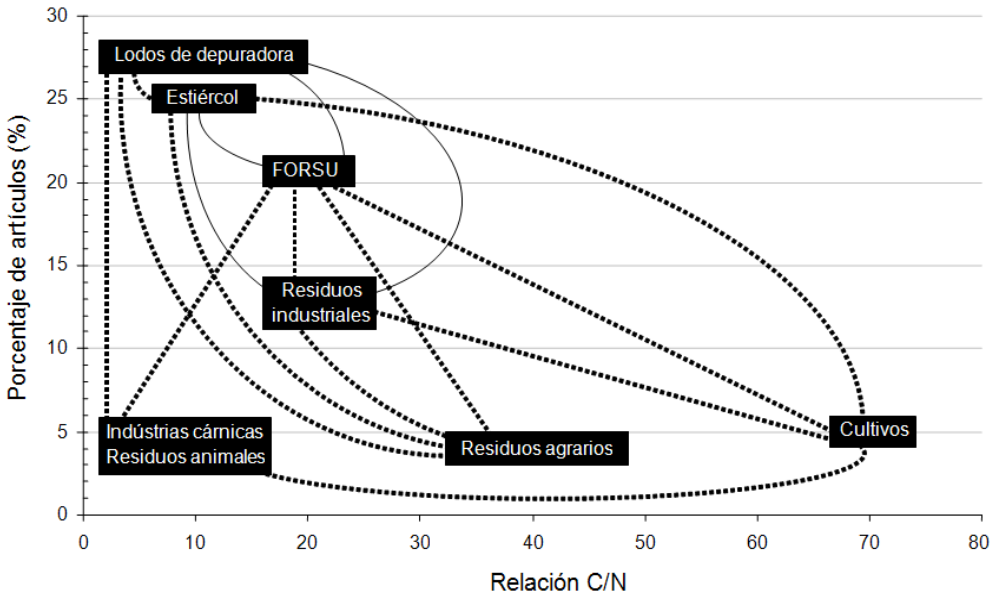

Figura 1. Artículos en revistas referentes a la codigestión anaeróbica (Mata-Álvarez y col., 2011). FORSU: Fracción orgánica de los residuos sólidos urbanos

Hay que tener en cuenta que, en muchos casos, el bajo rendimiento de producción de biogás no justifica la elevada inversión requerida en plantas de explotación agraria para la digestión del estiércol únicamente. Sin embargo, la producción de biogás puede incrementarse considerablemente con la adición de cosustratos ricos en carbono (Pavan y col., 2007). Uno de los países pioneros en la aplicación de la codigestión con deyecciones animales es Dinamarca, donde actualmente hay alrededor de 20 plantas de digestión anaeróbica centralizadas tratando 1,5 millones toneladas al año de estiércol aproximadamente, la mayoría de las cuales tratan conjuntamente residuos orgánicos, preferiblemente en condiciones termofílicas (Angelidaki y Ellegaard, 2003; Nielsen y Angelidaki, 2008). Otros países, como Suecia, también cuentan con un número significativo de plantas de codigestión, alrededor de 200 en total, entre las cuales 10 centralizadas. Los cosustratos para estiércol en estas plantas centralizadas proceden principalmente de la industria alimentaria, mientras que en las plantas individuales proceden de los residuos de los cultivos (Lantz y col., 2007). En cualquier tipo de planta, centralizada o no, hay que tener en cuenta que la adición de un cosustrato va a suponer una reducción del THR y un incremento de la velocidad de la carga orgánica (VCO) del digestor, factores que pueden empeorar la calidad del digerido, aunque esto va a depender mucho de la operación de la misma. Hay que considerar que la forma más sencilla de valorizar el digerido de un digestor anaeróbico es su aplicación directa al suelo como fertilizante o enmienda orgánica. En la actualidad, el empeoramiento de la calidad del digerido está propiciado por la prevalencia de la producción de biogás en decremento de la estabilización del residuo; factor que puede hacer imprescindible el post-tratamiento, por compostaje por ejemplo, del digerido antes de su aplicación al suelo. El post-tratamiento tendría como objetivo aumentar la estabilidad del digerido y, consecuentemente, reducir el riesgo de contaminación del ecosistema.

La CoAD también se ha estudiado desde el punto de vista de la modelización. El poder de los modelos radica en su capacidad para reproducir el comportamiento empírico de manera clara y cuantificable, a través de la simulación de los procesos físicos, químicos y biológicos (Esposito y col., 2008; Galí y col., 2009). Los primeros trabajos que estudiaron el modelado de procesos de codigestión aparecieron en 1996, 1997 y 1999, a pesar de que la mayoría de ellos han sido publicados más recientemente, y se basan en el estándar de la IWA, el *Anaerobic Digestion Model 1* (ADM1). La Tabla 1 resume la

evolución de la modelización de la codigestión de residuos desde 1996 hasta la actualidad.

