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INFLUENCE OF MICRONUTRIENTS CONCENTRATION ON SLUDGE ANAEROBIC DIGESTION

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Influencia de la concentración de micronutrientes en la digestión anaerobia de lodos

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Llegó la noche, y no encontré un asilo,
¡y tuve sed!... mis lágrimas bebí,
¡y tuve hambre! ¡Los hinchados ojos
cerré para morir!
¿Estaba en un desierto? Aunque a mi oído
de las turbas llegaba el ronco hervir,
yo era huérfano y pobre... ¡El mundo estaba
desierto... para mí!

Rima LXV

Gustavo Adolfo Bécquer. Poeta del Romanticismo castellano.

A María, siempre serás mi mal vicio.

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Y llegó el final. Una fase de la vida que pasa y obliga al recuerdo de quienes han pasado en mi vida para que el texto aquí escrito haya alcanzado no sólo su motivo de ser; sino que además, facetas humanas. Detrás de cada trabajo, tanto académico como industrial, es latente una parte humana. El romanticismo de estudiar por el amor a crecer en el conocimiento rodeado de intelectuales; o la lucha familiar de encontrar en el trabajo el sustento de asegurar el bien estar familiar.

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defraudarte, porque sin duda eres lo más parecido a la figura paternal que he tenido en Chile. Muchas gracias de corazón por todo tu tiempo.

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''El verdadero valiente no es el que siempre está lleno de coraje, sino el que se sobrepone a su legítimo miedo. ''

Mario Benedetti; escritor uruguayo de la Generación del 45.

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Abstract

The management of secondary sludge is complicated point by being a costly matter in wastewater treatment plants. Before their final disposal, normally it is treated inside anaerobic reactors to reduce the total amount of biosolids and therefore their management cost. Any improvement of this process with low cost strategies is an important research item to reduce the energy demand and the cost balances in these plants.

In the first part of this master thesis the thermoelectric fly ash was used as a source of micronutrients for anaerobic microorganisms. It was found that it was possible to triplicate the methane generation rate in thermal pre-treated (120 ºC) sludge with ash supply; while in case of no addition, the methane generation rate was 1.4 ml/d while with 150 mg of ash per liter this generation changes to 4.48 ml/l. Also the hydrolytic matter degradation kinetic was obtained in both cases using batch reactors; for the case of no addition the first order kinetic parameter was 0.019±0.002d^{-1}, when ashes were added this value was 0.046±0.000 d^{-1}. Therefore, the addition of these compounds is able to improve the methane generation and hydrolytic kinetics together.

In the second part of this master thesis how to reduce de sampling time of this assays was studied by combination of advanced statistical methods with the background experiment reported in this assays. These experimental tests are the analytical key techniques to assess the implementation and optimization of anaerobic biotechnologies. This chapter develops a mathematical strategy using sensitivity functions for early prediction of first-order methane generation model parameters. The minimum testing time for early parameter estimation showed a potential correlation with the substrate degradation kinetic constant rate: (i) slowly biodegradable substrates ($k \leq 0.1\ d^{-1}$) with minimum testing times of at least 15 days, (ii) moderately biodegradable substrates ($0.1 < k < 0.2\ d^{-1}$) with minimum testing times between 8 and 15 days, and (iii) rapidly biodegradable substrates ($k \geq 0.2\ d^{-1}$) with testing times lower than 7 days.

1. Introduction

Waste water treatment plants (WWTP) need high requirements of energy for removing pollutants. Activated sludge systems used in WWTP generate as byproduct large quantities of biosolids which are generally disposed for agricultural use, composting, landfilling or incineration (Stasta et al., 2006). The cost of treatment of this biosolids can represent the 50% of operation cost in WWTP (Appels et al., 2008). Taking into account the high energy demand and the high cost of biosolids management (around 500 €/Ton; Van Lier et al., 2008), the anaerobic digestion (AD) technology presents a great contribution in this sense (Bolzonella et al., 2005). Feeding anaerobic reactors with the generated sludge, the total amount of biosolids for disposal and odors emissions are reduced. Also using the generated biogas the energy balance of this treatment system could be equal to zero, or even positive (Perez-Elvira et al., 2012; Fernández - Polanco and Tatsumi, 2016).

Anaerobic digestion is a competitive treatment technology for the management of organic material-rich wastes since it transforms organic matter to renewable energy. As research field anaerobic digestion had shown an important relevance in science by the increment in publication rate; based on Scopus. The production rate of anaerobic digestion papers was around 483 papers in 2007 while in 2015 this quantity growth up to 1382 papers. This holds the importance of this technology as a pillar in wastewater research fields.

Several alternatives have been developed with the aim of replacing the energy consuming steps of typical sewage treatment plant. The pillars for improving the performance of anaerobic digestion in the waste management sector were the reactor design with pre-treatment methods (Appels et al., 2008; Stamatelatou and Tsagarakis, 2015). In the case of pre-treatments the more suitable, by economic balance, is the thermal pre-treatments when the hydrolysis is the limiting step; like secondary sludge digestion case. Beside the pre-treatments, the quality of biogas production keeps strong dependence with feedstock characteristics (solid content, carbohydrates or proteins concentration, trace metals …) so several authors (Romero-Güiza et al., 2016; Huiliñir et al., 2015) investigated about additives in anaerobic digestion (salts, enzymes among others); in order to improve the

reactor behavior stability and increase the methane productivity: e.g. additives to prevent inhibition by ammonia or volatile fatty acids.

Biomethane potential (BMP) tests are the most used methodology by academic and technical practitioners to determine the maximum methane production (B_0) of a certain substrate (Raposo et al., 2011). This batch assay determines B_0 by recording the methane produced when the substrate is mixed with an active anaerobic inoculum until no further methane is produced (Holliger et al., 2016; Ward, 2016). BMP testing is today the most reliable method to determine the ultimate methane production (Lesteur et al., 2010; Ward, 2016), which is a key parameter to assess the implementation feasibility of a full-scale anaerobic digester plant as well as its optimization (e.g. co-digestion, pre-treatment) (Angelidaki et al., 2009; Carrere et al., 2016; Mata-Alvarez et al., 2014). Moreover, BMP tests can also be used to estimate the kinetic parameter of the rate limiting step, i.e. hydrolysis rate for highly particulate substrates which is needed to achieve and optimal design and operation of anaerobic digesters (Angelidaki et al., 2009; Batstone et al., 2003; Koch and Drewes, 2014; Batstone and Jensen, 2011). However, BMP test of highly particulate substrates are very time consuming with testing time ranging from 30 days to over 100 days (Raposo et al., 2011). The high testing time makes sometimes BMP testing not practical for consulting companies and AD plants operators, in which decision-making processes cannot be hold for a month.

1.1 Research goals.

The goals of this research thesis have been focused on improving the feasibility and profitability of solid waste in the anaerobic digestion technology.

The effect of fly ash from thermoelectric plant in anaerobic digestion of secondary sludge from meat factory the addition was studied. When a stimulatory dosage was identified, the impact of it in hydrolytic anaerobic step was valued.

The possibility of reduction sampling time in BMP assays was studied. Here, the sensitivity functions from first order model were applied in order to define an identifiability criteria which leads the required sampling time around to 25 % of traditional sampling time.

2. Literature review

The anaerobic digestion technology is a complex process. In order to improve the minimum understanding that leads to have an idea about this technology and realize the contribution that this thesis brings to de AD. Therefore, it is necessary to introduce the reader to the general concepts about Anaerobic Digestion related to the research work developed in next chapters.

2.1 Anaerobic digestion. Importance.

Anaerobic digestion technology is closely related to energy production since the major final product of the process is a gaseous mixture of methane and carbon dioxide. From this aspect, anaerobic digestion is by default an energy producing technology and, depending on the substrate and the operating conditions, the energy produced may exceed any energy required so that the net energy balance may be positive (Stamatelatou and Tsagarakis , 2015).

Anaerobic digestion is essential an 'energy transfer' process. The energy captured in the chemical bonds of the organic compounds of solid or liquid media is transferred mainly to the C-H bonds of the gaseous methane where the carbon atom is at its utmost reduced state, meaning that methane is the highest energy density organic compound. This energy transfer is carried out by microorganisms growing in the absence of oxygen by degrading a great range of organic substrates or feedstocks. The microorganisms belong to groups of different physiology and substrate affinity; they co-operate to break complex compounds into successively smaller ones in a balanced anaerobic environment of natural ecosystems or robust bioreactors.

The common application of the anaerobic digestion is the treatment of sewage sludge and slurries in digesters. Landfills are also gigantic bioreactors where biogas emissions indicate the anaerobic biological activity upon the organic fraction of the municipal solid wastes. Other feedstock's considered to be ideal substrates for anaerobic microorganisms are the high organic loaded liquid or solid wastes of industries such as food, pulp, petrochemical

and similar industries, livestock establishments. Recently, dedicated crops or crop residues after harvesting and several waste streams of bio-refineries may be directed to centralized biogas units for producing biogas, fertilizers and a nutrient rich liquor which can be sold or become a part of the crop nutrient management plan. The anaerobic digestion represents a core technology role in the integrated resource recovery systems (Batstone and Virdis, 2014).

Traditionally, municipal wastewater treatment plants (Figure 2.1) are based on aerobic processes as the main biochemical conversion process and anaerobic digestion was restricted to sewage sludge treatment. Anaerobic digestion of municipal wastewater has been carried in warm climate countries but stability and other problems have arisen and there are doubts about the suitability of this technology on this type of wastewater (Chernicharo, 2007). In terms of energy aerobic sewage sludge treatment consumes around 1 kWh in the aeration system per 1 kg of chemical oxygen demand removed (COD). While in anaerobic digestion systems, around 13.5 MJ CH_4 energy per 1 kg of COD removed is generated; giving 1.5 kWh of electricity under assumption of 40% of energy recovery efficiency (Van Lier et al., 2008). These energetic values led to an increasing effort in researching who to increase the energetically recovery from waste by anaerobic digestion in order to reduce the energy demand in wastewater treatment plants.

