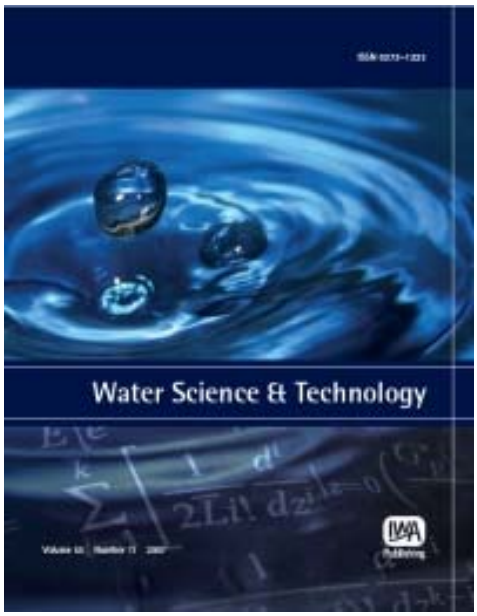

Influence of conditioning agents and enzymic hydrolysis on the biochemical methane potential of sewage sludge

Elena Marañón, Luis Negral, Yolanda Fernández-Nava and Leonor Castrillón

ABSTRACT

Biochemical methane potential (BMP) tests have been carried out on sewage sludge from two wastewater treatment plants to assess the effect of additives ($FeCl_3$ and two cationic polyelectrolytes) used in sludge dewatering. BMP tests were also carried out on the concentrated solid phase from the enzymic hydrolysis pre-treatment (42 °C, 48 h). $FeCl_3$ had no significant effect on specific methane production, obtaining 242–246 $LCH_4/kgVS_o$. The effect of the combination of polyelectrolyte and $FeCl_3$ depended on the polyelectrolyte and the sludge, but generally led to an increase in specific methane production (25–40%). When enzymic hydrolysis was applied as a pre-treatment, specific methane production increased from 6.8% in the sludge containing $FeCl_3$ to 20% in the sludge without $FeCl_3$, although the increases were not statistically significant. In terms of $LCH_4/kgVS_{rem}$, a general improvement was achieved both by means of additives and by enzymic hydrolysis. However, this improvement was only significant in the case of sludge which had undergone previous enzymic hydrolysis (62%) and in the untreated sludge containing a polyelectrolyte and $FeCl_3$ (24%). Cationic polyelectrolytes inhibited solid–liquid separation during enzymic hydrolysis and, although the presence of only $FeCl_3$ did not affect this separation, a significant decrease (32%) in $LCH_4/kgVS_{rem}$ was observed.

Key words | biochemical methane potential, cationic polyelectrolyte, ferric chloride, inverted phase fermentation, sewage sludge

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INTRODUCTION

Sewage sludge management has pursued better economic efficiency of sludge treatment for decades. The European Union (EU) is currently fostering the development of more sustainable sludge management. One way to reduce costs is via a decrease in sludge production. According to Gendebien *et al.* (2010), 10 million tonnes of sludge (expressed as dry matter) was produced each year in the EU at the end of the last decade. Reducing costs is a challenge promoted by the deceleration in sludge production and energy recovery (Murray *et al.* 2008).

Sewage sludge is a heterogeneous material rich in organic matter whose composition is influenced both by the characteristics of the influent and the processes employed at wastewater treatment plants (WWTPs). However, sewage sludge presents a considerable organic load due to the growth of the microorganisms that enable water treatment (Ucisik & Henze 2008). If this organic load is not sent to landfill for disposal (in accordance with restrictions introduced by the EU Landfill Directive 1999), other alternatives such as incineration or anaerobic digestion must be considered. The main differences between the two techniques are that when applying incineration, the sludge needs to be conditioned and ash is obtained as a final residue, whereas conditioning is optional when applying anaerobic digestion (depending on whether the digester is on-site or off-site) and the final products are biogas and a stabilized digested material (digestate) that can be used for agricultural purposes (Appels *et al.* 2008).

The biomethanation of sludge is characterized by poor bioavailability of substrates for the bacteria involved in biogas production. In particular, biogas production is slow due to the hydrolysis of the substrate, the first and

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rate-limiting stage for sewage sludge (Skiadas *et al.* 2005). Siegrist *et al.* (1993) studied the kinetics of sewage sludge at 35 °C. These authors estimated a hydrolysis rate constant of 0.25 d^{-1}, versus 5.0 d^{-1} for acidogenesis, 0.8 d^{-1} for acetogenesis, and 0.5 d^{-1} for methanogenesis of acetate-utilizing methanogens and 2.5 d^{-1} for hydrogen-utilizing methanogens. Most of the organic matter in sewage sludge comes from cell walls trapping nutrients inside cells or macromolecules such as extracellular polymeric substances (EPS). These fractions are not easily assimilated by microorganisms (Appels *et al.* 2008). The major interest in accelerating hydrolysis is thus understandable. The best hydrolysis strategy will entail economic efficiency and feasibility and will lead to the upgrade of sludge valorization at WWTPs. Many enzymic pre-treatments add hydrolases as exogenous enzymes to the substrate (Burgess & Pletschke 2008), resulting in an up to 10-fold improvement in hydrolysis constants (Yang *et al.* 2010). Since anaerobic microorganisms produce their own enzymes (Burgess & Pletschke 2008), an alternative would be to promote this enzymic activity. This means using bacteria strains present in the sludge. Le *et al.* (2008) proposed an enzymic hydrolysis method (inverted phase fermentation, IPF) that favours endogenous activity in sludge. The technique consists of keeping the sludge at 42 °C for 48 h under anaerobic conditions. As time passes, the nascent CO_2 drags solid particles to a new solid phase (SP) which floats over a clarified layer, the liquid phase or liquor. The SP reaches concentration factors for total solids (TS) of up to 2.8 (defined as TS in the SP versus TS in the initial sludge). Due to the enzymic hydrolysis, the SP and the liquor present a higher content in soluble organic matter than the sludge. The liquor, rich in soluble species such as volatile fatty acids, is characterized by an almost complete hydrolysis, as most of its total chemical oxygen demand (tCOD) is present in the soluble form (sCOD) (Negral *et al.* 2013). This particular form of enzymic hydrolysis is called IPF. The achievement of these distinct phases has several advantages: different treatments for the phases, thickening of sludge in the SP without the use of additives, enhancement of enzymic hydrolysis to increase sCOD, and 99.9% destruction of *Escherichia coli*.