Tabla 1. Evolución de la modelización de los procesos de codigestión

Año	Autor	Modelo	Corriente residual
1996	Bozinis y col.	- Cinética de Monod - Muchos parámetros	- AR industrial
	Gavala y col.	- Cuatro etapas - Tres grupos bacterianos	- RO, purín de cerdo y AR de la industria láctea
1997	Kiely y col.	- Dos etapas - Inhibiciones por amoniaco y ác. acético	- FORSU y lodo primario
	Angelidaki y col.	- Una etapa enzimática - Seis grupos bacterianos - Inhibiciones por amoniaco y ác. acético	- Deyecciones ganaderas y RO
1999	Angelidaki y col.	- Una etapa enzimática - Seis grupos bacterianos - Inhibiciones por amoniaco y ác. acético	- Purín con glicerina - Purín con gelatina - Purín con AR industrial
2007	Lübken y col.	- ADM1 incluyendo bacterias y metanógenos en la entrada	- Deyecciones ganaderas y cultivos energéticos
2008	Fezzani y Ben Cheikh,	- ADM1 incluyendo inhibición por AGV totales en la eliminación de acetato	- RO
	Espostio y col.	- ADM1 incluyendo la degradación de compuestos con azufre en la etapa de hidrólisis	- FORSU y lodo de EDAR
2009	Derbal y col.	- ADM1	- FORSU y lodo de EDAR
	Fezzani y Ben Cheikh,	- ADM1 incluyendo degradación de compuestos fenólicos	- RO
	Galí y col.	- ADM1 incluyendo inhibición por sulfito en la eliminación de acetato	- Combinación de residuos agropecuarios
	Zaher y col.	- ADM1	- Combinación de residuos agropecuarios

AR: Aguas residuales; EDAR: Estación depuradora de aguas residuoales; RO: Residuo de la oliva; FORSU: Fracción orgánica de los residuos sólidos urbanos

Objetivos

La presente tesis doctoral tiene como finalidad estudiar y modelizar los mecanismos que permiten mejorar el rendimiento de la producción de metano de un digestor anaeróbico cuando se utiliza la codigestión, es decir, la adición de un cosustrato que compense las carencias del sustrato principal.

Para la consecución de este objetivo, se ha trabajado con dos tipos de reactores anaeróbicos: digestores discontinuos, donde se realizaron los ensayos de biodegradabilidad, y digestores continuos. La modelización se ha empleado para mostrar de forma clara y cuantificable las interacciones entre sustratos.

Los objetivos específicos de este proyecto son:

- Realizar una caracterización físico-química de todos los residuos utilizados en el desarrollo de la tesis. Asimismo se pretende determinar su biodegradabilidad y potencial de metanización.

- Desarrollar una metodología que permita obtener parámetros, constantes y variables de estado para la modelización de la digestión anaeróbica.

- Identificar las sinergias y los antagonismos que tienen lugar durante la codigestión.

- Evaluar el comportamiento, producción de biogás y calidad del digerido, de un digestor operado en continuo cuando se le añade un cosustrato.

- Comparar, en los parámetros anteriormente descritos, la codigestión anaeróbica de dos residuos en condiciones mesofílicas (37 ºC) y termofílicas (55 ºC).

- Comparar los resultados experimentales con los de la modelización, y de este modo probar la robustez y precisión de los modelos utilizados.

Materiales y métodos

Métodos analíticos

Los métodos analíticos de la tesis doctoral se han realizado siguiendo los procedimientos del *Standard methods for the examination of water and wastewater* (APHA, 2005) tal y como se detalla en la Tabla 2.

Tabla 2. Métodos analíticos

Parámetro	Método
Sólidos totales y volátiles	Método estándar 2540G
Demanda química de oxígeno	Método estándar 5220D
Demanda bioquímica de oxígeno	Método estándar 5210D
Alcalinidad	Método estándar 2320B
Ácidos grasos volátiles	Cromatógrafo HP 5890-Serie II
Iones	Cromatógrafo 863 Advanced Compact Metrohm ionic
Nitrógeno amoniacal y Kjeldhal	Método estándar 4500-NH3D y 2500-NorgB 2320B
Proteínas	Galí y col., 2009
Lípidos	Método estándar 5520E
Fibras	Goering y Van Soest, 1970
Composición del biogás	Cromatógrafo Shimadzu GC-2010+

Dispositivo experimental

Ensayos de biodegradabilidad

El ensayo de biodegradabilidad utilizado a lo largo de este estudio está basado en el procedimiento descrito por Angelidaki y col., 2009. El dispositivo experimental consta de diferentes digestores de 115 mL equipados de un septo y un cierre hermético. La proporción $SV_{sustrato}$ /$SV_{inóculo}$ se fijó en 0,5. El digestor "blanco" solo contiene inóculo. Una vez introducido el inóculo y el sustrato el digestor se enrasa con agua desionizada hasta alcanzar un volumen final de 80 mL, se borbotea nitrógeno durante un minuto y se sella con el objetivo de asegurar que el proceso sea estrictamente anaeróbico. Para determinar el metano acumulado durante el ensayo se mide la sobrepresión generada entre mediciones mediante un vacuómetro de precisión Ebro VAM 320 (Figura 2A) y se analiza la muestra en el cromatógrafo de gases.