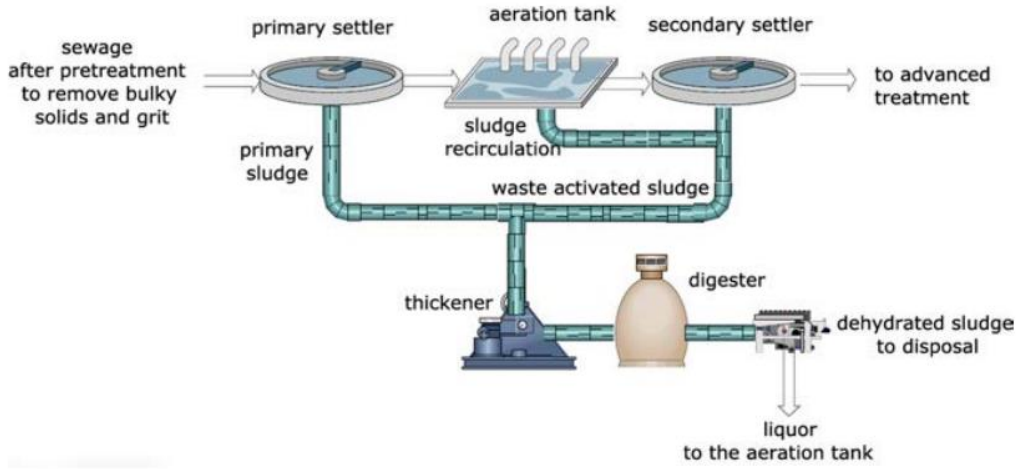

Fig. 2.1. Sewage sludge route in a typical layout of a sewage treatment plant (Stamatelatou and Tsagarakis, 2015).

2.2 The process. Biochemistry of anaerobic digestion.

Anaerobic digestion is a bioprocess consisting of a complex network of individual steps carried out by different groups of microorganisms. The microorganism groups generally grow at different rates and become sensitive at a different degree when exposed to certain environmental conditions (such as: pH, ammonia, concentration of metabolites such as volatile fatty acids, hydrogen…).

The basic conversion steps of the process and the breakdown of the organic content of the initial feedstock are sketched in Figure 2.2.

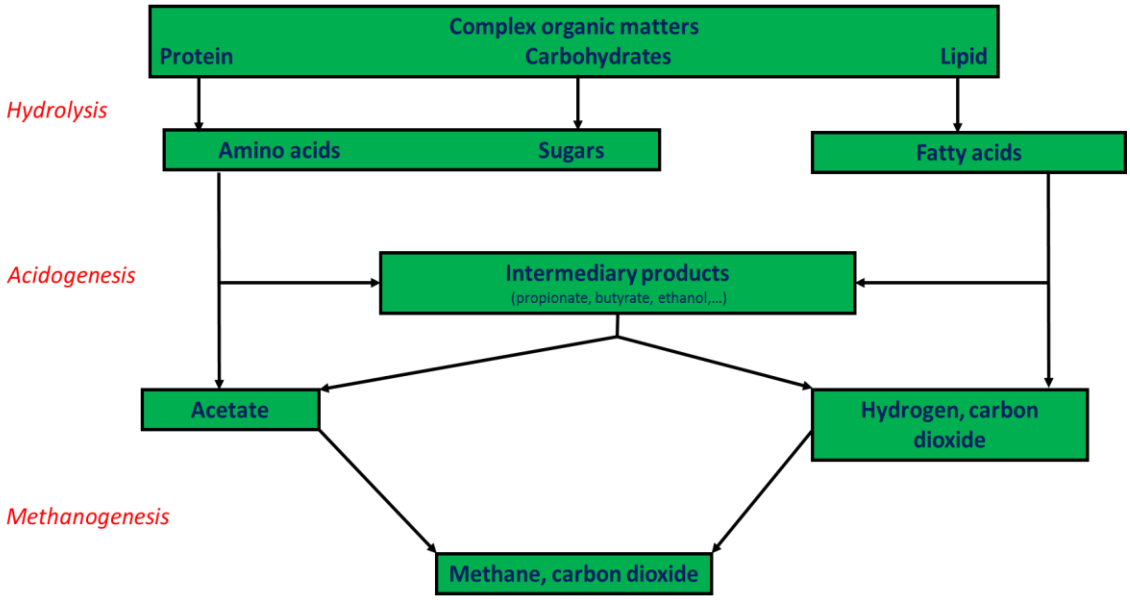

Fig. 2.2. Conversion steps in anaerobic digestion of complex organic matter. Adapted from Batstone et al, 2002.

A brief description of the anaerobic digestion steps follows:

Hydrolysis. This stage is related with the activity of hydrolytic enzymes. They are excreted by microorganisms to breakdown the organic polymers (carbohydrates, proteins and fats) into their respective monomers (sugars, amino acids, lipids), in order to be taken up by the microorganisms for further degradation.

Acidogenesis. Following hydrolysis, the monomers can be converted to a mixture of volatile fatty acids, alcohols and other simpler organic compounds by a versatile group of

microorganisms called acidogens. The electron donors and acceptors come from the organic compounds degraded. What is produced during acidogenesis (propionate, butyrate, valerate, lactate, alcohols, etc.) are transformed to acetic acid, carbon dioxide and hydrogen. Some authors call this stage for acetogensis and this is in general assumed as independent stage. But in this work is assumed this subcategory inside the acidogenesis category because all chemical species from acidogenesis are converted to acetid acid.

Methanogenesis. Methane can be produced from acetate or hydrogen utilization by the acetoclastic and the hydrogen utilizing methanogens respectively. The methane content of biogas is about 60% in most cases but depends on the oxidation state of the organic carbon in the initial substrate; the more reduced the carbon in the initial substrate is, the more methane will be produced (Gujer and Zehnder, 1983). Acetoclastic methanogens, which produce almost 70% of the total methane) are slow growing microorganisms and sensitive to pH, lack of nutrients (Romero-Güiza et al., 2016), certain compounds and so on.

Any unbalance among the anaerobic digestion steps may influence the methanogenesis adversely. It has been recognized that the most important factors that may cause such an unbalance are the pH, the temperature, the nature of the feedstock (composition, nutrients), the presence of toxic or inhibitory substances and the organic loading rate. Depending on the feedstock characteristics (solid content, carbohydrate or protein concentration etc.), the rate limiting step of the process may be the disintegration/hydrolysis or methanogenesis.

2.3 Micro and macro nutrients.

Supply of micro- and macro-nutrient supplements has become an important topic for agricultural biogas digestion plants (i.e. energy crops, animal manures, and crop residues), since the lack of some micro-nutrients has been identified to be the main reason behind poor process performance and failures (Demirel and Scherer, 2011; Schattauer et al., 2011; Nges and Björnsson, 2012). Several studies concluded that dosing nutrients can stimulate methane production as well as improve process stability (e.g. keep pH within optimum

values by avoiding volatile fatty acids accumulation and/or providing a minimum alkalinity level) (Nges and Björnsson, 2012; Zhang and Jahng, 2011; Gerardi, 2003). Macro-nutrients (e.g. P, N and S) are indispensable constituents of biomass but they also play a necessary role as buffering agents, while micro-nutrients (e.g. Fe, Ni, Mo, Co, W, and Se) are crucial cofactors in numerous enzymatic reactions involved in the biochemistry of methane formation (Schattauer et al., 2011; Lo et al., 2011[a]). However, excessive concentrations of some macro- and micro-nutrient can lead to inhibition of the AD process (Lo et al., 2012[b]).

In Table 2.1, some reported stimulatory and inhibitory concentrations of selected metals and their role in methanogenesis is shown. The concentrations varies significantly from one study to another, which can been related to a number of factors, including: (i) the abundance, structure and adaptation periods of the anaerobic niche; (ii) the chemical form of the metals (dependant on pH, redox potential and presence of chelating compounds), which may change their bioavailability for stimulatory and inhibitory purposes; and (iii) the antagonistic and synergistic effects between elements (Vintiloiu et al., 2013;Chen et al., 2008).

Process stability is a major concern in commercial full-scale AD plants, since poor process stability normally leads to unsteady methane productions. Even more, prolonged instability episodes may result in process failure. Accordingly, several research efforts have been carried out to overcome source of AD instability (Schmidt et al., 2014; Banks et al., 2014; Zhang and Jahng, 2012; Zhang et al., 2012). For instance; Nges and Björnsson (2012) observed that the addition of a concentrated solution of micro- and macro-nutrients stimulated and stabilized a digester fed with a mixture of energy crops. NS addition also allowed to reach higher methane yields at relatively short hydraulic retention times (HRT, 30 – 40 days). Similarly, Zhang et al. (2011) concluded that the micro-nutrients provided by a piggery wastewater were the main reason behind the improved methane yield of a food waste digester, whose control (without piggery wastewater addition) presented low methane yields and high levels of volatile fatty acids (VFA).

Table 2.1. Reported stimulatory and inhibitory concentrations of metals on anaerobic biomass and their role in methanogenesis (Romero-Güiza et al., 2016). CODH: carbon monoxide dehydrogenase; SODM: superoxide dismutase; ACS: acetyl-CoA synthesis; FDH: formate dehydrogenase.

Metal	**Stimulatory concentration (mg L^{-1})**	**Inhibitory concentration (mg L^{-1})**	**Role in Methanogenesis**
Al		1000<Al<2500	
Ca	100<Ca<1035	300<Ca<8000	
Cd	<1.6	36<Cd<3400	
Co	0.03<Co<19	35<Co<950	• Methyltransferase
Cr	0.01<Cr<15	27<Cr<2500	
Cu	0.03<Cu<2.4	12.5<Cu<350	
Fe	<0.3		• Formyl-MF-dehydrogenase • CODH, ACS • Hydrogenases
K	<400	400<K<28934	
Mg	<720		
Mn	<0.027		
Mo	<0.05		• Format-dehydrogenase • Formyl-MF-dehydrogenase
Na	100<Na<350	3500<Na<8000	
Ni	0.03<Ni<27	35<Ni<1600	• CODH • Methylreductase • Hydrogenases
Pb	<0.2	67.2<Pb<8000	
S			• CODH • Hydrogenases
Se	<0.04		• Format-dehydrogenase • Formyl-MF-dehydrogenase • CODH/ACS
W	<0.04		
Zn	0.03<Zn<2	7.5<Zn<1500	

Ni, Co and Fe are the most studied NS, since they are essential cofactors of carbon monoxide dehydrogenase, acetyl-CoA decarbonylase, methyl-H_4SPT: HS-CoM methyltransferase, methyl-CoM reductase and other enzymes involved in the acetoclastic methanogenesis pathway (Kida et al., 2001; Hoelzle et al., 2014). Furthermore, these metals have also shown to be essential for the acetotrophic pathway of methanogenesis (acetate oxidation to carbon dioxide and hydrogen). Pobeheim et al. (2011) who digested maize silage at mesophilic conditions, reported that Ni and Co deficit (<0.1 mg Ni^{2+} kg^{-1} and <0.02 mgCo^{2+} kg^{-1} in wet-basis) had a negative impact on process stability (i.e. accumulation VFA) at organic loading rate (OLR) above 2.6 g TS L^{-1} d^{-1}. However, enhancing Ni and Co levels to 0.6 and 0.05 mg kg^{-1} respectively, allowed stable digester performances until the system reached an OLR of 4.3 g TS L^{-1} d^{-1}. Contrariwise, Zandvoort et al. (2003), who analyzed the impact of Fe, Ni and Co on a methanol UASB reactor, noted that only Fe had significant effect on the methanol degradation rate. Specifically, increasing influent Fe concentration from 0.056 mg L^{-1} to 0.56 mg L^{-1} allowed improving the methanogenic activity from 152 mg CH_4-COD g VSS^{-1} d^{-1} to 291 CH_4-COD g VSS^{-1} d^{-1}, respectively. Coates et al. (2005) showed that adding amorphous Fe_2O_3 at a concentration of 16 g L^{-1} diminished the concentration of malodorous compounds (H_2S and VFA) and enhanced the methane production of a pig manure digester. The positive effect of Fe(III) supplementation was attributed to the Fe(III)-reducing capacity, which favors redox processes alleviating the thermodynamic limitations on VFA degradation. Furthermore, Fe(III) can precipitate H_2S minimizing related inhibition phenomena (Cherosky and Li, 2013). In a full-scale OFMSW digester, Romero-Güiza et al. (2014) observed that reducing the H_2S concentration from 1900 to 50 mg L^{-1} through a $FeCl_3$ solution (2.5 kg of $FeCl_3$ per ton of organic matter fed), led to a prompt reduction of the propionate concentration.