The additives usually employed at WWTPs include coagulants such as Al and Fe salts and lime (Thistleton *et al.* 2001; Smith & Carliell-Marquet 2008) and flocculants such as synthetic acrylamides and natural polymers (polyelectrolytes) (El-Mamouni *et al.* 1998; Degrémont 1979). The aim of these products is to clarify the effluent to which they are added, whether this be the wastewater or the sludge itself. As regards sludge, the purpose of additives is to destroy its structure, thus freeing the bacteria, particles and water from the EPS of the sludge. Altering the state of the EPS-based matrix decreases the volume as a result of destroying this structure. The structure of sludge consists of a mucilaginous EPS matrix in which bacteria are embedded (Burgess & Pletschke 2008). EPS are made up of a variety of organic substances such as carbohydrates, proteins, humic compounds, lipids, uronic acids and deoxyribonucleic acids (Tchobanoglous *et al.* 2003) which weave a structure that gives volume to the sludge. Destabilization of the colloidal structures that form EPS is carried out using coagulants (e.g. $FeCl_3$), while flocculants (e.g. high molecular weight polyacrylamides) favour the growth of flocs that cause these structures to settle. Q1 The ζ potential explains the work of sludge colloid destabilization by means of coagulants, since positive charges (i.e. Fe^{3+}) are added to the medium in which the EPS have negative charges. However, the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory, which predicts this behaviour, seems to require a combination of hydrophobic forces and interactions, due to the entanglement of the flocs, to more adequately explain some deviations from the predictions of experiments carried out with sludge (Mikkelsen & Keiding 2002). The attack by additives on the structure of sludge has potential importance for digestion on account of both altering hydrolysis (Wawrzynczyk *et al.* 2008) and mobilizing nutrients (Erden & Filibeli 2010). In terms of sludge disintegration, enzymes that can act on its matrix, i.e. extracellular enzymes, are of importance. These may be exoenzymes, which are free in the medium, or ectoenzymes, which are bound to the bacterial surface (Cadoret *et al.* 2002). There are doubts as to the location and operation of exoenzymes; they are most likely found in the EPS and not in the water (Boczar *et al.* 1992; Goel *et al.* 1998). Excessive EPS thus impair the hydrolytic activity of these exoenzymes, the dehydration of the sludge and its sedimentation (Liu & Fang 2003; Tchobanoglous *et al.* 2003). Regardless of the degree of functionality and location of exoenzymes, the key seems to lie in the level of contact between microbial cells and substrates (Burgess & Pletschke 2008). As a result of nutrient mobilization, additives can either inhibit or promote anaerobic digestion. There has been much debate in the literature regarding this issue for decades (Dentel & Gosset 1982; Smith & Carliell-Marquet 2008). Several studies point to the decreased activity of hydrolytic enzymes (Dentel & Gosset 1982; Wawrzynczyk *et al.* 2008). Another possible route via which the addition of coagulants inhibits anaerobic digestion would lie in the precipitation of phosphorus, making it a limiting factor for anaerobic metabolism (Smith & Carliell-Marquet

2008). Nevertheless, the adaptation of bacterial strains to stressful situations is common (Chen *et al.* 2008). Moreover, Lee & Shoda (2008) found marked increases in methane when digesting sludge with high concentrations of iron. As regards flocculants, their benefits have been reported in the granulation of sludge (El-Mamouni *et al.* 1998), with consequent advantages for biogas production (Hulshoff Pol *et al.* 2004).

The lack of consensus in the literature regarding the effect of conditioning agents in anaerobic digestion led us to design a series of tests to obtain the biochemical methane potential (BMP) of conditioned sludge (with $FeCl_3$, and with $FeCl_3$ plus polyelectrolytes) and also to study the SP that separates off when applying IPF to the sludge (with and without conditioning agents). To the best of our knowledge, the latter has not yet been studied. According to Angelidaki & Sanders (2004), biodegradability assays may be based on the measurement of either the formation of products or the measurement of substrate depletion. In this study, BMP tests were carried out using sludge from two WWTPs and the results are discussed in terms of both CH_4 production and substrate depletion (volatile solids (VS) removal). As coagulants and flocculants need to be added to the sludge for dewatering purposes, these products are likewise considered so as to assess their influence on the BMP. The BMP of the concentrated SP obtained after IPF pre-treatment were also tested.

MATERIALS AND METHODS

Materials

Sewage sludge

Experimental work was carried out with sewage sludge samples from two WWTPs. Table 1 provides the chemical composition of the sludge samples and of the SPs obtained after IPF pre-treatment. WWTP 'A' has an average flow rate of 3,210 m^3/h, with a high industrial contribution. At this plant, activated sludge secondary treatment is carried out directly after the pre-treatment, with no primary treatment being performed. This secondary sludge is conditioned with $FeCl_3$ (final concentration up to

Table 1 | Characterization of initial sludge samples (A and B), solid phases (SP), inoculum (I) and polyelectrolytes (Poly)

Sample	TS (g/L)	VS (g/L)	VS/TS	sCOD (g/L)	tCOD (g/L)	sCOD/tCOD	tCOD/VS	pH	$FeCl_3$ (g/L)
Sludge									
A1	37.23	29.52	0.79	4.87	52.73	0.09	1.79	5.41	0.14
A1 (SP)	85.08	66.70	0.78	10.24	127.58	0.08	1.91	5.63	
A2	55.94	44.56	0.80	8.66	96.96	0.09	2.18	5.14	0.10
B1	32.61	24.07	0.74	4.95	39.41	0.13	1.64	5.87	
B1 (SP)	64.31	43.78	0.68	16.75	85.41	0.20	1.95	6.30	
B2	48.93	35.90	0.73	4.34	66.93	0.06	1.86	3.31	5.60
B2 (SP)	90.68	68.89	0.76	6.05	94.07	0.06	1.37	3.13	
B3	75.11	39.61	0.53	2.52	54.44	0.05	1.37	5.16	5.60
Inoculum									
I1	25.06	9.89	0.39	1.96	12.62	0.16	1.28	7.98	
I2	20.21	8.81	0.44	1.49	11.14	0.13	1.26	7.85	
I3	21.60	9.20	0.43	1.61	18.52	0.09	2.01	7.61	
I4	19.78	9.49	0.48	1.38	17.83	0.08	1.88	7.59	
I5	18.02	7.92	0.44	1.58	17.02	0.09	2.15	7.79	
Polyelectrolyte									
Poly 1	3.90	3.47	0.89	0.026	0.031	0.84	<0.01	6.34	
Poly 2	6.70	6.06	0.90	0.012	0.018	0.67	<0.01	3.14	

Sludge: A1, A2, B1, B2 and B3 refer to sludge samples taken from two WWTPs (A and B) on different dates. '(SP)' after the name of a sample means that an aliquot of the corresponding sludge was used for enzymic pre-treatment. Inoculum: I1 [used in tests B1, B1 (SP)]; I2 [used in tests A1, A1 (SP)]; I3 [used in tests B2, B2 (SP)]; I4 [used in test B3]; I5 [used in test A2]. Polyelectrolyte: Poly 1 = Chemifloc CH80. Poly 2 = Chemifloc CH50.