A

B

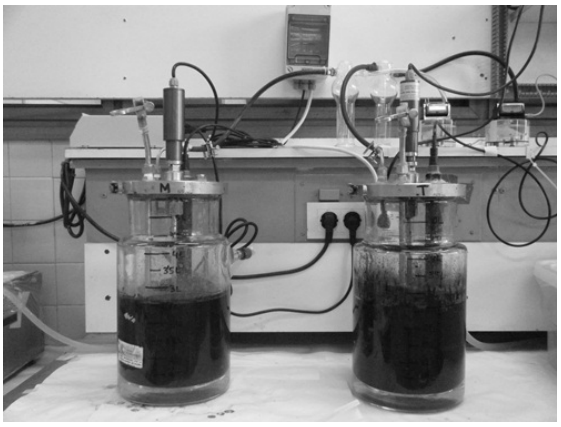

Figura 2. Dispositivo experimental de los: (A) ensayos de biodegradabilidad y (B) digestores continuos de mezcla perfecta a escala laboratorio

Digestores continuos de mezcla perfecta

Se han utilizado dos digestores de mezcla completa idénticos: digestor de referencia (D1) y digestor de codigestión (D2). Estos digestores anaeróbicos tienen una capacidad total de 5 L y un volumen operativo de 4 L; donde la temperatura de operación se mantiene en condiciones mesofílicas (37 ºC) o termofílicas (55ºC) gracias a un baño termostático que hace circular agua a través de la camisa de los reactores. El medio de reacción es agitado a 60 rpm. Los digestores fueron operados con un tiempo de residencia hidráulico (TRH) de 20 días en condiciones mesofílicas y con un TRH de 15 días en condiciones termofílicas. Asimismo, los reactores disponen de un conducto de alimentación y purga, un pH-metro y un contador de biogás milligascounter (Ritter MGC-1) (Figura 2B).

Programa de la Universidad de Barcelona para el modelado de la digestión anaeróbica

El programa de la Universidad de Barcelona para la modelización de la DA y la CoAD está basado en la metodología descrita por el ADM1 (Batstone y col., 2002) y se desarrolló en el marco del proyecto europeo AGROBIOGAS (Galí y col., 2009). El modelo incluye formado por 32 procesos (20 biológicos, 8 equilibrios y 4 de transferencia de gases) y 41 variables de estado (24 solubles, 13 particuladas y 4 gases). El modelo está desarrollado en Matlab/Simulink, aunque los parámetros y valores estequimétricos se obtienen de una hoja Excel.

Resultados y discusión

Digestión anaeróbica de lodos de depuradora: biodegradabilidad y modelización

Siete lodos de diferentes estaciones depuradoras de aguas residuales del Área Metropolitana de Barcelona fueron estudiados con el objetivo de proporcionar una amplia caracterización físico-química de este residuo e intentar correlacionar esta con su biodegradabilidad y cinética de solubilización; etapa limitante de la digestión anaeróbica de este residuo.

Los resultados mostraron potenciales de metanización de entre 188 y 214 mL CH_4 g^{-1} DQO (Figura 3) y eliminaciones de DQO entre 58 y 65%. Asimismo, se pudieron determinar dos grupos en lo que respecta a la constante de solubilización: (1) baja velocidad entre 0,23 y 0,35 $días^{-1}$; y (2) alta velocidad entre 0,27 y 0,43 $días^{-1}$. Constante obtenida ajustando la curva de metano acumulado a una cinética de primer orden. No se pudo determinar ninguna relación estadísticamente significativa ($p < 0.05$) entre el potencial de metanización o la cinética de solubilización y la caracterización del lodo. De hecho, la relación con un mayor coeficiente de determinación ($R^2 = 0.83$) se obtuvo cuando todos macro compuestos (carbohidratos, proteínas y lípidos) fueron considerados.

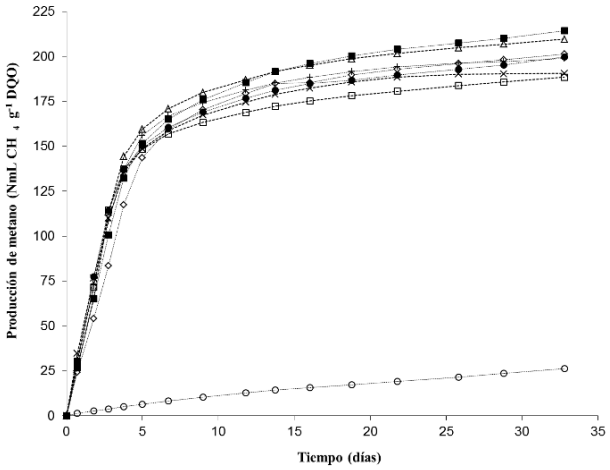

Figura 3. Producción acumulada de metano de los siete lodos en estudio: SS_A (◊), SS_B (■), SS_C (+), SS_D (×), SS_E (▲), SS_F (□), SS_G (●) y blanco (○).

Posteriormente se desarrolló una metodología, basada en la caracterización del lodo antes y después del ensayo de biodegradabilidad, para calcular los coeficientes de biodegradabilidad, concentración de partículas del lodo y otros coeficientes

esteoquimétricos. Estos datos son necesarios para la correcta modelización de los lodos cuando se utiliza la estructura y metodología descrita por el ADM1, aunque en la actualidad no existe un procedimiento definido para determinarlos. La metodología propuesta en este estudio relaciona la caracterización del lodo con los análisis de DQO total, soluble y particulada, y permite obtener los coeficientes de fraccionamiento de la materia particulada del lodo: inertes solubles ($f_{Si,XC}$), inertes particulados ($f_{Xi,XC}$), carbohidratos ($f_{Si,XC}$) proteínas ($f_{Xpr,XC}$) y lípidos ($f_{Xli,XC}$) (Tabla 3), así como los compuestos orgánicos e inorgánicos solubles del lodo.

