Chemical Engineering Journal 160 (2010) 488–496

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Estimating anaerobic biodegradability indicators for waste activated sludge

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ARTICLE INFO

Article history:
Received 22 September 2009
Received in revised form 25 March 2010
Accepted 26 March 2010

Keywords:
Anaerobic digestion
Biodegradability indicators
Characterisation
Waste activated sludge
Methane

ABSTRACT

The aim of this study was to show the link between the initial characteristics of waste activated sludge (WAS) samples and their thermophilic anaerobic biodegradabilities, as determined by biochemical methane potential (BMP) tests, in order to develop relevant prediction indicators. Macroscopic parameters, biochemical composition and a fractionation of total solids by the Van Soest method were carried out on WAS samples which were taken from the inlet and outlet of full-scale sludge anaerobic digesters. Biodegradability was expressed as a function of WAS characteristics by the partial least square (PLS) regression technique. Among several PLS models, the most appropriated model was based on biochemical characterisation (carbohydrates, lipids and proteins) and two macroscopic parameters (soluble organic carbon and the ratio of chemical oxygen demand to total organic carbon). The biodegradability indicators developed in this study permitted the prediction of the methane production from WAS samples.

1. Introduction

The European Union seeks to increase the share of energy from renewable sources in the Union total energy consumption from the 2005 level of 8.5% to 20% in 2020 [1]. This ambitious objective will contribute both to the worldwide efforts to deal with climate change and towards better control by the EU of its dependency on energy from outside.

In this context, the anaerobic digestion of sewage sludge will therefore contribute to achieving the aim of the European directive. Indeed, the amount of sewage sludge is growing with the increase in the volume of the treated wastewater and the management of sewage sludge has thus become an environmental and economic issue. Thanks to anaerobic digestion, sewage sludge can be profitably used as a renewable energy resource because the organic matter it contains is converted into biogas made up of 60–70% of methane (CH_4) [2], which can be transformed into heat and/or electricity or biofuel. In 2006, the annual biogas production from sewage sludge in Europe was 949.5 kt of oil equivalent [3]. This potential is very significant since it represents 18% of the Europe's total biogas production. Thus, the anaerobic conversion of sewage sludge should become an essential process in the modern wastewater treatment plant.

Research on the optimisation of anaerobic digestion operating conditions has shown that several parameters clearly impact on the biological conversion rates. The anaerobic biomass is very sensitive to pH and each population has an optimal range of pH [4]. The temperature has a big effect on both the microbial growth rates [5] and diversity [6]. Mixing strategy and intensity significantly affect performances and thus the production of methane [7]. The retention time also impacts on the process performance and biomass growth [8]. Consequently, several strategies can be applied to improve anaerobic conversion: thermophilic digestion (55 °C) to increase degradation rates and methane production [9]; increasing sludge retention time [2]; introducing a pretreatment step such as thermal pretreatment [10], sonication [11], enzymatic hydrolysis [12] or chemical pretreatment [13] to make the organic matter more readily available by the anaerobic biomass.

However, the mechanisms of anaerobic digestion are not well understood. In the case of sewage sludge, the process performances are limited with a mean conversion of organic matter from 30% to 50% [14]. Moreover, methane production depends on the sludge type. Indeed, the biodegradability of waste activated sludge (WAS) is lower than that of primary sludge [15]. The WAS matrix is more complex because of its biological origin and lower availability to the anaerobic biomass [16–18]. WAS biodegradability may be affected by operating conditions of the aerated tank in the wastewater treatment line [19]. Sludge originating from an extended aeration process is less biodegradable than sludge from a high-load process [20]. Moreover, Ekama et al. [19] showed that unbiodegradable organics of WAS, as determined from aerobic conditions, remain unbiodegradable under anaerobic digestion conditions. The anaerobic biodegradability of sludge has thus been shown to be linked

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doi:10.1016/j.cej.2010.03.059

to its aerobic biodegradability which is generally assessed by biological oxygen demand (BOD).

In the field of wastewater treatment, the COD/TOC ratio indicates the complexity of substrates [21]. This ratio can be used as a degradation indicator because it represents the availability of organic carbon as an energy resource [22]. A low COD/TOC ratio indicates a more oxidised state of organic carbon which is less available as an energy resource. Thus, WAS samples with a high COD/TOC ratio can be assumed to have a higher biodegradability.