0.10–0.14 g $FeCl_3$/L sludge) and a cationic polyelectrolyte before being dewatered in a centrifuge. $FeCl_3$ is added, not only for dewatering purposes, but also to enhance clarification in the settling tank. Therefore, it was not possible to carry out a BMP test on this sludge without the presence of this coagulant. Two sludge samples were taken on different dates, 'A1' and 'A2', and an aliquot of the former was also enzymically pre-treated, 'A1 (SP)'.

WWTP 'B' has an average flow rate of 900 m^3/h and produces primary and secondary sludge that is mixed before dewatering. Due to the use of a filter press instead of a centrifuge, $FeCl_3$ (up to 6 g/L) and lime (up to 22 g/L) are added prior to dewatering, no polyelectrolyte being added at present. Three sludge samples, 'B1', 'B2' and 'B3', were taken on different dates. Aliquots of B1 and B2 were also used for enzymic pre-treatment, 'B1 (SP)' and 'B2 (SP)'.

Inoculum

The inoculum used was mesophilic digestate from the continuous stirred-tank reactor (CSTR) co-digesting mixtures of sewage sludge, food waste and cattle manure. The digestate was kept in a closed recipient at 37 °C until being mixed with the substrate. The digestate was allowed to stand for a minimum of 2 d before being mixed with the sludge for the BMP tests to ensure degasification of the inoculum before making up the mixtures (Wan *et al.* 2011). Table 1 shows the chemical characterization of the inoculum samples. The VS content was 9.1 ± 0.8 g/L, the TS content 20.9 ± 2.6 g/L, and the total and soluble COD 15.4 ± 3.3 and 1.6 ± 0.2 g/L, respectively. As not all the BMP tests could be carried out simultaneously, different inoculum samples had to be used.

Additives

$FeCl_3$ and two cationic polyelectrolytes were employed for these experiments. Due to the high pH in the samples conditioned with lime, this additive was not considered for the BMP tests. Lime acts as a stabilization agent, thus preventing the biological degradation of the sludge. Solid $FeCl_3$ (as provided by the manufacturer) was diluted with Milli-Q water to a solution (40% w/v). This solution was poured into the corresponding sludge sample at a dosage of 5.6 g/L (Table 1). Two cationic polyelectrolytes were studied, both being high molecular weight polyacrylamides (Chemifloc CH80 and Chemifloc CH50). Each solid polyelectrolyte (as provided by the manufacturer) was diluted with Milli-Q water to a solution (0.6% w/v). The solution was poured into the sludge at a dosage of 88 mL/L. The content in solids, COD and pH of each flocculant solution is presented in Table 1. Although the composition of these polyelectrolytes is mostly organic, they are not easily oxidized due to their great stability. Therefore, the values of COD are very low. A homogeneous mixture between reagents and substrate was ensured through mechanical stirring: 200 rpm for 3 min.

Analytical methods

Both tCOD and soluble sCOD were determined following Method 5220 (closed reflux colorimetric method) of the *Standard Methods for the Examination of Water and Wastewater* (APHA 1998) on a Perkin Elmer Lambda 35 Visible-UV system. Samples were centrifuged (3,500 rpm for 15 min) and filtered through 1.2 μm pore filter paper for sCOD determination (Le *et al.* 2008). NH_4-N was determined using an Orion 95–12 selective electrode for ammonium. TS and VS were determined following Method 2540 of the *Standard Methods for the Examination of Water and Wastewater* (APHA 1998) and pH was determined using a Crison 25 pH-meter. All analytical determinations were performed in triplicate.

Biogas composition was monitored on an Agilent 7890A gas chromatograph using a thermal conductivity detector (TCD) and a Porapack N packed column plus a molecular sieve. The temperature ramp was: starting 35 °C (1.5 min), increasing up to 55 °C at a rate of 1.5 °C/min. Biogas volume was measured with a gas meter. All the gas volumes in this paper have been converted to standard temperature and pressure (273.15 K and 101.3 kPa).

Experimental procedure

All the sludge samples were characterized on reception at the laboratory and were kept under refrigeration at 4 °C for a maximum of 2 d before being used in the experiments so as to prevent biodegradation. To carry out the IPF, two 25 L plastic bottles were filled with fresh sludge and an outlet hose connected the bottles with a large flask containing water to achieve anaerobic conditions. The sludge was heated to 42 °C and the SP at the top was removed after 48 h and characterized (Table 1).

The mixtures of sludge (or SP from the IPF) and inoculum for the anaerobic biodegradability tests were made up maintaining a ratio of VS contribution from the substrate to VS contribution from the inoculum of 2:1. Subsequently, 1,750 g of the corresponding mixture were poured into glass bottles sealed with rubber stoppers and silicone. To study

the effect of the conditioning agents on the BMP, both the coagulant ($FeCl_3$) and the flocculants (poly 1 and poly 2) were added to the sludge in similar proportions to those used at the WWTPs. The 40% $FeCl_3$ solution was added to sludge B (the sludge A samples already contained $FeCl_3$) to achieve a concentration of 5.6 g/L. Solutions of polyelectrolytes containing 0.6% w/v were added to sludge A and sludge B to achieve a concentration of 88 mL polyelectrolyte solution/L substrate. No surplus nutrients were added to the mixtures (Yang *et al.* 2010). In this respect, Wan *et al.* (2011) observed that the addition of micronutrients to the co-digestion of thickened activated sludge did not improve biogas production or digestion stabilization. Once capped, bottles were purged with nitrogen to remove air from the headspace of the bottle. Although these experiments were batch tests, the bottles were shaken every time the volume and composition of the gas were determined (Luste *et al.* 2009). Biogas was collected in Tedlar bags and measured for volume and composition. To monitor the biogas production from the inoculum, two blanks were prepared with bottles solely filled with 1,750 g of inoculum. Consequently, their biogas production and chemical measurements were proportionally subtracted in the other experiments.

The biogas production rate was negligible after 25 d of anaerobic digestion at 37 °C; the bottles were then unlocked and the digestates analysed. Two replicates per experiment were simultaneously performed. The standard deviation of the replicates remained around or below 5% for the specific production ($LCH_4/kgVS_o$), with the exception of experiment B1 (standard deviation = 18%).

Statistical analysis

Analysis of variance (ANOVA) was applied to the variables $LCH_4/kgVS_o$ and $LCH_4/kgVS_{rem}$ to test the null hypothesis among the substrates. The Tukey test was then used to determine the significant differences (p-value < 0.05) in pairwise comparisons of the substrates. R-project software was employed for this purpose.