Tabla 3. Coeficientes de fraccionamiento de los lodos

	SS_A	**SS_B**	**SS_C**	**SS_D**	**SS_E**	**SS_F**	**SS_G**	**Promedio**
$f_{Xch,XC1}$	0.19	0.24	0.20	0.17	0.14	0.07	0.10	0.16
$f_{Xpr,XC1}$	0.15	0.15	0.19	0.18	0.17	0.21	0.18	0.17
$f_{Xli,XC1}$	0.24	0.21	0.20	0.20	0.31	0.26	0.26	0.24
$f_{Xi,XC1}$	0.41	0.40	0.41	0.44	0.38	0.45	0.46	0.42
$f_{Si,XC1}$	0.008	0.005	0.005	0.007	0.002	0.009	0.008	0.006

La comparación de los resultados experimentales con los simulados para los ensayos de biodegradabilidad mostró la consistencia de la metodología desarrollada en lo que refiere al balance de materia. Sin embargo, se detectó una subestimación de la constante de solubilización obtenida mediante el ajuste de primer orden. Este fenómeno se relacionó con la acumulación de compuestos intermedios durante el ensayo. Finalmente, la metodología desarrollada se validó con los resultados obtenidos en un digestor continuo a escala laboratorio (Tabla 4).

Tabla 4. Comparación de los resultados experimentales y modelados para un digestor a escala laboratorio de lodos de depuradora

	Unidades	**Experimental**	**Simulación**
DQO total	g O_2 L^{-1}	23.7 (21.0 – 25.6)	24.6
Eliminación de DQO	%	42.4 (52.2 – 34.4)	40.3
pH	-	7.7 (8.0 - 7.5)	7.2
Nitrógeno amoniacal	mg N L^{-1}	610 (580 – 627)	550
Producción de biogas	L g SV	1.1 (1.3 – 0.8)	1.1
Methane content	%	-	68

Identificación de las sinergias durante la codigestión anaeróbica

El objetivo del presente estudio fue analizar las interacciones (sinergias y antagonismos) entre carbohidratos, proteínas y lípidos cuando estos son tratados en un mismo digestor. Para ello, se realizaron dos tandas de ensayos de biodegradabilidad. La primera se realizó con substratos sintéticos donde celulosa, caseína y aceite de oliva se utilizaron como fuente de carbohidratos, proteínas y lípidos, respectivamente. En cambio, en la segunda tanda se utilizaron residuos de matadero siendo rumen, sangre y grasas de la unidad de flotación (DAF) los residuos elegidos como fuente de carbohidratos, proteínas y lípidos, respectivamente. En cada uno de los ensayos se ha realizado la monodigestión de los tres sustratos seleccionados y siete mezclas entre ellos (en base a los sólidos volátiles); esta últimas fueron diseñadas para abarcar todas las mezclas posibles entre ellos (Figura 4).

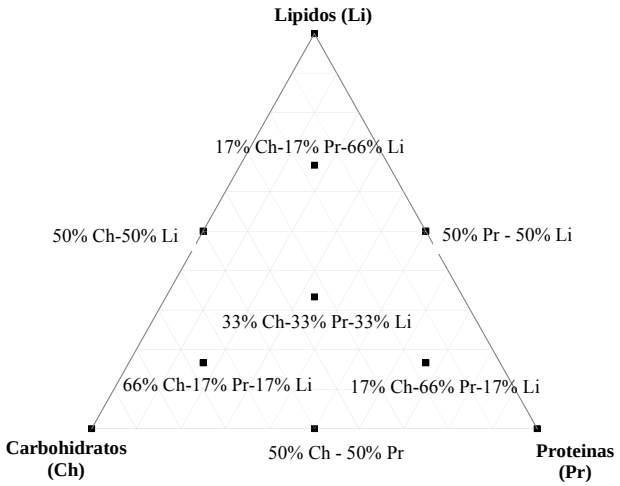

Figure 4. Diseño de las mezclas entre carbohidratos, proteínas y lípidos

La curva de metano de la celulosa y la caseína presentó un perfil correspondiente a una cinética de primer orden con potenciales de metanización de 355 y 480 mL CH_4 g^{-1} SV, respectivamente. Por otro lado, el aceite de oliva presentó un perfil sigmoidal, seguramente debido a la inhibición de los microorganismos metanógenos por la presencia de ácidos grasos de cadena larga (AGCL), con un potencial de metanización de 915 mL CH_4 g^{-1} SV. Teniendo en cuenta los valores teóricos de DQO de los sustratos en estudio queda claro que la eliminación de materia orgánica fue casi del 100%. Respecto a las mezclas, si se compara la curva de metano real con la calculada matemáticamente a partir de los resultados de monodigestión se observa claramente un