2.4 Ashes from waste incineration and thermoelectric residues. Biogas stimulation.

Municipal solid waste incineration (MSWI) is a widespread technology to treat municipal solid waste (MSW) as it produces energy and reduces MSW volume up to 90%. However, MSWI generates two types of solid ash: (i) bottom ash (BA), and (ii) fly ash (FA); the latter is

also known as air pollution control residue. BA, classified as a non-hazardous waste, is generally rich in calcium oxide and silica with a low heavy metals content, while FA, classified as a hazardous waste, is mainly composed of heavy metals, soluble salts, chlorinated organic compounds and lime (Valle-Zermeño et al., 2014). The addition of BA and/or FA to an anaerobic digester might increase the metals concentration resulting in beneficial or detrimental effects on the AD process (Lo et al., 2010; Banks and Lo, 2003; Lo, 2004). The beneficial impact of MSWI ashes on AD performance has been mainly related to alkalis and trace metals, wich can be leach out from the ash at the pH values in AD (6.5-8.0). For instance, under this pH range, CaO provides alkalinity to the system. Moreover, at reasonable dosing rates, it is unlikely that light metal ions would reach inhibitory levels when adding MSWI ashes to anaerobic digesters (Banks and Lo, 2003; Lo, 2004)

Lo et al., (2012)[c] studied the effect of ashes particle size on MSW anaerobic digestion through a series of biomethane potential test (BMP). Specifically, different doses of milled BA (68% 0.4-106 nm; 32% 1110-10000 nm), milled FA (75% 0.4-106 nm; 25% 1110-10000 nm) as well as non-milled BA and FA were tested. Results clearly indicated that both BA and FA (milled and not-milled) were able to improve biogas yields of OFMSW digestion (controls seemed inhibited as pH values around 6 were recorded during the test). Regarding particle size, milled BA and FA showed slightly better performance than non-milled ashes. This was related to their higher capacity to immobilize microorganisms. The authors concluded that the improvement of digester performance was mainly related to the increased levels of alkali metals, heavy metals and trace metals (i.e. Ca, Mg, K, Na, Fe, Si, Mn, B, Al, Ta, Ba and W). In a subsequent study, Lo et al., (2012)[a] analyzed the effect of two BA (12 and 24 g d-1) and two FA (1 and 3 g d^{-1}) additions on OFMSW continuous digesters (5 L), each operated at four different hydraulic retention times (i.e. 40, 20, 10 and 5 days). Results showed that, after an adaptation period, both BA dosing allowed to improve digesters performance (stability and biogas yields), but only when operated at high HRT (20 and 40 days). FA (1 g d^{-1}) also led to minor biogas production improvements. This phenomenon was again related to the released levels of alkalis (i.e. Ca, K, Na and Mg) and other metals (i.e. Co, Mo and W) (Lo et al., 2012) [a]. Huiliñir et al., 2015 used fly ash from thermoelectric plant and they

reported the possibility to enhance around 30% the methane generation. That result shows a promising alternative use of this residue as source of nutrients for anaerobic digestion with low cost.

2.5 Batch assays. Their role in anaerobic digestion.

The growing interest for production of biogas by anaerobic digestion has led to an increased demand for finding and evaluating new types of suitable feedstock. To evaluate the feasibility of one feedstock as suitable and reliable biodegradable substrate in anaerobic digester a batch test called as biochemical methane potential (BMP) is commonly used. This test is actually not standardized but since the work from Angelidaki et al. (2009) the guidelines for their star-up with reasonable reproducibility were established.

BMP's are the most used methodology by academic and technical practitioners to determine the maximun methane production (B_0) of a certain substrate (Raposo et al., 2011). This batch assay determines B_0 by recording the methane produced when the substrate is mixed with an active anaerobic inoculum until no further methane production. BMP testing is today most reliable method to determine the ultimate methane production (Lesteur et al., 2010; Ward, 2016), which is a key parameter to assess the implementation feasibility of a full-scale anaerobic digestion plant as well as its optimisation (e.g. co-digestion, pre-treatment) (Angelidaki et al., 2009; Carrere et al., 2016; Mata-Alvarez et al., 2014).

BMP test are quite simple but they take a lot of time; essentially they consist in putting together the waste with inoculum (Figure2.3). Using simple liquid displacement, pressure measurements and or chromatography techniques the methane generation in time can be measured. Using statistical tools the maximum methane recovery form proposed waste and kinetic parameters can be identifiable from a proposed model.

However, BMP test of highly particulate substrates are very time consuming with testing time ranging from 30 days to more than 100 days (Raposo et al., 2011). The large testing

time makes sometimes BMP testing not practical for consulting companies and AD plants operators, where decision-making processes cannot be hold for a month (Strömberg et al., 2015).

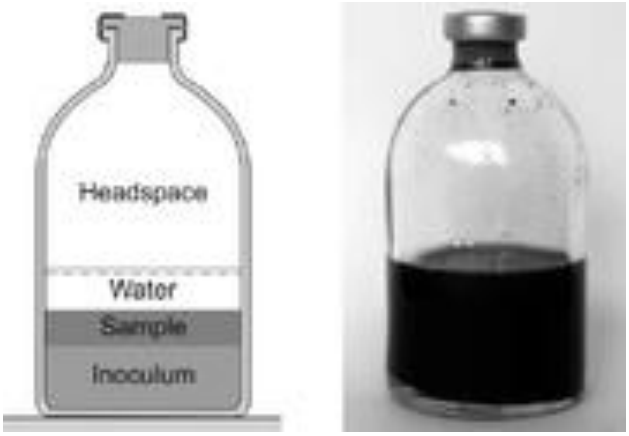

Fig. 2.3. Image of a bottle for a BMP type test along with a sketch of the parts that are requested in the bottle.

3. Anaerobic digestion of secondary sludge from wastewater meat factory: improvement with fly ash and kinetic organic matter biodegradation.

3.1 Abstract

The management of secondary sludge or biosolids is a critical point by being a costly matter in wastewater treatment plants. Before their final disposal, normally they are treated by anaerobic digestion with the aim of reducing their total amount and, therefore, their management cost. The improvement of the anaerobic digestion process of this waste with low cost strategies is an important research item wiyh the aim to decrease the energy demand and improve the cost balances in these plants. In the present work, thermoelectric fly ash was used as a source of micronutrients for microorganisms in the anaerobic digestion process of thermally-pretreated secondary sludge (1 hour, 120 °C). The maximum methane production rate increased from 1.40 mL/d to 4.48 mL/d when fly ashes were added at a dosage of 150 mg/L in BMP tests compared with tests without ash addition. Also, the kinetic constants of the hydrolysis of particulate organic matter were obtained in both cases (without and with ash addition) in batch reactors using a first-order kinetic model; in the case of no addition, the first-order kinetic parameter was 0.019±0.002 d^{-1}, while when ashes were added this value was found to be 0.045±0.000 d^{-1}. Therefore, the addition of fly ashes allowed improving both the methane generation and hydrolytic kinetics.

3.2 Introduction

Secondary sludge or biosolids are a byproduct from aerobic biological treatment systems installed in wastewater treatment plants (WWTPs). In general, the management of this waste is a serious and difficult problem to solve due to it has a slow biodegradation rate, poor dewaterability, it produces odour problems as well as it is generated in large quantities in aerobic reactors. In spite of these problems, this residue is an energy source in WWTPs when it is digested in an anaerobic reactor (Stamatelatos and Tsagarakis, 2015). Also, its treatment by anaerobic digestion (AD) reduces the sludge management costs for generating

energy, improving dewaterability, reducing pathogen content and solving odour problem emissions. The improvement of this process with low cost action strategies is an important challenge to decrease the energy demand and improve the cost balances in these plants.

The WWTPs need high requirements of energy for removing pollutants. Given that the activated sludge systems are used in WWTPs, large quantities of secondary sludge or biosolids are generated, which are generally used for agricultural purposes, composting, landfill or incineration. The treatment of these biosolids could get to represent the 50% of operation costs of these plants. Taking into account the high energy demand and the high management cost, the AD technology represents a great contribution in this sense (Appels et al., 2008). Digesting the generated sludge by anaerobic processes, the total amount of solids to be disposed and odour emissions are considerably reduced. Also, the use of the biogas generated can make the energy balance of this treatment system equal to zero, or even positive (Pérez-Elvira and Fernandez-Polanco, 2012; Fernandez – Polanco and Tatsumi, 2016).

The AD is a natural process in which the biodegradable organic matter is converted into biogas (mainly methane and carbon dioxide) by a microorganism consortium. In spite of AD is complex, it is always explained by three general steps: macromolecules hydrolysis in which the complex matter is converting into fermentable molecules, hydrolytic product acidification in which previous molecules are transformed into volatile fatty acids (VFA) and, finally, a methanogenic step in which VFA are converted to biogas. Generally, the biogas is composed by around 75-65% of methane and 35-25% of carbon dioxide with low levels of other gases, such as hydrogen sulfide or hydrogen. The AD process, in practice, works out in two ranges of temperature; mesophilic conditions at around 30–38ºC and thermophilic conditions at around 49–57 ºC. The mesophilic conditions are the most common and most stable temperature operation range for anaerobic reactors. AD can take place in single stage reactors at fixed temperature either in a combination of reactors and conditions. The last strategy is performed to improve favourable conditions in AD so as it optimizes organic removal, biomass retentions and biogas production (Stamatelatos and Tsagarakis, 2015; Stamatelatos et al., 2012).