Kinetic modelling

The first-order kinetics for the digestion of the substrates studied in this paper was modelled. The degradation of substrate 'S' with time may be expressed as:

$$-\mathrm{d}S/\mathrm{d}t = k_1 S \tag{1}$$

where k_1 is the kinetic constant. Integrating Equation (1):

$$\begin{aligned} &\mathrm{Ln}(S_{(0)}/S_{(t)}) = k_1 \cdot t \\ &S_{(t)} = S_{(0)}\ \exp(-k_1 \cdot t) \end{aligned} \tag{2}$$

where $S_{(t)}$ is the concentration of substrate at time t and $S_{(0)}$ is the concentration of substrate at the initial moment. Moreover, the concentration of substrate at the initial moment is the sum of the concentration of the substrate at a given time plus the concentration of degraded substrate, '$S_{(d)}$', which can be expressed as:

$$\begin{aligned} &S_{(0)} = S_{(t)} + S_{(\mathrm{d})} \\ &S_{(\mathrm{d})} = S_{(0)} - S_{(t)} \end{aligned} \tag{3}$$

In anaerobic digestion, the substrate is transformed into CH_4, so a coefficient of the yield of substrate into CH_4, 'α', can be introduced:

$$\begin{aligned} &\alpha \cdot S_{(\mathrm{d})} = \mathrm{CH}_{4(t)} \\ &\alpha \cdot S_{(0)} = \mathrm{CH}_{4(0)} \end{aligned} \tag{4}$$

where $CH_{4(t)}$ is the methane production at time 't' and $CH_{4(0)}$ is the methane production at the end of the anaerobic digestion of the substrate.

Substituting Equation (3) into Equation (4), we obtain:

$$\alpha(\mathrm{S}_{(0)} - \mathrm{S}_{(t)}) = \mathrm{CH}_{4(t)} \tag{5}$$

Substituting Equation (2) into Equation (5) and operating, we obtain:

$$\mathrm{CH}_{4(0)}[1 - \exp(-k_2 \cdot t)] = \mathrm{CH}_{4(t)} \tag{6}$$

where the new kinetic constant, k_2, now refers to methane production rather than substrate degradation. Matlab software was used to test each model.

RESULTS AND DISCUSSION

The characteristics of the different sludge samples and the inoculum are shown in Table 1. The two samples from WWTP A have different solid and COD contents. However, the VS/TS ratios are quite similar (0.79–0.80). With respect to samples from WWTP B, note that sample B3 had a higher inorganic content than the other two samples, with a low VS/TS ratio (0.53).

As expected, the SP presents a higher concentration in solids and COD after the IPF pre-treatment compared to the sludge (Le *et al.* 2008; Negral *et al.* 2013), achieving solids concentration factors of around 2 in the three sludge samples tested.

The determination of COD in such complex residual matter as sludge is more prone to analytical errors (Angelidaki & Sanders 2004) than the determination of VS. This is due to the presence of certain organic compounds that are difficult to oxidize, as well as to the presence of reduced inorganic compounds that can be oxidized (Fe^{2+}, Cl^-, S^{2-} …). Accordingly, and bearing in mind the high amounts of iron chloride present in some of the samples, the results of the BMP tests are given in Table 2 as specific methane production with respect to initial VS ($LCH_4/kgVS_o$) and to removed VS ($LCH_4/kgVS_{rem}$). The standard deviation of the replicates remained around 5% for specific production ($LCH_4/kgVS_o$), with the exception of experiment B1 (standard deviation = 18%).

Samples of the inoculum presented the lowest concentrations in solids and COD and also the lowest VS/TS ratios (0.39–0.48), as most of the organic biodegradable matter has been mineralized during bacterial metabolism (Carrère *et al.* 2008; Marañón *et al.* 2012).

CH_4 concentrations during the stable period ranged from 63% in sample B2 plus $FeCl_3$ and 72% in B1 (SP). Of the negligible biogas produced by the inoculum, only 22% was CH_4.

After the analysis of variance, the Tukey test was used to search for significant differences in $LCH_4/kgVS_o$ and $LCH_4/kgVS_{rem}$ among the substrates. Table 3 presents the results of the Tukey test for those pairwise comparisons with significant differences (p-value < 0.05). This table does not show the pairwise comparisons with the inoculum, as this is not relevant for testing the effect of the conditioning agents and the pre-treatment.

Table 2 | Degradation of solids and specific methane production in substrates after 25 d of biodegradation

Sludge sample	TS (%)	VS (%)	$LCH_4/kgVS_o$	$LCH_4/kgVS_{rem}$	LCH_4/kg_{subs}
A1 + $FeCl_3$	35	52	255	493	7.52
A1 (SP) + $FeCl_3$	65	70	233	338	15.55
A1 + $FeCl_3$ + poly 1	16	38	248	662	6.80
A2 + $FeCl_3$ + poly 2	43	58	358	612	14.85
B1	35	50	246	496	5.92
B1 (SP)	17	37	296	804	12.98
B2 + $FeCl_3$	14	45	242	541	8.69
B2 (SP) + $FeCl_3$	28	52	276	545	18.99
B3 + $FeCl_3$ + poly 1	14	52	302	582	11.08
B3 + $FeCl_3$ + poly 2	12	40	269	672	9.92
Inoculum	12	1	7	548	0.07

Table 3 | Results of the Tukey test when significant differences (p-value <0.05) were found between substrates for $LCH_4/kgVS_o$ and $LCH_4/kgVS_{rem}$

$LCH_4/kgVS_o$ (pairwise comparison, substrates)		p-value
Sludge A1 + $FeCl_3$	Sludge A2 + $FeCl_3$ + poly 2	0.0025
Sludge A1 (SP) + $FeCl_3$	Sludge A2 + $FeCl_3$ + poly 2	0.0008
Sludge A1 (SP) + $FeCl_3$	Sludge B3 + $FeCl_3$ + poly 1	0.0499
Sludge A1 + $FeCl_3$ + poly 1	Sludge A2 + $FeCl_3$ + poly 2	0.0002
Sludge A1 + $FeCl_3$ + poly 1	Sludge B3 + $FeCl_3$ + poly 1	0.0177
Sludge A2 + $FeCl_3$ + poly 2	Sludge B1	0.0017
Sludge A2 + $FeCl_3$ + poly 2	Sludge B2 + $FeCl_3$	0.0001
Sludge B2 + $FeCl_3$	Sludge B3 + FeCl3 + poly 1	0.0118
$LCH_4/kgVS_{rem}$ (pairwise comparison, substrates)		**p-value**
Sludge A1 + $FeCl_3$	Sludge B1 (SP)	0.0000
Sludge A1 + $FeCl_3$	Sludge B3 + $FeCl_3$ + poly 2	0.0004
Sludge A1 (SP) + $FeCl_3$	Sludge A1 + $FeCl_3$ + poly 1	0.0000
Sludge A1 (SP) + $FeCl_3$	Sludge A2 + $FeCl_3$ + poly 2	0.0004
Sludge A1 (SP) + $FeCl_3$	Sludge B1 (SP)	0.0000
Sludge A1 (SP) + $FeCl_3$	Sludge B2 + $FeCl_3$	0.0446
Sludge A1 (SP) + $FeCl_3$	Sludge B2 (SP) + $FeCl_3$	0.0125
Sludge A1 (SP) + $FeCl_3$	Sludge B3 + $FeCl_3$ + poly 1	0.0000
Sludge A1 (SP) + $FeCl_3$	Sludge B3 + $FeCl_3$ + poly 2	0.0000
Sludge B1	Sludge B1 (SP)	0.0001
Sludge B1	Sludge B3 + $FeCl_3$ + poly 2	0.0014
Sludge B1 (SP)	Sludge B2 + $FeCl_3$	0.0005
Sludge B1 (SP)	Sludge B2 (SP) + $FeCl_3$	0.0026
Sludge B2 + $FeCl_3$	Sludge B3 + $FeCl_3$ + poly 2	0.0082
Sludge B2 (SP) + $FeCl_3$	Sludge B3 + $FeCl_3$ + poly 2	0.0310