However, few studies have focussed on the accurate characterisation of the organic matter in input sludge and the relation between this organic matter and the anaerobic conversion performance. Recently, some work has concluded that the organic matter composition in the substrate has a strong impact on anaerobic digestion performance, in the same way as do the operating conditions [23]. Indeed, the co-digestion of municipal sludge with fresh and readily available organic matter, such as organic waste from domestic refuse, can improve biogas production by 80% in comparison to a digester fed with a mixture of municipal sludge [24]. Neves et al. [15] studied the anaerobic digestion of restaurant waste and showed that the highest methane production rate and the lowest volatile fatty acid (VFA) accumulation were obtained in the case of a waste with a high carbohydrates content. In the same way, the C/N ratio can influence VFA production and the distribution patterns of individual VFAs. An optimal carbon-to-nitrogen ratio (C/N) was beneficial for the acidogenesis biomass activity and improved the conversion of organic matter into VFAs [25]. Bengtsson et al. [26] concluded that the differences observed among four wastewaters regarding the degree of acidification, and the composition of the resulting VFAs are most likely due to differences in the composition of organic matter in the wastewaters. Demirel and Yenigun [8] showed that substrate composition impacts on the microbial diversity and the conversion pathways in the digester. The composition and the availability of organic fractions present in a substrate are the key factors that determine methane production.

In a recent work, Schievano et al. [27] created regression models between biogas potential and certain chemical and biological parameters of both feed and digested mixtures of energy crops, pig manure and the organic fraction of municipal solid waste. They found a linear regression between the four most significant variables, in particular volatile solids content (VS), oxygen demand during 20 h of respirometry assay (OD20), total organic carbon (TOC) and soluble fraction (CS according to Van Soest fractionation). These parameters provided a good representation of both the content and the quality of the organic matter in the matrices and made it possible to predict biogas potential with a mean error of 16%.

On the topic of sewage sludge mineralisation in soil, several studies have tried to establish a relationship between the characteristics of organic matter of the sludges (chemical and biochemical composition) and their decomposition and C and N mineralisation in the soil. By statistical analysis, Hattori and Mukai [28], Gilmour and Skinner [29], Parnaudeau et al. [30] found that the N mineralisation is generally related to the C/N ratio and the N concentration of organic residues. Moreover, the decomposition rates of sludge in soil are negatively linked to the lignin-“like” fraction from Van Soest characterisation.

However, the relationship between the initial organic composition and the anaerobic digestion performance has not been studied for sludges. Thus, it is of great interest to carry out such work in order to define the relevant initial indicators for predicting anaerobic digestion performance. Such research necessarily involves studying the substrate composition with great accuracy by first defining a methodology for its characterisation. This will result in a topology of the sewage sludge and thus better knowledge of the input sludges quality.

The aim of this study was to propose different methods for characterising the solid matter in the WAS, before and after full-scale anaerobic digestion. These methods have enabled us to determine the major constituents of the organic matter contained in the WAS and their removal during full-scale anaerobic digestion. At a later stage in the research, in order to find prediction indicators, the initial sludge characterisation was linked to sludges thermophilic anaerobic biodegradability as determined by biochemical methane potential (BMP) tests.

2. Materials and methods

2.1. Waste activated sludge (WAS) samples

WAS samples were taken from various full-scale sludge anaerobic digesters which were selected in function of the load type at the wastewater treatment plant and the temperature range of its anaerobic digester. One wastewater treatment plant (F) was equipped for the Biothelys® process which involves a thermal hydrolysis at 150–180 °C by steam injection at 8–10 bar with a retention time of 30–60 min, applied as a pretreatment for anaerobic digestion. Table 1 presents the wastewater treatment plant's main characteristics. The samples were collected from the influent and effluent sludge of reactors. Three samples were collected at plant F: before and after thermal hydrolysis (F pretreatment input and F pretreatment output) and at the output of anaerobic digestion (F output). It must be noted that “F pretreatment output” corresponds to “F input” of the digester.

2.2. Methodology for the characterisation of sludge samples

The chemical characterisation was performed on influent (input) and effluent (output) sludge samples. The potential methane was evaluated for the influent sludge samples in order to link the sludge samples initial composition with their anaerobic biodegradability.

All the analyses were performed in triplicate according to standard methods (APHA, 1997). The particulate fraction was separated by centrifugation at 50 000 × g for 15 min at 5 °C. The supernatant was then filtered through a cellulose acetate membrane with a pore size of 0.45 μm to obtain the soluble fraction. For Van Soest fractionation and the determination of lipids, samples were freeze-dried and sieved with a 1 mm mesh.