incremento de la cinética del proceso. Al ser el fenómeno repetitivo en todos los ensayos se ha concluido que la diversificación de la materia orgánica a digerir genera una sinergia que queda reflejada en una mejora de la cinética del proceso. Asimismo, en las muestras con una mayor concentración de aceite de oliva (50%Ch-50%Li, 50%Pr-50%Li, 33%Ch-33%Pr-33%Li y 17%Ch-17%Pr-66%Li) se pudo observar como la curva de metano presentaba un perfil de primer orden y no sigmoidal. Consecuentemente, se pudo concluir que una diversificación de la composición de la materia orgánica también puede ser utilizada para disminuir la inhibición de los metanógenos, en este estudio causada por los AGCL. Finalmente remarcar que ninguna de las mezclas ensayadas presenta un incremento o disminución significativa del potencial de metanización.

Los ensayos de biodegradabilidad de los residuos de matadero muestran resultados parecidos a los obtenidos en los ensayos de biodegradabilidad de los sustratos sintéticos. De hecho, los perfiles de la caseína y sangre, y los de aceite de oliva y DAF son coincidentes. Consecuentemente, la DA del DAF también estuvo inhibida por la presencia de AGCL. Por otro lado, el rumen presenta, debido a la elevada concentración de lignina, una cinética y un potencial de metanización menor al de la celulosa. Los resultados de las mezclas también mostraron, al comparar la curva de metano real con la calculada matemáticamente, un incremento de la cinética del proceso. Para ser precisos las mezclas ricas en DAF (50%Ch-50%Li, 50%Pr-50%Li, 33%Ch-33%Pr-33%Li y 17%Ch-17%Pr-66%Li) presentaron una mejora mayor a la obtenida con los sustratos sintéticos, mientras que las ot ras mezclas presentados valores parecidos. La menor inhibición por AGCL se relacionó con la adsorción de estos compuestos en las partículas del rumen y de la sangre, factor que hizo disminuir la adsorción de los AGCL sobre los microorganismos metanógenos. Para finalizar es importante resaltar que tres mezclas (50%Ch-50%Li, 33%Ch-33%Pr-33%Li y 17%Ch-17%Pr-66%Li) presentan una diferencia significativa entre el potencial de metanización real y el teórico, este hecho ha sido relacionado con la presencia de biomasa hidrolítica en el rumen que es capaz de degradar más los lípidos presentes en el DAF.

Codigestión anaeróbica de purín de cerdo y glicerina: estudio experimental y de modelización

La producción de biodiesel en Europa ha aumentado de forma exponencial durante los últimos años (EBB, 2010), donde la glicerina representa aproximadamente un 10% es peso de la producción obtenida en una planta de biodiesel. Este subproducto, una vez refinado se puede emplear en la industria química, sin embargo, este tratamiento no es económicamente viable para plantas de tamaño medio y pequeño. En la actualidad, la gran cantidad de glicerina industrial producida ha despertado el interés de la comunidad científica para encontrar otras utilidades sin necesidad de alcanzar elevada pureza. En el presente estudio se propone utilizar la glicerina como cosustrado de la digestión anaeróbica de purines de cerdo. Con el objetivo de analizar la viabilidad de las mezclas entre purín de cerdo y glicerina se analizó un amplio rango de mezclas. Estas abarcaban del 100% hasta el 20% en peso de purín de cerdo. Esto diseño experimental permitió evaluar cómo afecta al proceso de digestión el déficit de nitrógeno cuando grandes cantidades de glicerina son añadidas. Finalmente se utilizó una versión modificada del ADM1 para simular todas las mezclas y evaluar la capacidad de predicción del modelo desarrollado.

Como se puede observar en la Figura 5, la mezcla que contenía un 80% de purín de cerdo fue la que presentó un mayor producción específica de metano con 215 mL CH_4 g^{-1} DQO, lo que representa un 125% más que la obtenida cuando el purín de cerdo fue monodigerido (96 mL CH_4 g^{-1} DQO). Aunque la mezcla que presentó un menor potencial de metanización fue que contenía un 20% de purín de cerdo. Esta mezcla, como mostraron los análisis al final de ensayo, estuvo claramente inhibida por la acumulación de ácidos grasos volátiles y la limitación de nitrógeno en el medio de reacción. La mayor o menor producción de metano de las mezclas se correlacionó con el ratio carbono-nitrógeno de cada una de ellas, así como el nitrógeno disponible en el medio de reacción. La comparación entre los resultados experimentales y los simulados muestra claramente que los modelos son una herramienta eficaz para predecir el comportamiento de un digestor anaeróbico cuando se emplea la codigestión anaeróbica (Figura 5). Aunque estos resultados también ponen en evidencia que hay factores que el presente modelo no puede reproducir como la inhibición en la mezcla del 80% de purín o la velocidad de inhibición en la mezcla de 40 y 20% de purín.