Table 4 presents the kinetic parameters for methane production after adjusting the experimental data to a first-order reaction model. The experimental data were adjusted to these kinetics with an acceptable fitting of the curve (R^2 = 0.9407–0.9914). A 2-d lag period was observed in some trials with sludge samples from WWTP B.

Effect of $FeCl_3$ on the methane potential of sludge

The solids degradation in sample B1 (without $FeCl_3$) remained within the usual range observed by other authors (Mottet *et al.* 2010). A 50% reduction in VS was achieved,

Table 4 | Parameters for the first-order model of the methane production, equation: $CH_{4(t)} = CH_{4(0)}[1-\exp(-k_2 \cdot t)]$

Sludge sample	$CH_{4(0)}$ ($LCH_4/kgVS_o$)	k_2 (d^{-1})	R^2	Lag period (d)
A1 + $FeCl_3$	270	0.1250	0.9751	
A1 (SP) + $FeCl_3$	247	0.1513	0.9731	
A1 + $FeCl_3$ + poly 1	272	0.1071	0.9743	
A2 + $FeCl_3$ + poly 2	406	0.0998	0.9766	
B1	280	0.1135	0.9407	
B1 (SP)	314	0.1495	0.9719	2
B2 + $FeCl_3$	250	0.1751	0.9839	2
B2 (SP) + $FeCl_3$	270	0.2353	0.9864	2
B3 + $FeCl_3$ + poly 1	309	0.1723	0.9820	
B3 + $FeCl_3$ + poly 2	271	0.1925	0.9892	
Inoculum	6.46	0.1612	0.9914	

producing 246 $LCH_4/kgVS_o$ (Table 2). There was no significant effect on methane potential or biodegradation due to the presence of $FeCl_3$, obtaining specific productions of 255 $LCH_4/kgVS_o$ and 242 $LCH_4/kgVS_o$ for sludges A1 and B2, respectively. Note that the low pH in sample B2, due to the high concentration of $FeCl_3$ (5.6 g $FeCl_3$/L), did have a slight effect on biodegradation; the VS removal being 45% instead of 50% (the value obtained in the sample not containing $FeCl_3$). Regarding the specific production per VS removed, it should likewise be noted that, although the VS removal was higher in the sludge sample without $FeCl_3$, the conversion of these VS to methane improved when the sludge had $FeCl_3$ added (from 496 to 541 $LCH_4/kgVS_{rem}$), although the difference was not statistically significant (Table 3).

Dentel & Gosset (1982) obtained lower biogas productions when adding $FeCl_3$ to the substrate. They stated that, working with concentrations of up to 800 mg$FeCl_3$/L, the decrease was not due to deleterious effects of the salt on the inoculum. These authors pointed to reduced extracellular enzymic activity as the reason for the decrease. Wawrzynczyk *et al.* (2008) studied the influence of metallic cations on the hydrolytic enzyme activity of sewage sludge. In their study, they reported that hydrolytic activity decreased with increasing Fe^{3+} concentration. Fe^{3+} would bind EPS, which in turn would hinder contact between enzyme and substrate.

Many basal mineral media in biodegradability assays incorporate iron in the $FeCl_2 \cdot 4H_2O$ form from 2 g/L mother solutions (Ferreiro & Soto 2003; Skiadas *et al.* 2005). The samples from WWTP B containing $FeCl_3$ have a three-fold higher iron concentration than these mother solutions. There is much discussion in the literature regarding the benefits and drawbacks of coagulation for anaerobic digestion of sewage sludge (Dentel & Gosset 1982; Smith & Carliell-Marquet 2008). However, in line with the present study, Lee & Shoda (2008) showed that concentrations of up to 6 gFe/L increased CH_4 production in sludge digestion. In fact, after the 2-d lag period, the kinetic constant was higher when $FeCl_3$ was present in the sludge (Table 4).

Smith & Carliell-Marquet (2008) justified the worsening of methane production in the anaerobic digestion of sewage sludge conditioned with $FeCl_3$. According to these authors, the probable cause is the precipitation of the phosphorus contained by iron in the sludge. The phosphorus would thus not be bioavailable to the microorganisms, which would have a deleterious effect on their metabolism and hence worsen methane production. However, the explanation for the different findings in the literature regarding the effects of the metal may lie in the handling of the sedimented flocs themselves. Vlyssides *et al.* (2009) argued that iron favoured granulation in the digester, thereby resulting in an improved methane yield. Once the flocculated nutrients settle, they remain in contact with the granules that have already formed in the digester, thus facilitating contact between bacteria and nutrients. Johnson *et al.* (2003), on the other hand, reported that the addition of iron decreased H_2S. That H_2S removal would be the result of the formation of FeS (Vlyssides *et al.* 2009) and, under anaerobic conditions in the reactor, the reduction of Fe^{3+} to Fe^{2+} is totally feasible (Novak & Park 2010). This iron would originate from the nutrient flocs. Several benefits would thus be obtained by the formation of the inorganic salt (FeS):

(a) Decrease in the concentration of H_2S, which is a counterproductive agent in biomethanization.
(b) The iron that the nutrients had taken to the bottom of the digester, where granules naturally accumulate, would free those nutrients in direct contact with the granules. Nielsen & Keiding (1998) reported that 10% of the organic matter of the flocs is solubilized due to their weakening as a result of the formation of FeS.
(c) The formation of the FeS salt would constitute a new inorganic support matrix to enable the formation of new granules. In fact, the granules constitute a symbiosis of bacterial communities in which anaerobic digestion is synergistic (Hulshoff Pol *et al.* 2004).