Analyses were carried out to obtain three types of characterisation: macroscopic characterisation which comprises methods generally used in sludge or waste management, biochemical characterisation and Van Soest fractionation.

2.2.1. Macroscopic characterisation

Organic solids were assimilated to volatile solids. Total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were measured for the total and particulate fractions.

Chemical oxygen demand (COD) was measured for the total and soluble fractions. The particulate fraction COD was determined by the difference in COD values between the total and soluble fractions.

Organic carbon (OC) in the total and soluble fractions was analysed with a Carbon TOC-V module (Shimadzu).

2.2.2. Biochemical characterisation

The biochemical analysis was done to identify the volatile solids composition. The biochemical compounds (i.e. proteins, carbohydrates, lipids and VFA) were analysed. The proteins and carbohydrates were measured, respectively, by Lowry's and the

Table 1
Characteristics of wastewater treatment plants.

WWTP	A	B	C	D	E	F[a]
Size of the plant (PE)	5 500 000	260 000	250 000	470 000	160 000	62 000
Wastewater	Urban	Urban and industrial (70/30)	Urban	Urban	Urban	Urban
Aerobic process	High load	High load	Very high load	Very high load	Extended aeration	Extended aeration
Sludge age (days)	2	1.5	0.36	0.6	11	21
Anaerobic process	Mesophilic	Mesophilic	Thermophilic	Thermophilic	Thermo/mesophilic	Mesophilic
Sludge retention time (days)	21	27	14	16	2/13	15

[a] Wastewater treatment plant F: Biothelys® process as pretreatment of anaerobic digestion (thermal hydrolysis at 165 °C).

anthrone methods [31,32]. Concentrations are expressed in equivalent bovine serum albumin (BSA) and in equivalent glucose (Glu). The lipids were measured by accelerated solvent extraction (ASE) (ASE 200, Dionex), using petroleum ether. VFA concentrations were measured in the soluble fraction by gas chromatography (GC 800, Fisons Instruments).

2.2.3. Van Soest fractionation

The Van Soest method [33] enables the organic matter of sludge samples to be fractionated with sequential extraction by neutral and acid detergents, followed by strong acid extraction. This method is usually used for materials of plant origin and fractionates the organic matter into soluble components, hemicellulose, cellulose and lignin. In the case of WAS, these fractions will be designed as fractions "like".

2.2.4. Anaerobic biodegradability measurement

The methane potential of the various WAS samples was determined by adapting the biochemical methane potential (BMP) test originally proposed by Owens and Chynoweth [34] and Angelidaki and Sanders [35].

The batch anaerobic tests were carried out in triplicate in plasma bottles of 500 mL at a thermophilic temperature (55 °C). The inoculum was taken from a full-scale industrial plant. It was thus adapted to treat WAS. The plasma bottles were inoculated at a load of 0.5 g_{COD} of substrate per g_{VS} of anaerobic inoculum with a growth medium containing nutrients and trace elements. Experiment duration was 24 days. A blank test and a test with a fully-biodegradable compound (ethanol) were carried out to determine endogenous activity and inoculum activity, respectively.

The biogas production was measured periodically by a movement of liquid (distillated water, pH = 2). Biogas composition was determined by gas chromatography.

Methane specific production was calculated from the volume of methane produced divided by the quantity of sludge sample COD introduced. It was expressed in $mL_{CH4}\, g_{CODintro}{}^{-1}$.

Biodegradability (BD) was determined by dividing the methane specific production by the theoretical maximum methane yield of 350 $mL_{CH4}\, g_{COD}{}^{-1}$ in standard conditions of temperature and pressure (0 °C, 1013 hPa) [36]:

$$\text{Biodegradability}\ (\%) = \frac{\text{BMP}}{350\, \text{mL}_{\text{CH}_4}\, \text{g}_{\text{COD}}^{-1}} \times 100$$

2.3. Partial least square analyses

The partial least square (PLS) regression technique is based on the construction of PLS factors (also called principal components) by minimising the covariance between the dependent variables (Y block) and the explicative variables (X block). Then, the prediction of the Y block was calculated with a multivariable linear regression on X block through PLS1 models using the R version R 1.2.2 software for Windows along with PLS functions developed by Durand et al. [37]. The algorithm constructs orthogonal PLS factors in each block by minimising the covariance between the X and Y blocks. The first PLS factor contains the highest percentage of variance, and the following factors account for decreasing amounts of variance. The number of PLS factors (also called dimensions, dim) of the model was determined by minimising the mean squared prediction error (PRESS) through a leave-out cross-validation procedure. The prediction quality was evaluated by two methods: cross-validation and model validation from external data.