The composition of the generated biogas has strong relation to the macronutrients feed. In fact, there are strategies to estimate the composition of biogas from molecular formula of substrate. The presence of micronutrients has more impact on the kinetic process. Several micronutrients are part of enzymes which protagonist an important role in methanogenic metabolic routes (Lier et al., 2008; Kida et al., 2001; Romero-Güiza et al., 2010). Authors like Kayhanian and Rich (1995), Ma et al. (2009) and Huiliñir et al. (2015) give information about the importance of different metals which are used as a part of enzymes and other compounds in AD. Elements like Co, Ni and Fe are involved in the process of methane formation and they are part of enzymes to produce acetate in acetogenic step or they are a part of co-factors of some enzymes involved in the methanogenic step (Ma et al., 2009; Kayhanian and Rich, 2009).

In addition, the application of anaerobic digestion to treat these biosolids is limited by high hydraulic retention times, 20-30 days, and low degradation efficiency of solids around 30-50 %. These limiting factors are associated with the hydrolytic step. For this reason, the secondary sludge anaerobic digestion is a biological process, in which the hydrolytic stage is widely assumed as controlling step in the global stabilization process. There are physical and chemical strategies to improve the particulate matter solubilization such as thermal and alkali treatments (Appels et al., 2008; Lou et al., 2012; García-Gen et al., 2015; Trzcinski et al., 2015; Nava-Valente et al., 2016). Several authors (Tomei et al., 2008; García-Gen et al., 2015) have reported how to model this limiting step using a first-order kinetic equation in spite of there are other models with better statistical responses but more complex and less useful for practice. These complex models take into account parameters like biomass concentration inside the reactors. Biomass is a difficult variable to identify in continuous reactors, especially when they are digesting secondary sludge. Table 3.1 shows some values of hydrolytic kinetic constant found by different authors.

When comparing the values from Table 3.1 with 0.5 d^{-1} as maximum specific growth rate for methane-forming archaea (Henze et al., 1997) it can be explained why hydrolytic step is the controlling stage, since the hydrolysis kinetic constant is much lower. In order to enhance hydrolytic kinetic behaviour, several methods have been proposed and tested in

lab scale but few mechanical, thermal and thermochemical methods have been successfully applied at full scale for having high cost and energy requirements (Ariunbaatar et al., 2014; Pérez-Elvira and Fernandez-Polanco, 2012). The selection and success of any strategy keeps a strong dependence with mass, energy and economically balances for the global process.

Table 3.1. First-order kinetic constant values obtained in the AD of biosolids and other substrates by different authors.

Substrate	k (d-1)	Temperature (ºC)	Reference
Primary sludge	0.25-0.40	35-55	Siegrist et al. 2002
Waste activated sludge	0.12	35	Zhang et al. 2010
Waste activated sludge	0.026-0.035	35	Tomei et al.2008
Waste activated sludge	0.1-0.4	50-65	Ge et al.2011
Waste activated sludge	0.106-0.215	40-70	Luo et al. 2012
Carbohydrates	0.025-0.2	55	Chris et al. 2000
Proteins	0.015-0.075	55	Chris et al. 2000

Thermal pre-treatments are those where biomass or complex substrate is solubilized by applying heat. Their performance may be influenced both by temperature and exposure time. However, temperature seems to be the most influencing factor on biomass disintegration and anaerobic biodegradability. Temperature ranging from 60 ºC to 180 ºC is generally used for thermal pre-treatment although a wide range of temperatures has been assayed. The optimal temperature depends on the substrate characteristics (Carrere et al., 2010).

On the other hand, the positive effect of metal addition in AD has been widely reported by some authors like Gonzalez-Gil et al., (1999) and Fermoso et al. (2008). They added metals like Ni and Co in their AD studies and found that the bioconversion was improved when metals were added, while limitation of them caused a deficient performance of the reactor and even failure by acidification. It is important to mention that these authors used salts as

chlorides for their experiments; this fact makes difficult the scale up their uses as a supplement by their costs. Other authors used other sources of metals like modified zeolites and they had an improvement in the methane generation from synthetic wastewater as well as with fatty acids as substrate (Milan et al., 2010). Lo et al. (2004) studied the co-digestion of municipal solid wastes with ashes from their incineration; they also improved the biogas generation with ashes in batch processes. Kida et al. (2001) studied the variation on corrinoids, F430 and methanogenic activity with addition of Ni and Co; they found an improvement of the methanogenic activity after their addition. Romero-Guiza et al. (2010) reported a profound review about additives to anaerobic microorganisms; furthermore, they also reported the inhibition effects by heavy metals; in general, there is a threshold beyond which the inhibition of anaerobic process occurs. Taking into account that each residue contains different concentrations of heavy metals and the capability of inhibition in AD process by them above certain threshold (different in each metal: Co, Ni, Cu), they give importance to previous experiments that define a stimulatory dosage in each residue, if it exits. In this sense, a protocol to find this dosage has not been defined up to now.

According to the information presented, the main objective of this research was to study the possibility of improvement the methane generation from secondary sludge when this was thermally pre-treated at 120 ºC using fly ash addition as a source of micronutrients. For this purpose, biochemical methane potential (BMP) tests with different fly ash dosages were made and compared. Once the stimulatory dosage was found, the hydrolytic kinetic behaviour was modelled and studied, comparing the results obtained without ash addition with those achieved when fly ash at the optimal dosage was added.

3.3Materials and methods

3.3.1 Characteristics of the anaerobic inoculum, substrate and fly ash used

Purge of an anaerobic mesophilic reactor property of sanitary WWTP “La Farfana”, Chile was used as anaerobic inoculum. This reactor digests secondary sludge with 13.3 ± 2.4 g VSS (volatile suspended solids) /L and 23.5 ± 1.3 g TSS (total suspended solids)/L in the purge

line. Secondary sludge was used as substrate, and this substrate was collected from the purge line of an activated sludge reactor. This system belongs to a meat factory located in La Calera, Chile. At the moment of collection the system was operated with 25 days of solid retention time. The substrate was thermally pre-treated at 120 ºC during one hour in autoclave, as soon as the sludge was received at the laboratory from factory's WWTP. This thermal treatment permits to distinguish the concentration of volatile suspended solids that belongs to the substrate, under the assumption of a constant concentration of biomass during the batch assays. Another reason is to study how to improve the AD process using a common pre-treatment used in the management of biosolids (Table 3.2).

Table 3.2. Characterization of secondary sludge.

Parameter		Value	EM
COD	Total (mg/l)	14797	503
	Soluble (mg/l)	230	58
Solids	TSS (g/l)	12.6667	2.54
	VSS (g/l)	10.1167	1.0583
	ISS (g/l)	2.5500	1.4817
	TS (g/l)	15.2250	2.8575
	VS (g/l)	10.9000	1.9050
	IS (g/l)	4.3250	0.9525
Solids post thermal treatment	TSS (g/l)	12.6667	2.5400
	VSS (g/l)	10.1167	1.0583
	ISS (g/l)	2.5500	1.4817
	TS (g/l)	15.2250	2.8575
	VS (g/l)	10.9000	1.9050
	IS (g/l)	4.3250	0.9525
pH		7.10	
COD_{total} / N-total		15	

EM is the error margin

The used fly ash came from the thermoelectric plant "Guacolda Energía", Chile. The mean particle size of ashes was 0.09 mm and their composition was determined by inductively coupled plasma (ICP) (Table 3.3).

Table 3.3. Chemical composition of thermoelectric fly ash used.

Component	Unit	Value
SiO_2	% weight	29.060
Ca	% weight	1.070
Fe	% weight	5.500
Ti	mg/kg	4.334
V	mg/kg	356.600
Cr	mg/kg	131.000
Mn	mg/kg	554.200
Co	mg/kg	70.600
Ni	mg/kg	52.800
Cu	mg/kg	109.400
Zn	mg/kg	97.000
Rb	mg/kg	30.200
Sr	mg/kg	267.600
Zr	mg/kg	217.000
Pd	mg/kg	163.800
Hg	mg/kg	0.005
Ba	mg/kg	1.109

3.3.2 Experimental procedure for determination of optimum dosage of fly ash

The accumulative methane production was measured in BMP tests. These assays allowed corroborating the improvement of anaerobic digestion after ash addition and to find the best ash dosage. These tests were similar to those done to determine the specific methanogenic activity values by Lemos (2008). Each flask contained the same concentration of substrate (2 g COD/L) and inoculum (2.5 g VSS/L) but different concentrations of fly ashes (0, 30, 90, 150, 180, 200 mg/L). These reactors were immersed in a thermal bath at 35 ºC.

All assays were carried out in triplicate. The reactors were operated without micronutrients supplement because the objective of addition of ashes was precisely to supply the micronutrients necessary for an optimal performance of the process. A phosphate buffer was used to fix the pH at 7.3. All changes were planned with the purpose to study the methane generation rate from secondary sludge in common conditions of pH and unbalanced nutrients such as occurs in industrial WWTPs. The produced methane was measured by displacement of a NaOH (2.5 %) solution in an inverted graduated cylinder.

The total volume of the glass flasks used was 250 mL with an effective volume of 200 mL. Inside flasks were introduced the buffer solution, the substrate, the inoculum and the corresponding amount of fly ash. For ensuring anaerobic conditions in each flask, nitrogen gas was introduced in each head space before closing it and starting the experiment. Each flask was manually shacked once daily.

A mean accumulative curve was done with the triplicate values. The representation of mean data has been used as visual criteria to find the stimulation effect on the methane generation and to select the best dosage. Also, these data were correlated through linear regression; their slopes could be used to choose the best dosage with numerical criteria. The slope from regression lines represents the rate of methane generation, and it shows if the anaerobic digestion inside the flask was improved or deteriorated with respect to the flask without ash addition. This is the simplified proposed form to corroborate how to find the stimulation dosage and compare with the case of no ash addition.

3.3.3 Kinetic estimations of particulate matter hydrolysis.

Once the stimulation dosage was found and fixed, the degradation of organic particulate material with and without ash supplementation was studied. To estimate the particulate material in this research the volatile suspended solids content was used directly. The degradation of particulate organic matter with the time was adjusted to a first-order kinetic model. Once the kinetic constants were obtained, it is easy to compare the biodegradation improvement in terms of an increase in the rate constant.

Starting at the same time, eight batch reactors were operated with the stimulatory quantity of ashes and other eight batch reactors without them. At days of operation 8, 19, 23 and 27 the VSS contained in two reactors from each serial line (two with and two without ash) were analysed to determine the mean solids concentration reduction with time. In this way, the variation of the organic particulate content was obtained with time from the substrate in anaerobic batch conditions with or without ash addition.