A review of the scientific papers that lean towards the benefit (or neutrality) of iron in anaerobic digestion reveals that digestion takes place under no mechanical stirring

conditions in these studies (e.g. upflow anaerobic sludge blanket (UASB) reactors and digesters stirred once a day, as in our study). In contrast, the presence of iron in the sludge tends to be deleterious when digestion is carried out under vigorous stirring conditions, as stirring destroys the granules.

Effect of polyelectrolytes in combination with $FeCl_3$ on the methane potential of sludge

The combination of polyelectrolyte 1 with $FeCl_3$ had a detrimental effect on solids degradation in sludge A, with VS removal decreasing from 52 to 38% (Table 2), although the effect on specific methane potential was not statistically significant, decreasing from 255 to 248 $LCH_4/kgVS_o$. In sludge B, however, the combination of polyelectrolyte 1 with $FeCl_3$ led to an increase in both VS degradation (from 45 to 52%) and specific methane production (242–302 $LCH_4/kgVS_o$, p-value < 0.05). The combination of polyelectrolyte 2 with $FeCl_3$ led to a higher specific methane potential in both types of sludge, resulting in a significant increase from 255 to 358 $LCH_4/kgVS_o$ in sludge A, although the increase from 242 to 269 $LCH_4/kgVS_o$ in sludge B was not significant. The effect of this combination of polyelectrolyte 2 with $FeCl_3$ was also observed in the organic matter biodegradation of sludge A, with VS removal increasing from 52 to 58%. The combination of polyelectrolytes with $FeCl_3$ always enhanced the conversion of VS into CH_4 for both sludge types, being statistically significant for polyelectrolyte 2.

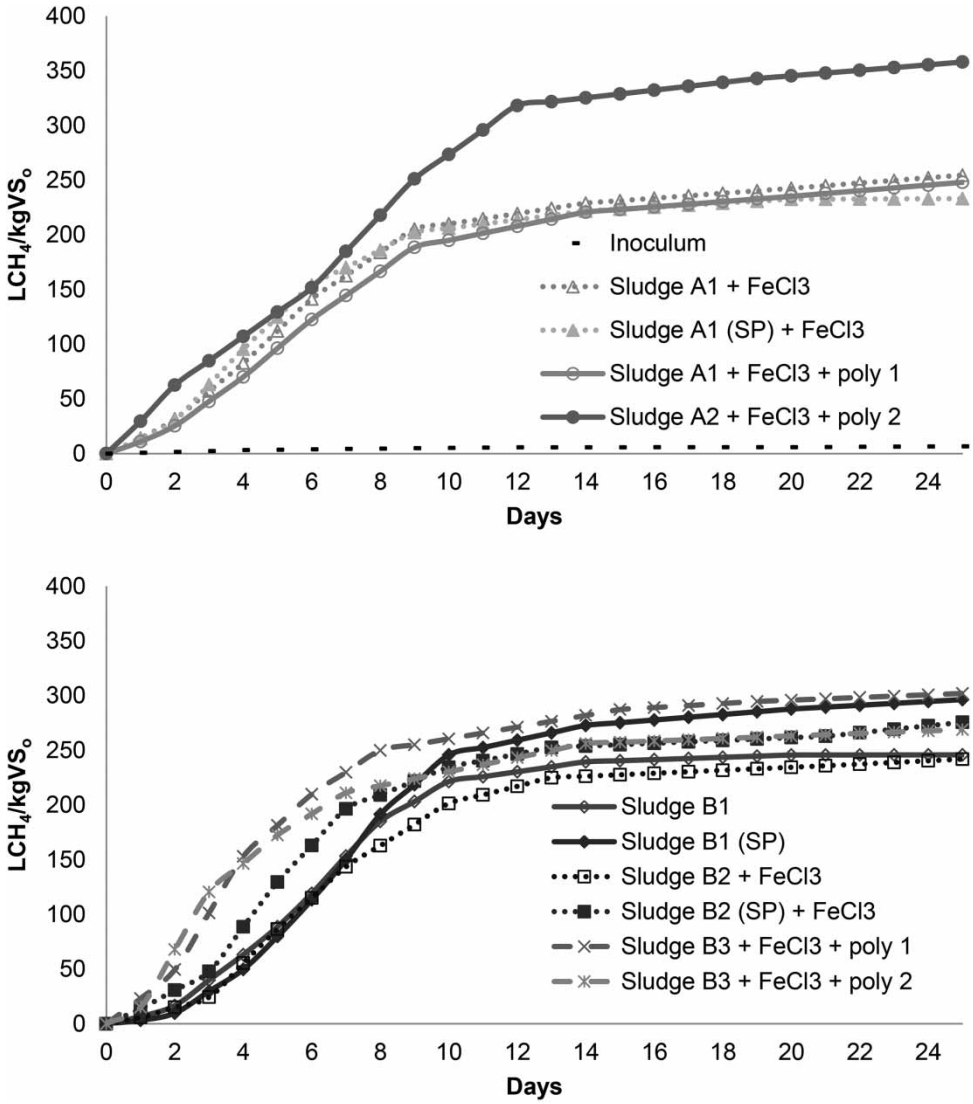

Figure 1 | Specific methane production values refer to initial volatile solids.

These effects can be observed in Figures 1 and 2 and Table 4. It is worth noting that, with the exception of the combination of polyelectrolyte 1 with $FeCl_3$ added to sludge A, an acceleration in CH_4 production was observed, even leading to the avoidance of the lag period in sludge B.

Along these same lines, Chu *et al.* (2003) reported that the addition of cationic polyelectrolytes in similar amounts to those added in this study accelerated CH_4 production in the initial stages. The upgrade in CH_4 production by adding coagulants and flocculants was somewhat surprising, as they produce super-structures which, *a priori*, are less available to bacteria. In other words, the contact between substrate and bacteria/enzymes would be hindered in an initial stage. On the other hand, the formation of flocs may enable nutrients to come into contact with bacteria/enzymes. Thus, additives would enhance the 'transport' of nutrients to bacteria. In fact, El-Mamouni *et al.* (1998) observed that flocculants added to a UASB favoured granulation, with the known benefits for biogas production (Hulshoff Pol *et al.* 2004).