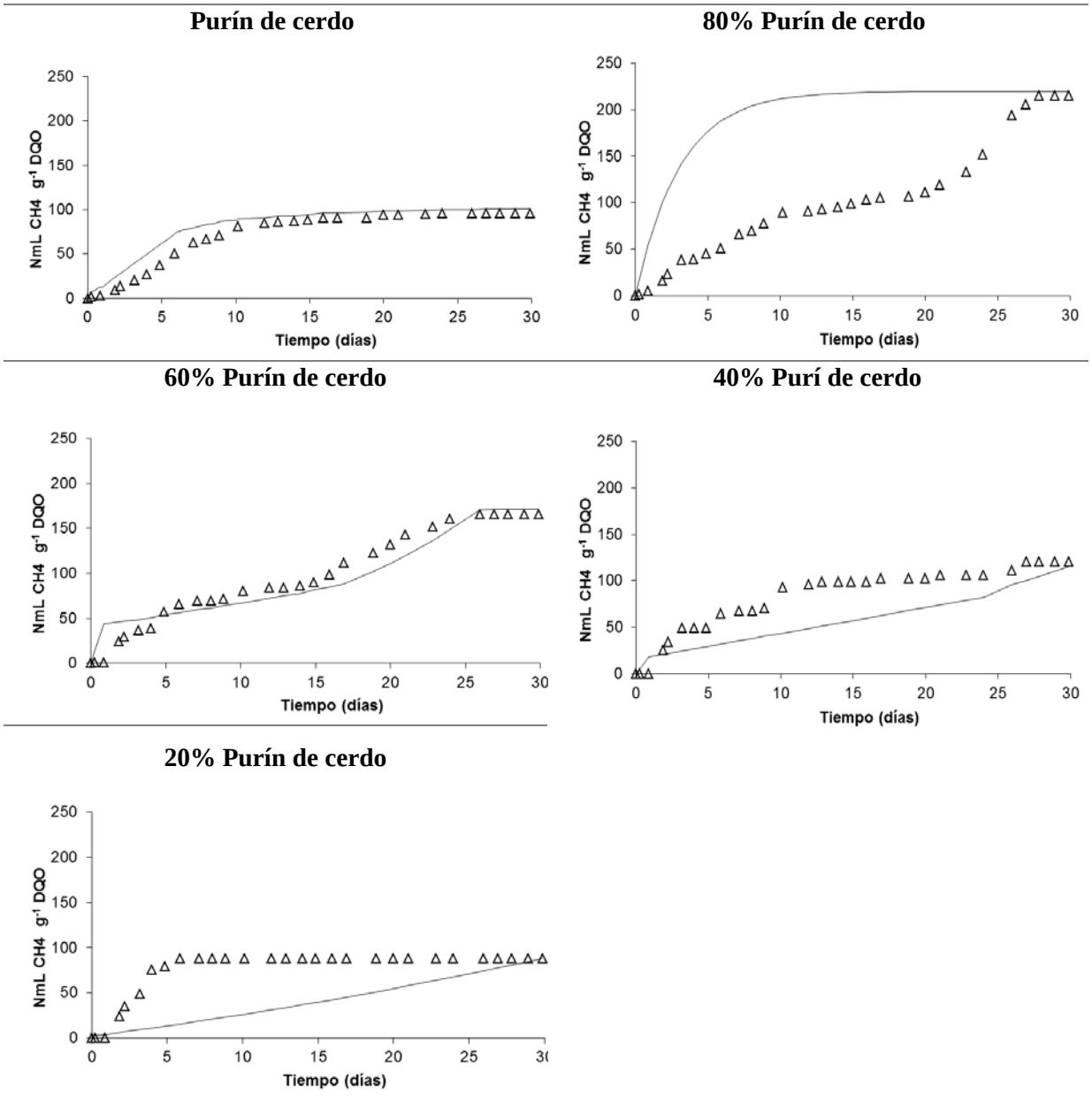

Figura 5. Producción acumulada de metano de los ensayos experimentales (Δ) y su correspondiente simulación (-)

Codigestión anaeróbica de lodo de estación depuradora de aguas residuales y glicerina industrial: estudio de las sinergias y las inhibiciones

La presencia de algunos compuestos (metanol, sales) y el riesgo de sobrecarga son las principales limitaciones cuando glicerina industrial se utiliza como cosustrato para la digestión anaeróbica. En este estudio, varias tandas de ensayos de biodegradabilidad se realizaron con el objetivo de estudiar los mecanismos de sinergia e inhibición que tienen lugar durante la codigestión de glicerina industrial y lodos de depuradora. Además, en

este estudio se utilizaron técnicas estadísticas no lineares para la cuantificar el efecto de la codigestión sobre la biodegradabilidad y la cinética de los sustratos empleados.

Los ensayos permitieron determinar que la glicerina empleada en el presente estudio tenía un potencial de metanización (550 mL CH_4 g^{-1} SV) superior al que cabría esperar (426 mL CH_4 g^{-1} SV). Este incremento seguramente se debió a la presencia de lípidos y otros compuestos que no han reaccionado durante la producción de biodiesel. Además en los ensayos de codigestión se determinó que el la glicerina presenta una biodegradabildad cercana al 100%. Esto no pudo ser corroborado en los ensayos que trataban únicamente glicerina, pues presentaron una inhibición severa. Los métodos estadísticos no lineares permitieron estimar que la constante de semi-saturación de la glicerina es de 1 g L^{-1} aunque los ensayos no se vieron fuertemente inhibidos hasta que la concentración de glicerina fue superior a los 3.5 1 g L^{-1}.