There are some references in the literature, in which anaerobic digestion is modelling paying attention to different response variables; biogas production or substrate consumption are the most common response variables used to identify the model parameters(Huiliñir et al., 2015; García-Gen et al., 2015; Tomei et al., 2008; Angelidaki et al., 2009; Donoso-Bravo et al., 2010). In the present work, the selected response variable was the VSS content. This concentration is an important parameter in secondary sludge anaerobic digestion because it represents the particulate organic matter to be removed; and as it was previously stated that the solubilization of particulate material is the controlling or limiting-rate step. A high degradation implies more methane generation and less cost for biosolids management. For these reasons the VSS concentrations were chosen to develop the kinetic model. The AD of solid organic matter can be described as a first-order kinetics (Ruiz-Hernando et al., 2014; Mata-Alvarez et al., 1992; Christ et al., 2000). The process kinetics can be formulated in two different forms; one only considers the variation of particulate matter concentration with time without taking into account the dependence of the reaction rate on the biomass concentration, while the second-order kinetics uses both the biomass concentration and particulate matter concentration and considers the dependence of the rate on the biomass content. The second-order model becomes to a pseudo-first order model on substrate by assuming there are no changes on biomass concentration during the experiment; so in that case the obtained apparent kinetic constant is the product of kinetic parameter and biomass concentration in the experiment. There are other authors that assume only first-order and divide the organic material degradation in two parallel reactions; rapidly degradable and slowly degradable material (Nava-Valente et al., 2016). In the present research, the simplest first-order model was used

(equation 1) for studying the organic matter degradation, in which k is the hydrolysis kinetic constant, X is the particulate substrate concentration at any time, t is the time and Xo is the initial particulate substrate concentration:

$$\ln\left(\frac{X}{X_0}\right) = -k \cdot t \qquad (1)$$

According to equation (1) a plot of Ln (X/X_0) versus t should give a straight line of slope equal to k with intercept zero. In the specialized literature equation (1) is the most used model for anaerobic sewage sludge degradation. According to Tomei et al., (2008) this empirical equation could consider the cumulative effects of many processes. Although some practical cases show that degradation kinetics depends on the biomass concentration, the common practice of the use of equation (1) to determine the kinetic constant could be attributed to the real difficult in the anaerobic digestion of sludge to distinguish the active biomass from the sludge volatile solids representing the substrate(Tomei et al.,2008).The biodegradable fraction of VSS was estimated based on equations and tables for activated sludge systems that can be found in Von Sperling (2008) or Bolzonella et al. (2005). These equations depend on solid retention time, and, in this case, with 25 days, this fraction was around 60%. Despite this, other authors like Lou et al. (2012) assume the totality of particulate matter and give no relevance to non-biodegradable fraction.

3.3.4 Statistical analyses

The variation of particulate organic solids concentration with time was described by equation 1. The kinetic constants were determined through a linear regression analysis following the least square strategy. A linear regression analysis permits finding easily the most important parameters for comparing slopes and explaining the linear tendency; the slope represents the kinetic constant, while the standard deviation of the kinetic constant is also determined. r is de correlation factor and it informs about the degree in which the experimental values fit to the proposed linear model as well as permits calculate the coefficient of determination (R^2) by multiplying r by itself. The estimation of coefficient of

determination via correlation coefficient can only be done in linear models like this; in other cases, different strategies should be used.

3.3.5 Analytical procedures

Total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS) and total volatile solids (VS), mineral suspended solids (MSS), total mineral solids (MS) and pH values were determined according to the Standard Methods (APHA, 2012). Soluble and total chemical oxygen demands (COD) were determined according to the semi-micro method proposed by Soto et al. (1989). All analyses of solids and COD were carried out in triplicate.

3.4 Results and discussion

Figure 3.1 shows the variation of the cumulative methane production with time for the different dosages (0, 30, 90, 150, 180 and 200 mg/L of ashes) applied. As can be seen comparing the experimental data plotted in this figure, the best dosage stimulant was found to be 150 mg/L. The slopes of the straight lines obtained by linear regression adjust of the experimental points are equal to the maximum methane production rates. As can be observed, there is a trend that the methane production rate increase with increased dosages up to a maximum at 150 mg/L of ash dosage. After this dosage, there was a decrease in the methane production rate compared to the mentioned maximum value reached. For the case in which there was no ash addition, the maximum methane productivity was 1.4 mL/d while for the optimum case of 150 mg/L of ash addition, the maximum methane production rate was 4.5 mL/d, which is three times higher than that obtained without ash addition (Table 3.4).

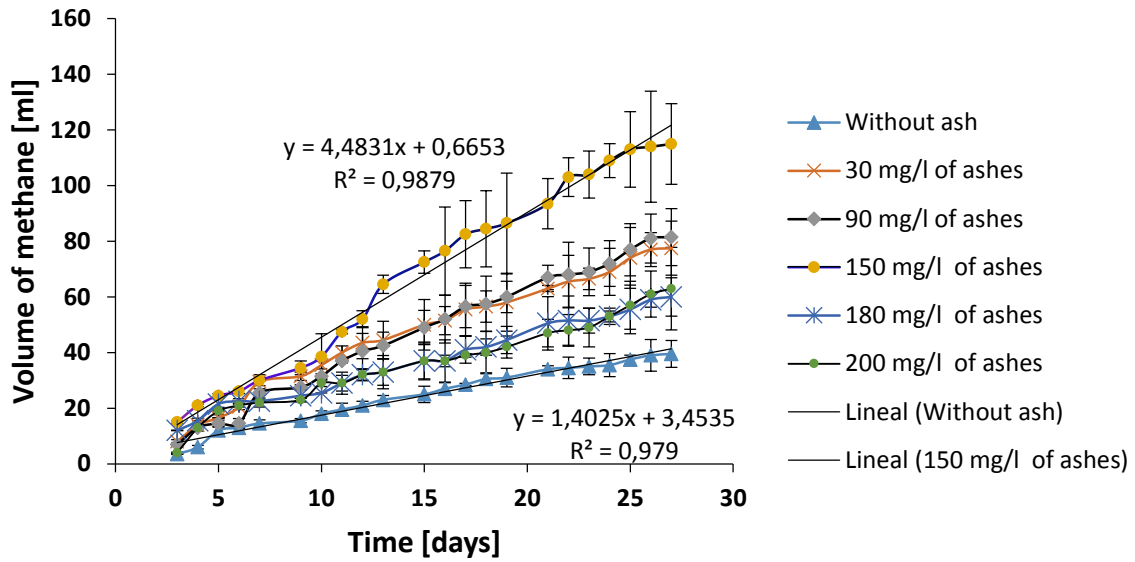

Fig. 3.1. Experimental data and kinetic model. Two cases: adding and no adding ash.

Table 3.4. Values of the maximum methane production rate (slope) from the BMP tests and values of determination coefficients of the linear regression fit.

Ash concentration (mg/L)	Slope	R^2
Without ashes	1.402	0.979
30	2.732	0.982
90	3.077	0.991
150	4.483	0.988
180	1.897	0.990
200	1.402	0.979

3.4.1 Kinetic parameters

Based on previous results, and choosing the optimal dosage of 150 mg/L of ashes, a kinetic study was made to compare the case of the highest stimulation in methane production with the case of no ash supply. Two batch reactors series were only used for measuring the mean variation of VSS with the time. Table 3.5 shows the mean variation of VSS concentration with time in these experiments. Two experimental series were made; one with the optimal ash dosage and the other without ash addition. For the case in which ash was used, the percentage of VSS reduction was 25% while in the other case, without ash supplementation, this percentage was 16% at the end of the experimental period tested.

Table 3.5. Experimental results for suspended volatile solids degradation modelling.

	Without ash supply			With ash supply		
Exp. day	Volatile Suspended Solids, X (g/L)	Error (g/L)	Ln(X/X_0)	Volatile Suspended Solids, X (g/L)	Error (g/L)	Ln(X/X_0)
0	6.31	Estimated	0.00	6.31	Estimated	0.00
8	6.05	0.21	-0.12	5.47	0.20	-0.46
19	5.33	0.13	-0.56	5.01	0.23	-0.84
23	5.83	0.20	-0.23	4.80	0.02	-1.08
27	5.30	0.17	-0.58	4.71	0.03	-1.20

Figure 3.2 shows the model and the experimental values of Ln(X/X0) versus time according to equation (1) to calculate the kinetic constants and obtain other statistical parameters. For the case without ash addition, the hydrolysis kinetic parameter was 0.019 ± 0.002 d^{-1} with correlation coefficient (r) equal to -0.939 and the coefficient of determination (R^2) equal to 0.881; while in the case with ash supplementation, the kinetic constant value was 0.045 ± 0.000 d^{-1} with r equal to -0.998 and R^2 equal to 0.997. Therefore, when ashes were supplemented, the kinetic constant increased by 2.3 times compared to the value obtained without ash addition.

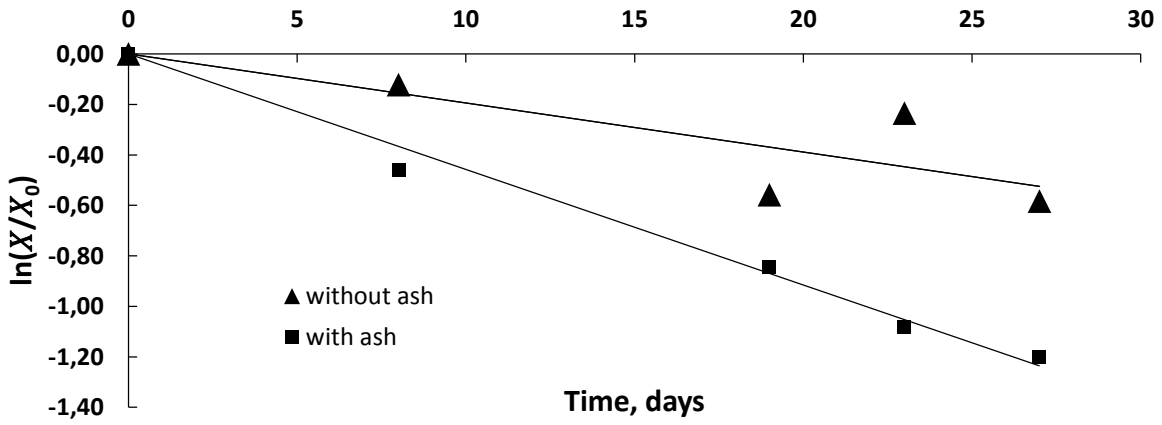

Fig. 3.2. Experimental data and kinetic model. Two cases: adding and no adding ash.

The fact that the correlation parameters (r and R^2) are very high and close to 1 means that there is strong linear dependence among experimental data given in equation (1). Moreover, the intercept, in both cases, achieved a value very close to zero (-0.001). Thus, all these data support the suitability of the first-order kinetic model chosen. As can be seen in Figure 3.2, in the case of no ash addition two outlier points that correspond to 19 and 23 days were observed. If these two experimental points were not taken into account for calculations, the kinetic parameter would be 0.021 and the R^2 would be equal to 0.997; this means that these outliers do not generate a significant difference in the kinetic constant value, but they give a variance that the model cannot explain. This caused a worst value of the previous calculated R^2 when all the experimental points were taken into account in the calculation.