Effect of IPF on the methane potential of sludge

When applying IPF to sludge B, the SP thus obtained increased the solids concentration by a factor of 1.9. Although the solids degradation achieved in the SP was lower than in the untreated sludge, the CH_4 conversion yield was significantly higher, increasing from 496 to 804 $LCH_4/kgVS_{rem}$. Note that this

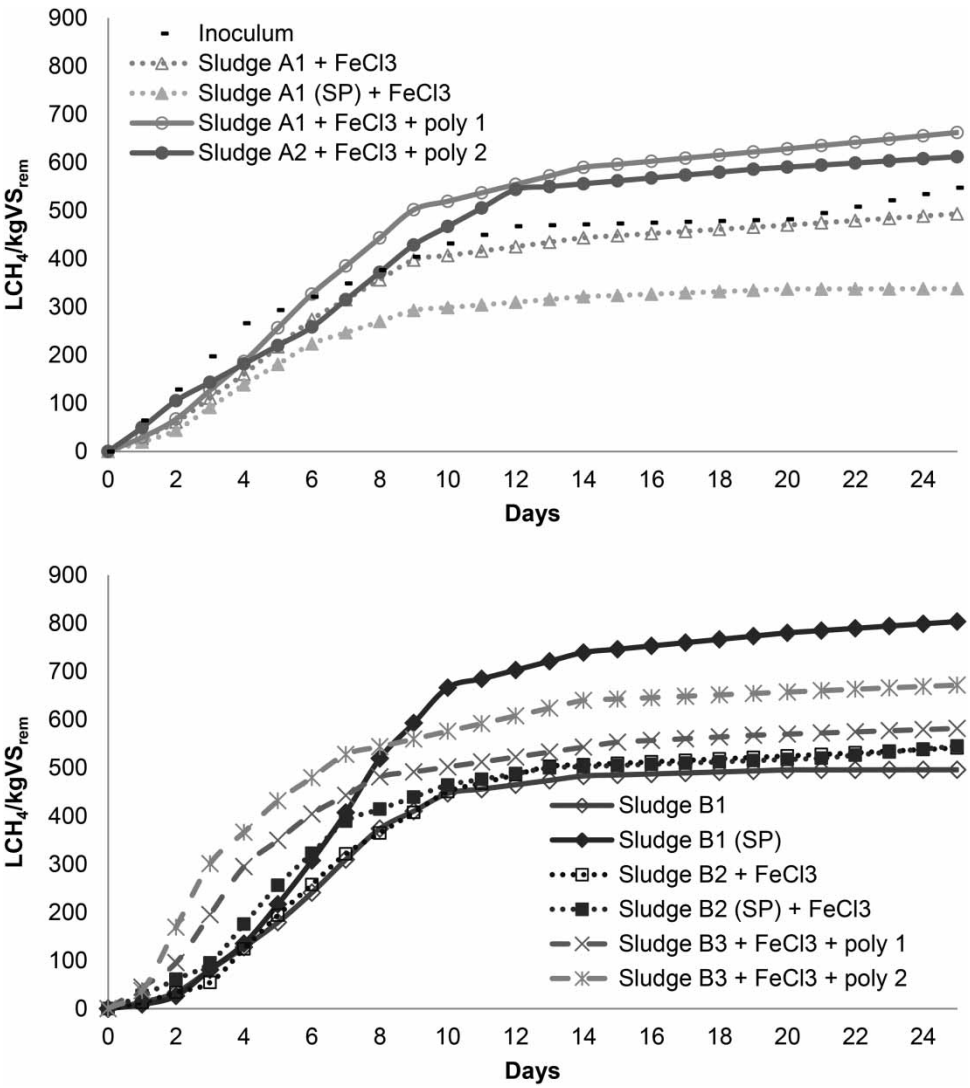

Figure 2 | Specific methane production values refer to removed volatile solids.

result considered only the SP as the substrate, although a liquor, rich in volatile fatty acids, is also obtained after IPF. Therefore, the enzymically hydrolyzed sludge would produce an even better methane yield than sludge. This research forms part of an on-going study.

Although the addition of $FeCl_3$ led to higher degradations in solids, the specific methane potential decreased, though not significantly, from 296 to 276 $LCH_4/kgVS_o$ in sludge B. A significant decrease was found with respect to the CH_4 conversion yield, from 804 to 505 $LCH_4/kgVS_{rem}$.

In contrast with the experiments carried out with sludge, in which the addition of $FeCl_3$ led to an increase in methane production per VS removed, no common pattern was observed with the SP. In fact, when applying IPF to sludge B, the SP achieved the highest result (804 $LCH_4/kgVS_{rem}$). When IPF was performed with the coagulant, this experiment achieved a significantly lower upgrade (545 $LCH_4/kgVS_{rem}$), somewhat similar to the enhancement achieved by the non-hydrolyzed sludge with an added coagulant (541 $LCH_4/kgVS_{rem}$). For sludge A, IPF yielded the poorest methane conversion in all experiments (338 $LCH_4/kgVS_{rem}$). These dissimilarities between the two types of sludge could probably be explained by the different working conditions at the WWTPs.

CONCLUSIONS

The addition of $FeCl_3$ had no significant effect on specific methane production, decreasing from 246 to 242 $LCH_4/kgVS_o$ in the sludge with a higher concentration of $FeCl_3$ (5.6 g/L). The combination of Chemifloc CH80 with $FeCl_3$ achieved a significant increase of 25% when working with sludge B. The combination of Chemifloc CH50 with $FeCl_3$ led to a significant increase of 40% in sludge A. When these results are compared with those in the literature, the reason for the absence of worsening observed in our experiments might lie in the absence of stirring of the reactor that might favour granulation with these additives, thereby overcoming the deleterious effects (e.g. the limiting phosphorus bioavailability) reported elsewhere.

When applying IPF as a pre-treatment, a 20% increase was observed in sludge B without $FeCl_3$. Although this increase was not statistically significant, these experiments only considered the SP, so the contribution from the liquor is expected to improve the yield. Cationic polyelectrolytes inhibited IPF.

First-order modelling was carried out on the methane production from all substrates. A 2-d lag period was observed for sludge B with $FeCl_3$, and for the IPF of this sludge, regardless of the presence of $FeCl_3$. The kinetic constant was always higher when employing IPF as a pre-treatment, although no common pattern was observed for this constant with respect to the additives employed in the study.

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REFERENCES

Angelidaki, I. & Sanders, W. 2004 Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science and Bio/Technology* **3**, 117–129.

APHA, AWWA & WEF 1998 *Standard Methods for the Examination of Water and Wastewater*. 20th edn, Washington, DC, USA.

Appels, L., Baeyens, J., Degrève, J. & Dewil, R. 2008 Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science* **34**, 755–781.

Boczar, B. A., Begley, W. M. & Larson, R. J. 1992 Characterization of enzyme activity in activated sludge using rapid analyses for specific hydrolases. *Water Environment Research* **64** (6), 792–797.

Burgess, J. E. & Pletschke, J. 2008 Hydrolytic enzymes in sewage sludge treatment: a mini-review. *Water SA* **34** (3), 343–349.

Cadoret, A., Conrad, A. & Block, J. C. 2002 Availability of low and high molecular weight substrates to extracellular enzymes in whole and dispersed activated sludges. *Enzyme and Microbial Technology* **31**, 179–186.

Carrère, H., Bougrier, C., Castets, D. & Delgenès, J. P. 2008 Impact of initial biodegradability on sludge anaerobic digestion enhancement by thermal pretreatment. *Journal of Environmental Science and Health Part A* **43**, 1551–1555.