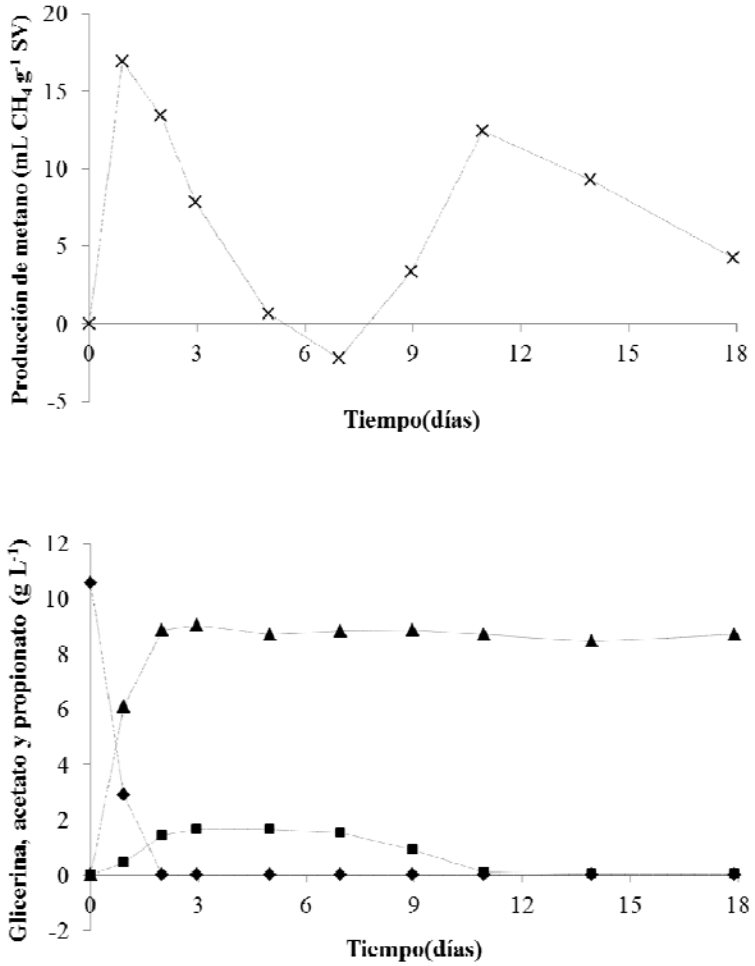

Figura 6. Producción acumulada de metano (arriba) y evolución de la concentración de glicerol (♦), acetato (■) y propionato (▲) (abajo).

Posteriormente, se realizó un ensayo de biodegradabilidad, tratando únicamente glicerina, para identificar el mecanismo de inhibición de esta. Para ello, no solo se siguió la producción de metano acumulada sino que también se analizó la evolución de glicerina, acetato, propionato, butirato, valerato, etanol, propanol, butanol, y 1,3 propandiol. Como muestra la Figura 6, el glicerol fue degradado muy rápidamente, de hecho los 10 g L^{-1} fueron convertidos a propionato y acetano en menos de dos días. Sin embargo, cuando la concentración de propionato alcanzó los 9 g L^{-1} la producción de acetato y metano paró. La baja o ausencia de los otros intermedios así como de H_2 hicieron concluir que la degradación de la glicerina se realiza a través de la producción de lactato y luego propionato. Consecuentemente, cuando elevadas dosis de glicerina añadidas como cosustrato esta es convertida rápidamente a propionato, compuesto que ha sido ampliamente reportado como inhibidor de la digestión anaeróbica.

Digestión anaeróbica de purín de cerdo y glicerina industrial en condiciones mesofílicas en un digestor en continuo de mezcla completa

Como se ha podido comprobar en los estudios detallados anteriormente la glicerina industrial es, debido a su elevada biodegradabilidad, potencial de metanización y solubilidad en agua, un cosustrato ideal para la codigestión anaeróbica y por ello se utilizó como cosustrato para el purín de cerdo en los ensayos en continuo.

La puesta en marcha de ambos digestores se realizó inoculando 4 L de purín digerido obtenido de una planta de DA de tratamiento de purines. A partir de ese momento ambos digestores fueron alimentados con purín fresco hasta que alcanzaron las mismas condiciones estacionarias de operación. A continuación, se introdujo progresivamente glicerina en el alimento del digestor de codigestión (D2). La mezcla glicerina/purín se fue enriqueciendo paulatinamente (1%, 3% y 5% en peso húmedo de glicerina). El incremento se realizaba a medida que el reactor mostraba signos de aclimatación hasta que se detectaron síntomas de inhibición. Es importante destacar que pequeñas adiciones de glicerina en el alimento supone: (1) un significante incremento de la carga orgánica del reactor, (2) un substancial incremento del porcentaje de la materia orgánica soluble del alimento y (3) un incremento de la relación C/N. Todos estos factores hacen necesaria una adaptación y redistribución de las poblaciones anaeróbicas y, en