The kinetic constant values obtained in the present work were lower than those obtained by other authors like Lou et al. (2012), which reported a value for the kinetic parameter of 0.17 d^{-1}. However, the kinetic constant values achieved in the present research were of the same order of magnitude than those achieved by Tomei et al. (2008) in the anaerobic digestion of untreated and previously sonicated sewage sludge, for which values of 0.026-0.035 d^{-1} with R^2 of 0.97- 0.84 were reported. In this case, the pre-treated secondary sludge used by Tomei et al. (2008) had a high sludge age too (around 20 d). These authors explain that the low values of the kinetic parameters can be attributed to the high solid retention time on activated sludge; this fact has sense due to the high sludge age, which implies a high stabilization inside the aerobic reactor, wherewith the secondary sludge is characterized by the presence of a high amount of slowly biodegradable matter (Bolzonella et al., 2005). Also, it is important to mention that Lou et al. (2012) reported kinetic constant values in secondary sludge hydrolysis between 0.10 and 0.40 d^{-1} at 55 ºC and 65 ºC respectively; hence, it would be expected that at 35ºC their values would be lower and close to the values obtained in the present work.

3.5 Conclusions

The addition of thermoelectric fly ashes improved the anaerobic degradation of thermally pre-treated secondary sludge from two points of view: the methane generation rate and hydrolysis kinetics. The maximum methane generation rate was increased three times, when comparing with the BMP tests with no ash addition, passing from 1.40 mL methane/d to 4.48 mL methane/d when an ash dosage of 150 mg/L was added. In the case of particulate organic matter degradation, the kinetic constant increased from 0.019 ± 0.002 d-1 in no addition case to 0.045 ± 0.000 d-1 when ashes were added at the optimal dosage (150 mg/L).

The improvement of the methanogenic activity and the hydrolytic conversion converts the addition of thermoelectric ashes as a low cost strategy to balance the lack of micronutrients and so the economic and energy balances on WWTPs with the obtained results it is possible design anaerobic reactors in which the required volume is less with higher solids percentage

removal if ashes are used at the optimal dosage to compensate micronutrients requirements.

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4. BMP testing: reducing testing time by early parameter estimation.

4.1 Abstract

Biomethane potential (BMP) test is a key analytical technique to assess the implementation and optimisation of anaerobic biotechnologies. However, this technique is characterised by large testing times (from 20 to >100 days), which are not suitable for waste utilities, consulting companies or plants operators whose decision-making processes cannot be hold for large time. This study develops a mathematical strategy using sensitivity functions for early prediction of BMP first-order model parameters (i.e. methane yield (B_0) and kinetic constant rate (*k*)). The minimum testing time for early parameter estimation showed a potential correlation with the substrate degradation kinetic constant rate: (i) slowly biodegradable substrates ($k \leq 0.1\ d^{-1}$) with minimum testing times of at least 15 days, (ii) moderately biodegradable substrates ($0.1 < k < 0.2\ d^{-1}$) with minimum testing times between 8 and 15 days, and (iii) rapidly biodegradable substrates ($k \geq 0.2\ d^{-1}$) with testing times lower than 7 days. This study also shows that a balanced regression (3 - experimental points: t=0, t=minimum testing time and t= average time of sampling interval) is the most suitable regression strategy as it allows minimising the influence of the experimental data from the proportional region in the regression analysis.

4.2 Introduction

Anaerobic digestion (AD) is a competitive treatment technology for the management of organic-rich wastes since it transforms organic matter to renewable energy in the form of methane-rich biogas and a stabilised organic mulch fertiliser (Appels et al., 2008; Batstone and Jensen, 2011). Biomethane potential (BMP) tests is the most used methodology by academic and technical practitioners to determine the maximum methane production (B_0) of a certain substrate (Raposo et al., 2011). This batch assay determines B_0 by recording the methane produced when the substrate is mixed with an active anaerobic inoculum until no further methane is produced (Holliger et al., 2016; Ward, 2016). BMP testing is today most

reliable method to determine B_0, which is a key parameter to assess the implementation feasibility of a full-scale AD plant as well as its optimisation (e.g. co-digestion, pre-treatment) (Lesteur et al., 2010; Ward, 2016; Angelidaki et al., 2009; Carrere et al., 2016; Mata-Alvarez et al., 2014). Moreover, BMP tests can also be used to estimate the kinetic parameter of the rate limiting step (e.g. hydrolysis rate for highly particulate substrates) which is needed to achieve and optimal design and operation of anaerobic digesters (Angelidaki et al., 2009; Batstone et al., 2003; Koch and Drewes, 2014; Batstone and Jensen, 2011). However, BMP test of highly particulate substrates are very time consuming with testing time ranging from 20 days to over 100 days (Raposo et al., 2012). The large testing time makes sometimes BMP testing not practical for waste utilities, consulting companies and AD plants operators, which decision-making processes cannot be hold for a month or longer time.

Two strategies have been evaluated to reduce the testing time needed to obtain reliable B_0 and kinetic constant values: (i) the development of new and faster methods such as near-infrared spectroscopy and aerobic respirometry (Lesteur et al., 2010; Ward, 2016), and (ii) the statistical treatment of BMP data for parameter's early prediction (Ponsá et al. 2011; Strömberg et al. 2015). The second strategy is a more conservative approach; however, it can be carried out using current BMP equipment and it is readily applicable. Strömberg et al. (2015) proposed the early prediction of B_0 by using an interactive programed algorithm with 6 different models. The algorithm returns the most suitable model when the experimental data reaches the established criterion. Strömberg et al. (2015) algorithm could predict B_0 in 6 days or less, with the exception of agricultural waste, which required 8 days. Similarly, Ponsá et al. (2011) found a strong correlation between the methane generated at 14 days and the B_0 of municipal solid wastes. However, these studies have only focus on B_0 early prediction, while little attention has been paid to degradation kinetics.

BMP kinetic constant rate (k) early prediction requires the selection of a mathematical model before the experiment is finished. Many kinetic models have been used to describe the methane production of BMP tests (Vavilin et al., 2008; Donoso-Bravo et al., 2010). Among them, the first-order kinetic model is the most widely used due to its simplicity, and

because it is able to reflect the cumulative effect of all the reactions occurring during the actual process (Gavala et al., 2003; Vavilin et al., 2008). However, the simultaneous early prediction of B_0 and *k* cannot be done during the period of time where both parameters are correlated, i.e. when B_0 and *k* are mathematically related by a functional relationship (changes in the value of one variable can be balanced by changes in the value of another variable) (Li and Vu, 2013). Therefore, the two parameters of the first-order model (B_0 and *k*) may only be identified after a certain period of time to ensure enough no-proportionality between sensitivity functions. In this aspect, sensitivity analysis is a suitable tool for assessing parameter identifiability for simple mathematical model, like the first-order model (McLean and McAuley, 2011; Li and Vu, 2013).

The present study aims to develop a mathematically sound methodology for biomethane potential test first-order model parameters early prediction, i.e. maximum methane production (B_0) and kinetic constant rate (*k*).

4.3 Materials and methods

4.3.1 BMP assays

BMP tests were carried out at mesophilic conditions following the procedure described by Angelidaki et al. (2009). BMP tests were performed in triplicate in 160 and 240 mL serum bottles sealed with rubber septa and aluminium caps. The serum bottles contained inoculum and the amount of substrate required to achieve an initial inoculum to substrate ratio of 2 (VS-basis). Blank assays containing only inoculum were used to correct for the background methane potential of the inoculum. Next, the headspace of each bottle was flushed with 99.9% N_2 for one minute (4 L min^{-1}). Finally, the bottles were placed in an incubator set at 37 °C. Serum bottles were manually mixed by swirling before each sampling event. Accumulated volumetric methane production was calculated of the headspace at each sampling event. Methane yields are reported at standard conditions (i.e. 0 °C and 1 bar).

Eight different substrates were selected for this study based on industrial interest and diversity criteria. Specifically, the BMP data set consist of a mix of already published data and genuine results including: two different sewage sludge (Astals et al., 2013), one primary sludge (Peces et al., 2016), two slaughterhouse wastes (paunch and blood) (Astals et al., 2014), two different pig manure (this study), and a mixture of sewage sludge with glycerol (0.25% of glycerol in weight-basis) (this study).

4.3.2 Sensitivity analysis of the first order kinetic

The BMP cumulative methane production can be typically described by a first-order model (Eq. 1):

$$B(t) = B_0\left(1 - e^{-kt}\right) \qquad \text{Eq. (1)}$$

Where B(t) is the methane production over time (mL/g VS); t is the independent variable (d); B_0 is the maximum methane production (mL/g VS); and *k* is the kinetic parameter (d^{-1}).

According to Beck and Arnold (1977), B_0 and k (model parameters) can only be estimated by using experimental data from operational time regions where the sensitivity functions are not proportional between them. The sensitivity functions are the partial derivative of the model equation with respect to each parameter. Taken into account Eq. 1, the sensitivity functions for B_0 (Lb_0) and *k* (Lk) are Eq. 2 and 3, respectively.

$$Lb_0 = \frac{dB(t)}{dB_0} = 1 - e^{-kt} \qquad \text{Eq. (2)}$$

$$Lk = \frac{dB(t)}{dk} = B_0 t e^{-kt} \qquad \text{Eq. (3)}$$

For the operational region where the sensitivity coefficients are proportional between them, the relationship can be expressed as for Eq. 4:

$$Lb_0 = C\ Lk \qquad \text{Eq. (4)}$$

Where *C* is the constant of proportionality. Eq. 4 can be also expressed as:

$$\left(1 - e^{-kt}\right) = C'\left(te^{-kt}\right) \qquad \text{Eq. (5)}$$

Where *C'* is $C \cdot B_0$.