Chen, Y., Cheng, J. J. & Creamer, K. S. 2008 Inhibition of anaerobic digestion process: a review. *Bioresource Technology* **99**, 4044–4464.

Chu, C. P., Lee, D. J., Chang, B. V., You, C. H., Liao, C. S. & Tay, J. H. 2003 Anaerobic digestion of polyelectrolyte flocculated waste activated sludge. *Chemosphere* **53**, 757–764.

Degrémont 1979 *Manual Técnico del Agua*. 4a edición, Bilbao, España.

Dentel, S. K. & Gosset, J. M. 1982 Effect of chemical coagulation on anaerobic digestibility of organic materials. *Water Research* **16**, 707–718.

El-Mamouni, R., Leduc, R. & Guiot, S. R. 1998 Influence of synthetic and natural polymers on the anaerobic granulation process. *Water Science and Technology* **38** (8–9), 341–347.

Erden, G. & Filibeli, A. 2010 Ultrasonic pre-treatment of biological sludge: consequences for disintegration, anaerobic biodegradability, and filterability. *Journal of Chemical Technology and Biotechnology* **85**, 145–150.

Ferreiro, N. & Soto, M. 2003 Anaerobic hydrolysis of primary sludge: influence of sludge concentration and temperature. *Water Science and Technology* **47** (12), 239–246.

Gendebien, A., David, B., Hobson, J., Palfrey, R., Pitchers, R., Rumsby, P., Carlton-Smith, R. & Middelton, J. 2010 Environmental, Economical and Social Impacts of the Use of Sewage Sludge on Land. Final report, Part III: Project Interim Reports, European Commission, Brussels.

Goel, R., Mino, T., Satoh, H. & Matsuo, T. 1998 Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. *Water Research* **32**, 2081–2088.

Hulshoff Pol, L. W., de Castro Lopez, S. I., Lettinga, G. & Lens, P. N. L. 2004 Anaerobic sludge granulation. *Water Research* **38**, 1376–1389.

Johnson, D. K., Carliell-Marquet, C. M. & Forster, C. F. 2003 An examination of the treatment of iron-dosed waste activated sludge by anaerobic digestion. *Environmental Technology* **24**, 937–945.

Le, M. S., Hu, R., Tyrrel, S. & Cartmell, E. 2008 Inverted Phase Fermentation for Digestion Intensification. *AquaEnviro 13th European Biosolids and Organic Resources Conference*, Manchester, UK.

Lee, H. & Shoda, M. 2008 Stimulation of anaerobic digestion of thickened sewage sludge by iron-rich sludge produced by the Fenton method. *Journal of Bioscience and Bioengineering* **106**, 107–110.

Liu, Y. & Fang, H. H. P. 2003 Influences of extracellular polymeric substances (EPS) on flocculation, settling and dewatering of activated sludge. *Critical Reviews in Environmental Science and Technology* **33** (3), 237–273.

Luste, S., Luostarinen, S. & Sillanpää, M. 2009 Effect of pre-treatments on hydrolysis and methane production potentials of by-products from meat-processing industry. *Journal of Hazardous Materials* **164**, 247–255.

Marañón, E., Castrillón, L., Quiroga, G., Fernández-Nava, Y., Gómez, L. & García, M. M. 2012 Co-digestion of cattle manure with food waste and sludge to increase biogas production. *Waste Management* **32**, 1821–1825.

Mikkelsen, L. H. & Keiding, K. 2002 Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. *Water Research* **36**, 2451–2462.

Mottet, A., François, E., Latrille, E., Steyer, J. P., Déléris, S., Vedrenne, F. & Carrère, H. 2010 Estimating anaerobic biodegradability indicators for waste activated sludge. *Chemical Engineering Journal* **160**, 488–496.

Murray, A., Horvath, A. & Nelson, K. L. 2008 Hybrid life-cycle environmental and cost inventory of sewage sludge treatment and end-use scenarios: a case study from China. *Environmental Science and Technology* **42**, 3163–3169.

Negral, L., Marañón, E., Fernández-Nava, Y. & Castrillón, L. 2013 Short term evolution of soluble COD and ammonium in pre-treated sewage sludge by ultrasound and inverted phase fermentation. *Chemical Engineering and Processing: Process Intensification* **69**, 44–51.

Nielsen, P. H. & Keiding, K. 1998 Disintegration of activated sludge flocs in presence of sulphide. *Water Research* **32**, 313–320.

Novak, J. T. & Park, C. M. 2010 The effect of iron and aluminium for phosphorus removal on anaerobic digestion and organic sulphur generation. *Water Science and Technology* **62** (2), 419–426.

Siegrist, H., Renggli, D. & Gujer, W. 1993 Mathematical modelling of anaerobic mesophilic sewage sludge treatment. *Water Science and Technology* **27** (2), 25–36.

Skiadas, I. V., Gavala, H. N., Lu, J. & Ahring, B. K. 2005 Thermal pre-treatment of primary and secondary sludge at 70 °C prior to anaerobic digestion. *Water Science and Technology* **52** (1–2), 161–166.

Smith, J. A. & Carliell-Marquet, C. A. 2008 The digestibility of iron-dosed activated sludge. *Bioresource Technology* **99**, 8585–8592.

Tchobanoglous, G., Burton, F. L. & Stensel, H. D. 2003 *Wastewater Engineering: Treatment and Reuse*. 4th edn, McGraw-Hill, New York, USA.

Thistleton, J., Clark, T., Pearce, P. & Parsons, S. 2001 Mechanisms of chemical phosphorus removal 1 – iron(II) salts. *Transactions of the Institution of Chemical Engineers* **79** (B), 339–344.

Ucisik, A. S. & Henze, M. 2008 Biological hydrolysis and acidification of sludge under anaerobic conditions: the effect of sludge and origin on the production and composition of volatile fatty acids. *Water Research* **42**, 3729–3738.

Vlyssides, A., Barampouti, E. M. & Mai, S. 2009 Influence of ferrous iron on the granularity of a UASB reactor. *Chemical Engineering Journal* **146**, 49–56.

Wan, C., Zhou, Q., Fu, G. & Li, Y. 2011 Semi-continuous anaerobic co-digestion of thickened waste activated sludge and fat, oil and grease. *Waste Management* **31**, 1752–1758.

Wawrzynczyk, J., Recktenwald, M., Norrlöw, O. & Dey, E. S. 2008 The function of cation-binding agents in the enzymatic treatment of municipal sludge. *Water Research* **42**, 1555–1562.

Yang, Q., Luo, K., Li, X. M., Wang, D. B., Zheng, W., Zeng, G. M. & Liu, J. J. 2010 Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes. *Bioresource Technology* **101**, 2924–2930.

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