consecuencia, el incremento del porcentaje de glicerina en el alimento se realiza paulatinamente. En este aspecto, cuando se incrementó el porcentaje de glicerina al 5% en peso, el digestor no mostró signos de adaptación. Con el objetivo de evitar el fallo del digestor se disminuyó la concentración de glicerina hasta el 4% en peso. Estas condiciones de operación (VCO 1,7 g SV L^{-1} $día^{-1}$, un 240% superior al reactor de referencia), mantenidas durante más de 120 días sin señales de inhibición o desestabilización, fueron las que mostraron los mejores rendimientos. Mientras que el digestor de referencia (D1) tenía una producción de biogás de 0,27 L L^{-1} $día^{-1}$ el reactor de codigestión producía 1,36 L L^{-1} $día^{-1}$. La sinergia generada entre ambos sustratos también se vio reflejada en la degradación de la materia orgánica que aumentó de un 41% SV, para D1, hasta un 77% SV, para D2. Al igual que en el estudio anterior, las significantes mejoras obtenidas en el reactor de codigestión pueden ser explicadas principalmente por el incremento de la VCO, aunque el aumento de la producción específica de biogás pone de relieve que el gran potencial de metanización de la glicerina y la optimización del ratio C/N han sido factores importantes para la optimización del proceso.

Por otro lado, el análisis de los compuestos orgánicos mayoritarios (proteínas, lípidos, carbohidratos y fibras) pone de manifiesto la gran cantidad de proteínas y carbohidratos que contiene el purín de cerdo, donde las fibras representan aproximadamente un 50% del total de los carbohidratos. En cambio, en la mezcla glicerina/purín, las fibras representan menos del 10% de los carbohidratos presentes. Fijándose en la eliminación de estos compuestos se puede observar como los microorganismos de D1 degradan mayores cantidades de proteínas, lípidos y fibras (compuestos principalmente particulados) que los microorganismos de D2. Esto es seguramente debido a que los microorganismos deban hidrolizar mayores cantidades de materia orgánica para obtener nutrientes mientras que D2 utiliza principalmente glicerina como fuente de carbono. Este fenómeno también queda reflejado en los valores respirométricos de los efluentes, que aun siendo inferiores a los límites de estabilidad sugeridos, eran menores para el digerido de D1 que para el de D2; poniendo de relieve que la materia orgánica en el digestor de codigestión no estaba tan estabilizada como consecuencia de la adición de glicerina.

Digestión anaeróbica de purín de cerdo y glicerina industrial en condiciones termofílicas en un digestor en continuo de mezcla completa

La puesta en marcha de ambos digestores se realizó incrementando la temperatura desde 37 a 55 ºC en un día; día sin alimentación. Sin embargo, D2 no mostro signos de adaptación por lo que el porcentaje de glicerina en el alimento se tuvo que reducir del 4 al 3%. La reducción del porcentaje de glicerina llevo al digestor a unas condiciones estables de operación. Cuando el digestor alcanzo el estado estacionario se realizaron los mismos análisis que en el estudio anterior.

Los resultados obtenidos en este estudio son parecidos a los reportados anteriormente donde el incremento de la producción de biogás de 0,17 a 0,47 L L^{-1} $día^{-1}$ fue relacionado con la biodegradabilidad de la glicerina, la reducción de la concentración de nitrógeno amoniacal y la optimización del ratio carbono-nitrógeno. Asimismo, el análisis de los macro-compuestos puso otra vez de manifiesto que los microorganimos degradaban el glicerol en decremento de los compuestos particulados presentes en el purín de cerdo. Sin embargo, en contra a lo reportado en condiciones mesofílicas, el digerido obtenido de D2 no cumple los valores mínimos de estabilidad necesarios para ser aplicado directamente al suelo. Consecuentemente, sería necesario incrementar el TRH del digestor, reducir el porcentaje de glicerina en el influente y/o realizar un post-tratamiento del digerido.

Conclusiones

Las conclusiones más destacadas de esta tesis son:

- La mejora del modelo *Anaerobic Digestion Model 1* ha permitido predecir con mayor exactitud el comportamiento de los digestores anaeróbicos de lodos de depuradora y de la codigestión anaeróbica de lodo o purí de cerdo con glicerina.

- El correcto desarrollo de nuevas ecuaciones para la codigestión así como de métodos estadísticos ha permitido mostrar de una forma clara y cuantificable el efecto de la codigestión sobre la biodegradabilidad, cinética, inhibición de los sustratos involucrados en el proceso de codigestion.

- En los diferentes estudios se ha cuantificado que la codigestión de residuos es una opción tecnológica que permite incrementar la producción de biogás de un digestor anaeróbico, mejorar la cinética de degradación y mitigar la inhibición de los microorganismos.

- La glicerina, subproducto de la producción de biodiesel, es debido a su elevada biodegradabilidad, potencial de metanización y solubilidad en agua, un cosustrato ideal para la codigestión anaeróbica, ya sea de lodos de depuradora o de purín de cerdo. Aunque conlleva riesgos de inhibición por sobrecarga relacionados con la acumulación de propionato en el medio de reacción.

- La codigestión de purines de cerdo y glicerina en condiciones mesofílicas y termofílicas mejoró un 180% la producción específica de biogás. En el codigestor mesofílico se obtuvo un digerido apto para ser aplicado directamente al suelo, mientras que el digerido obtenido en condiciones termofílicas no cumplía los mínimos de estabilidad.

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