In Eq. 5, a functional relationship between both sensitivity functions occurs from t=0 until a certain period of time (threshold time), which depends on *k*. In this study, a coefficient of determination (R^2) of 0.80 between both sensitivity functions was chosen as a criterion to define when the proportionality is lost. The combination of Eq. 5 and the $R^2<0.8$ criteria, allows obtaining the relationship between the kinetic rate and the threshold time (Figure 4.1). As can be seen in Figure 4.1, the relationship between *k* and threshold time is well represented by a potential model (Eq. 6). In this study, Eq. 6 will be used to determine the threshold time (i.e. the minimum testing time).

$$\text{Thresold time (day)} = 1.892\text{k}^{-0.908} \qquad \text{Eq. (6)}$$

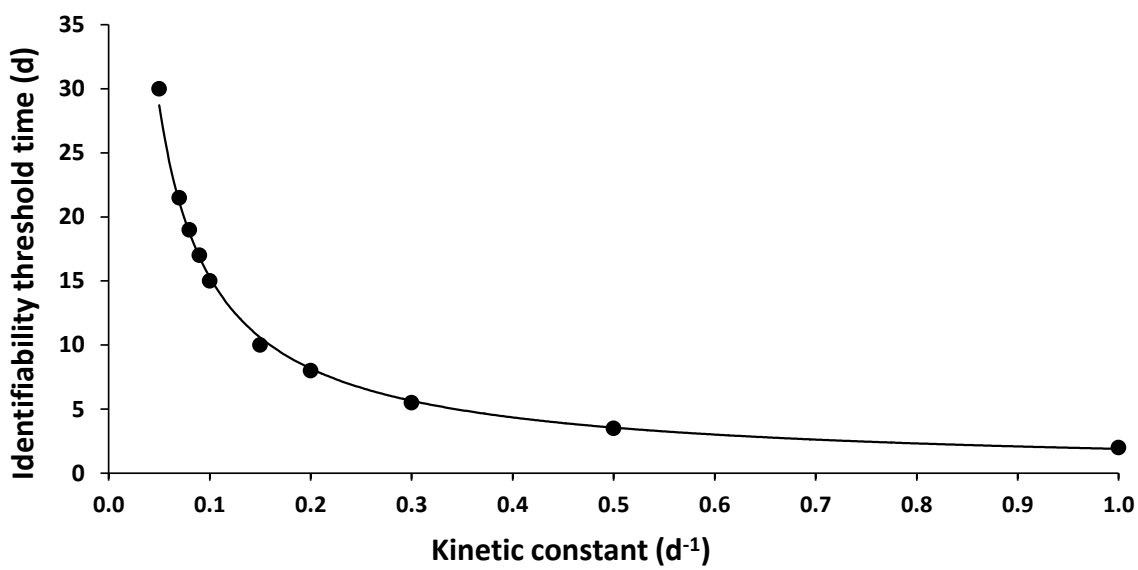

Fig. 4.1. Minimum testing time required to make both first-order model parameters identifiable

4.3.3 Parameters estimation

The average data from triplicates was used to estimate B_0 and *k* of each BMP. Matlab® function ''fitnlm'' was used to carry out the nonlinear regression of the first-order model (Eq. 1). This function minimises the mean squared differences between the experimental data and the model predictions.

For each BMP test, the parameters estimation was done by three different approaches: (1) using all the experimental data (common approach); (2) using all the experimental data between t=0 until the threshold time calculated; and (3) using three data points ("balanced threshold sampling"): the initial time, the threshold time and the average time between them. The latter strategy is chosen to reduce the contribution of the initial experimental data belonging to the proportional region, while aligning with the sampling strategy proposed by D-optimal (Grijspeert and Vanrolleghem, 1999; Valencia et al., 2013). Parameters confidence intervals were estimated at the 95% confidence level using a two-tailed t-test. Adjusted coefficient of determination (R^2_{adjust}) was used to describe "goodness of fit" between the experimental observations and the model predicted outcomes (Montgomery, 2012).

4.4 Results

4.4.1 Traditional regression

The parameter estimation carried out using all the experimental points (traditional regression analysis) of the eight substrates under study gave kinetic constants values from 0.08 to 0.39 d^{-1} (Table 1). $R^2_{adjusted}$ > 0.98 for all BMPs indicates that the first-order model fits well the experimental data. The lowest *k* value (0.08 d^{-1}) was obtained for the lignocellulose-rich paunch, followed by pig manure (0.14 and 0.20 d^{-1}) and mixed (primary and secondary) sewage sludge (0.18 and 0.23 d^{-1}). Primary sludge *k* value was estimated at 0.31 $days^{-1}$; while for blood, a protein-rich substrate, the *k* value was 0.28 d^{-1}. The highest *k* values (0.39 d^{-1}) was estimated for the co-digestion mixture between sewage sludge and glycerol. The difference on *k* values between sewage sludge and the sewage sludge co-digestion mixture is attributed to the addition of an easily biodegradable substrate like glycerol (Jensen et al., 2014).

4.4.2 Threshold regression

The minimum testing time required to make both first-order model parameters (B_0 and *k*) identifiable was obtained by applying the *k* values from the traditional regression to Eq. 6. Thus, the minimum testing time ranges from 4.5 days for the sewage sludge co-digestion mixture to 19 days for paunch. The minimum testing time for common substrates in anaerobic digestion such as sewage sludge and pig manure is around 10 days.

Table 4.1. Nonlinear regression results: traditional sampling, threshold sampling, and balanced threshold sampling.

	Experimental	Traditional regression				Threshold regression				Balanced threshold regression		
Substrate	B_0 (mL CH_4/g VS)	B_0 (mL CH_4/g VS)	k (d^{-1})	R^2_{adj}	Time (d)	B_0 (mL CH_4/g VS)	k (d^{-1})	R^2_{adj}	Threshold time (d)	B_0 (mL CH_4/g VS)	k (d^{-1})	R^2_{adj}
Sewage sludge 1	201±17	196±10	0.23±0.05	0.992	33	235±131	0.17±0.16	0.990	7.5	195±1	0.24±0.01	0.999
Sewage sludge 2	437±55	428±11	0.18±0.04	0.984	56	563±256	0.12±0.08	0.973	9.5	415±1	0.20±0.01	0.999
Primary sludge	337±22	330 ±11	0.31±0.04	0.991	24	430±151	0.20±0.22	0.992	5.5	362±1	0.27±0.01	0.999
Pig manure 1	228±8	307±16	0.14±0.02	0.992	67	299±36	0.15±0.03	0.998	11.5	288±1	0.16±0.01	0.999
Pig manure 2	148±14	141±10	0.20±0.04	0.988	37	148±43	0.19±0.09	0.990	8.0	134±1	0.23±0.01	0.999
Blood	422±25	419±6	0.28±0.08	0.994	38	512±127	0.20±0.08	0.993	10.0	446±1	0.25±0.01	0.999
Paunch	232±19	252±17	0.08±0.02	0.984	38	331±93	0.05±0.02	0.983	19.0	245±1	0.09±0.01	0.999
Sewage sludge and glycerol mixture	311±21	288 ±9	0.39±0.06	0.983	60	294±89	0.39±0.24	0.988	4.5	280±1	0.42±0.01	0.999

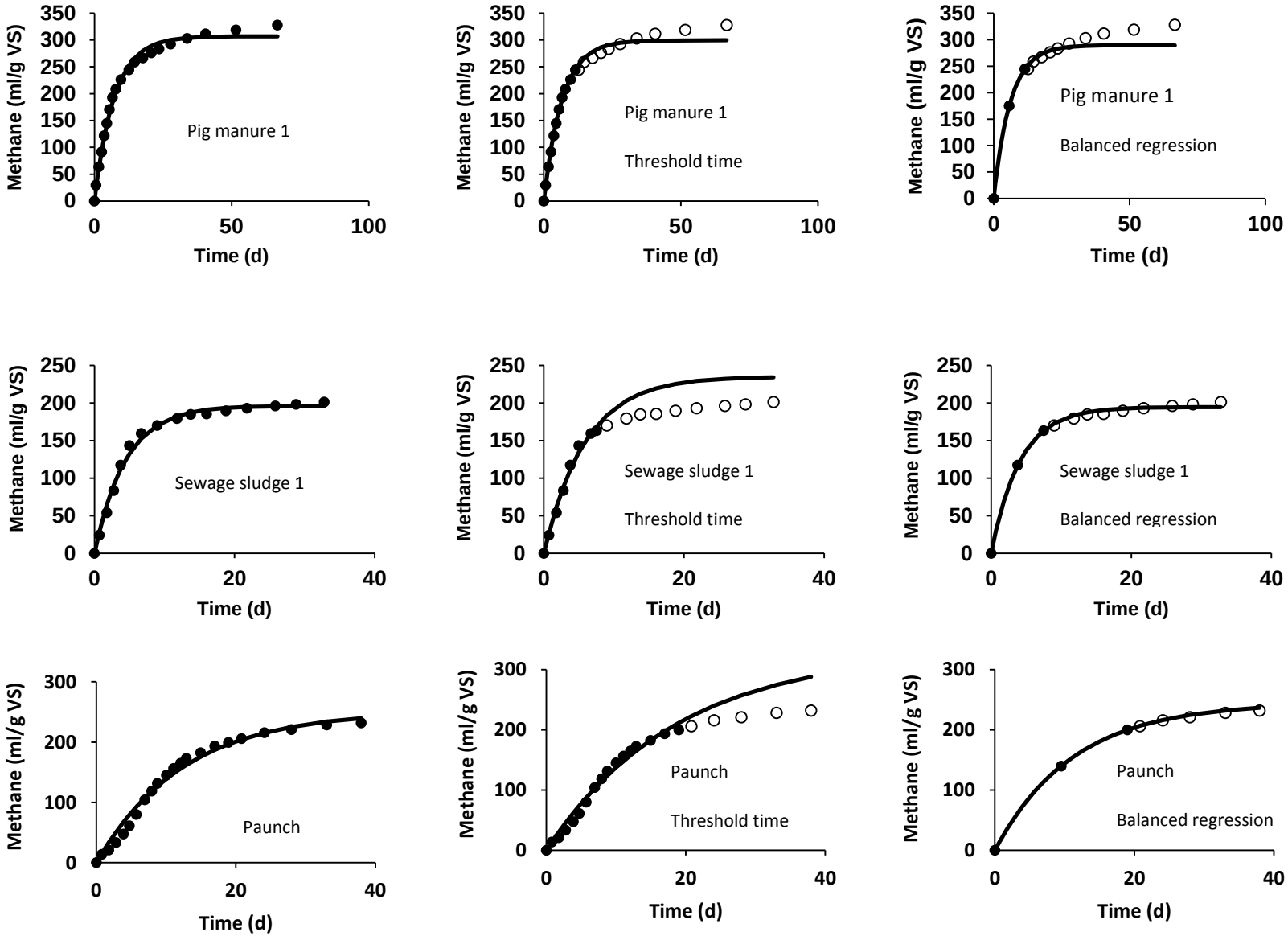

Fig. 4.2. Regression curves for pig manure 1, sewage sludge 1 and paunch by the three different sampling strategies. Data used for analysis regression (•).

4.5 Discussion

4.5.1 Parameters identifiability

High $R^2_{adjusted}$ values (>0.98) indicates that the first-order model (Eq. 1) is able to properly describe BMP experimental data, which is agreement with most published data (Vavilin et al., 2008; Angelidaki et al., 2009; Donoso-Bravo et al., 2010). The loss of proportionality (R^2<0.80) between both first-order model sensitivity functions (Eq. 2 and 3) was used to determine the minimum testing time needed to make both first-order parameters (B_0 and *k*) identifiable. The minimum testing time obtained under these conditions shows a strong potential relationship with *k*, with a R^2 close to 1 (Figure 4.1 and Eq. 6). Figure 4.1 shows that the minimum testing times for substrates with *k* values higher than 0.2 d^{-1} is less than a week, while for substrates with *k* values of 0.1 $days^{-1}$, and below, the minimum testing time is two weeks or more. The asymptotic behavior of Figure 4.1 to the y-axis indicates that small decreases in the degradation kinetics will led to significant increase of the minimum testing time. This fact would clearly explain why Strömberg et al. (2015) and Ponsá et al. (2011) needed lower testing times to predict the maximum methane yield for highly biodegradable substrates than for slowly biodegradable substrates.

Interestingly, the minimum testing times suggested by Strömberg et al. (2015) are lower than the ones obtained in this study. For instance, the minimum testing time needed for sewage sludge by Strömberg et al. (2015) is 4 days while in this study 8 and 10 days are recommended for sewage sludge 1 and 2, respectively. Although the difference can be attributed to the different models used, it is also true that the criterion used in this study to decide when both sensitive functions have lost their proportionality is somewhat conservative (R^2<0.80). For instance, if the R^2 criterion is increased to 0.90 the minimum testing time sewage sludge 1 and 2 is reduced to 5.5 and 7 days, respectively. However, a more conservative approach is preferred by the authors in order to avoid inaccurate predictions while still managing relatively low testing times.

4.5.2 Parameters predictions robustness

As can be seen in Table 4.1, there is no statistically significant difference between the experimental value of B_0 and that was predicted from the traditional regression. However, major differences are observed in five out of eight of the substrates when comparing the experimental value of B_0 and that predicted using all experimental data obtained at times lower than the threshold time calculated. Such difference appears when the BMP curve deviates from the exponential ideal behavior, which has been related to phenomena like tailing (e.g. pig manure 1) and sigmoidal shape (e.g. paunch). Contrariwise, in most cases, the difference between the experimental value of B_0 and that obtained from a balance threshold regression B_0 are insignificant (Table 4.1). The percentage difference between the predicted B_0 by the balanced threshold regression for the conflicting substrates is 7% for primary sludge and 12% for pig manure 1. This ~10% difference is considered acceptable from a practical point of view.

Regarding the *k* values, in most cases, there is no statistical difference between the *k* values obtained from the three different regressions approaches. However, the confidence interval provided by the threshold regression is much larger than the confidence region obtained from the traditional and the balanced threshold regression. These results highlight that the balanced threshold regression (3-points regression) gives better results than the threshold regression (using all data set between t=0 and t=threshold time). The balanced threshold regression allows minimising the influence of the experimental data from the proportional region in the regression analysis, while improving the quality of the parameters estimation (i.e. higher accuracy and lower confidence intervals). The present results clearly show that the balance threshold regression is a feasible tool for *k* and B_0 early prediction.

Finally, it is worth to mention that the methane yield (Raposo et al., 2012) and the kinetic constant rates reported in the literature for the substrates (Table 2) under study are highly

variable but in range with the values obtained in the present study. Based on these values, using *k* values from the literature, the minimum BMP testing time required for determining both k and B_o are suggested (Table 4.2).

Table 4.2. Literature kinetic constant rate values of some common anaerobic digestion substrates and the subsequent threshold time suggested to carry out the BMP test.

Substrate	k (d^{-1})	Threshold time (d)	Reference
Sewage sludge	0.17-0.60	9.5 – 3.5	Vavilin et al., 2008 Batstone et al., 2002 Donoso-Bravo et al., 2010
Primary sludge	0.23-0.40	7.5 – 4.5	Siegrist et al., 2002 Donoso-Bravo et al., 2010
Pig manure	0.07 – 0.17	21.5 – 9.5	Vavilin et al., 2008 Pham et al., 2013
Paunch	0.10 –0.23	15.5-7.5	Jensen et al., 2014 & 2016
Waste activated sludge	0.16-0.30	10 - 6	Wang et al.,2013 Ruiz-Hernando et al.,2014
Crops residues	0.009-0.094	136.5-16.5	Vavilin et al., 2008
Algae	0.032-0.11	43.5-14.5	Gavala et al., 2003 Passos et al., 2014
Slaughterhouse waste	0.35 - 0.28	5-6.5	Jensen et al., 2015 Vavilin et al., 2008

4.5.3 Proposal for BMP parameters early prediction

An iterative strategy to minimize the experimental effort in the determination of BMP first-order model parameters (B_0 and k) is proposed (Figure 4.3). Depending on the type of substrate, minimum testing time can be selected to set the first iteration (Table 4.2). In general, substrates can be merged in three mayor group depending on the kinetic constant rate value: (i) slowly biodegradable substrates ($k < 0.1$ d^{-1}) with minimum testing times of, at least, 15 days, (ii) moderately biodegradable substrates (0.1 <k< 0.2 d-1) with minimum testing times between 15 and 8 days, and (ii) rapidly biodegradable substrates ($k \geq 0.2$ d^{-1}) with minimum testing time lower than a week.

Once the BMP test is run for the minimum recommended time, a non-linear regression is applied to experimental data to estimate both model parameters. With the value of k obtained, the minimum time required for the BMP test is calculated by equation 6. If the time is lower than the sampling time used, early parameter estimation is achieved. On contrary, the BMP test should go on because the early prediction cannot be hold.

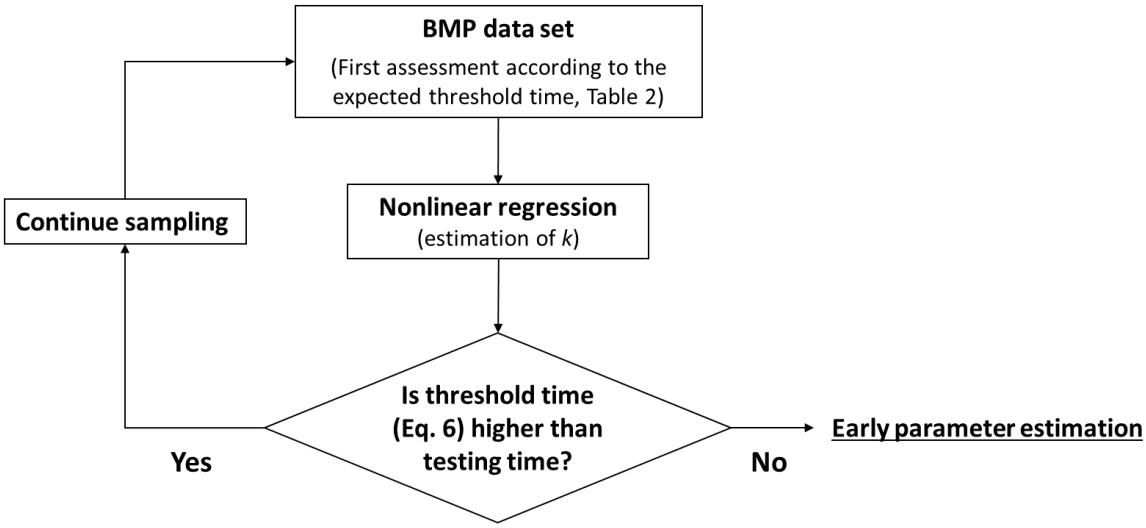

Fig. 4.3. Diagram flow for BMP first-order model parameters early prediction parameters.

4.5.4 Conclusions

A mathematically robust strategy using sensitivity functions for early prediction of BMP first-order model parameters (i.e. maximum methane production (B_0) and kinetic constant rate (*k*)) has been developed. The minimum testing time for parameters early prediction showed a potential correlation with the substrate kinetic constant rate: (i) slowly biodegradable substrates ($k \leq 0.1\ d^{-1}$) require minimum testing times of >15 days, (ii) moderately biodegradable substrates ($0.1 < k < 0.2\ d^{-1}$) require minimum testing times between 15 and 8 days, and (iii) rapidly biodegradable substrates ($k \geq 0.2\ d^{-1}$) require testing times <7 days. This study also shows that a balanced regression (3- experimental points: t=0, t=minimum testing time and t= average time of sampling interval) gives better results than strategy than the regression that uses all experimental data until t=minimum testing time. The balanced regression allows minimising the influence of the experimental data from the proportional region in the regression analysis, while improving the quality of parameters estimation (i.e. higher accuracy and lower confidence intervals). Finally, this methodology should be only applied to those substrates following a first order kinetic model.

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5 General conclusions

The presented research chapters contains new contributions to anaerobic digestion technology and profitability of its operation and technical evaluation.

The use of fly ashes as stimulatory trace material in anaerobic lab digesters was valued. For secondary sludge waste from meat factory by using BMP tests a stimulatory dosage of 150 mg/l fly ashes was identify. That dosage showed two stimulatory effects: 1) the methane rate generation was increased three times. Passing from 1.4 ml/d (case of no ash dosage) to 4.48 ml/d of methane (when stimulatory dosage was applied). 2) The kinetic hydrolytic constant of the particulate material was increased in twice. When no ashes were added the obtained first order kinetic hydrolytic parameter were 0.019 ± 0.002 d^{-1} while when stimulatory dosage was added, the kinetic parameter was improved to 0.046 ± 0.000 d^{-1}.

This results not only proves the use of fly ash as reliable low cost strategy when the anaerobic digester is unbalanced in trace elements, it also reports that not only methanogenic step is improved, the hydrolysis of particulate material shown also been improved.

The enhancing of methanogenic activity and hydrolytic conversion makes feasible the thermoelectric fly ashes as low cost strategy to balance the lack of micronutrients and therefore the energy and economic balances on WWTP. The hydrolytic stage enhancing by fly ashes is not reported in anaerobic digestion literature; in general the anaerobic digestion publications are focusing on how the heavy metals impacts on archea flora and activity (methanogenic step). That result open a new point of view for the necessary understanding of how trace metals impacts on anaerobic digestion process; more concretely in the hydrolytic step.

A mathematical strategy using sensitivity functions for early prediction of BMP first-order model parameters (methane yield and kinetic constant rate) was developed. BMP tests are the key analytical technique to assess the implementation and optimization of anaerobic digesters. However, this technique is characterized by large testing times; in magnitude of months. The first order kinetic model is well used in anaerobic field in order to model the methane generation in solid wastes.

An iterative routine which combines the sensitivity function and minimum least squares, was developed to define the minimum parameter identifiable time for the first order kinetic model. The identifiable time regions were compared with eight real solid BMP tests and the results holds the possibility to reduce the required incubation time around 20% of the time used to observe the null methane generation.

It is expected that this results will lead to improve early making decisions in anaerobic biotechnology; grade of mixture of residues in co-digestion and early technological feasibility of anaerobic digesters will be the anaerobic areas to get benefit with this strategy.

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