3. Results and discussion

3.1. Characterisation of organic matter

3.1.1. Input sludge composition

The distribution of macroscopic parameters and biochemical components in sludge samples is given in Tables 2 and 3, respectively. The total solids were mainly particulates, from 85.3$\%_{TS}$ to 97.5$\%_{TS}$. The volatile solid content in the input sludge ranged from 66$\%_{TS}$ to 81$\%_{TS}$ and was mainly composed of proteins between 0.34 and 0.47 $g_{BSA}\, g_{VS}{}^{-1}$. The carbohydrate and lipid content in the input sludge samples were around 0.12–0.30 $g_{Glu}\, g_{VS}{}^{-1}$ and 0.00–0.03 g $g_{VS}{}^{-1}$, respectively. Proteins are the main compounds of WAS because they originate from microbiological activity during wastewater treatment. Indeed, WAS is mainly composed of extracellular polymeric substances produced by microbial metabolism, cells and organic matter not degraded under aerobic condition and remaining in the sludge matrix [17].

The sum of proteins, carbohydrates, lipids and VFA concentrations represented around 76 ± 10% of organic matter in the input sludge and around 77 ± 16% in the output sludge. Thus, a part of the constituents was not measured by the applied biochemical characterisation. This could be due to measurement errors. Moreover, not all the biochemical constituents making up the organic matter were quantified: for example humic substances or nucleic acids. In addition, the concentrations of biochemical constituents have to be considered as indicators rather than real concentrations, because colorimetric methods were calibrated using referent compounds (bovine serum albumin and glucose). Finally, the structure of the sludge floc, which presents a high degree of cohesion [38] with several interactions [39] may render some compounds unavailable for chemical dosage. In particular, the sludge from an extended aeration process can show greater cohesion and stabilisation of the organic matter in the matrix and so its structure did not permit the exhaustive quantification of the biochemical compounds analysed [40]. Indeed, in the case of WAS from an extended aeration process (sludge E and F), the sum of proteins, carbohydrates, lipids and VFAs represented only 66% and 62% of the organic matter, respectively, as against 76% in average.

The ratio COD/TOC of WAS ranged from 2.58 to 4.98. It should be noted that thermal pretreatment led to the increase of this ratio.

The Van Soest method was used to define another fractionation of sludge solid matter. This method permits the determination of four fractions: soluble in neutral detergent, hemicellulose-"like", cellulose-"like" and lignin-"like". It may be assumed that these fractions have different biodegradability. Using this type of

Table 2
Macroscopic parameters of sludge samples.

Sludge	TS ($g\,L^{-1}$)	TSS ($\%_{TS}$)	VS ($\%_{TS}$)	COD/TOC ($g_{COD}\,g_C{}^{-1}$)	Sol OC ($g\,g_{VSsoluble}{}^{-1}$)
A input	38.0 ± 0.2	90 ± 1	76.1 ± 0.7	3.13 ± 0.30	0.23
A output	23.6 ± 1.3	53 ± 5	43.6 ± 5.0	6.06 ± 0.73	0.03
B input	59.7 ± 0.4	91 ± 1	72.0 ± 1.3	3.24 ± 0.19	0.55
B output	35.0 ± 0.1	97 ± 3	54.0 ± 0.7	3.50 ± 0.01	0.21
C input	51.0 ± 0.1	85 ± 1	73.5 ± 0.3	2.58 ± 0.14	0.22
C output	28.4 ± 0.2	90 ± 1	64.0 ± 0.8	4.15 ± 0.12	0.39
D input	29.9 ± 0.6	93 ± 3	81.0 ± 2.7	4.98 ± 0.12	0.46
D output	27.0 ± 0.4	88 ± 3	56.0 ± 2.3	3.18 ± 0.12	0.38
E input	31.6 ± 0.2	94 ± 2	75.3 ± 0.8	3.04 ± 0.19	0.18
E output	26.9 ± 0.1	90 ± 1	66.0 ± 1.0	3.32 ± 0.18	0.29
F pretreatment input	177.5 ± 1.4	97 ± 2	66.0 ± 1.1	2.95 ± 0.09	0.37
F pretreatment output	143.5 ± 0.7	77 ± 1	66.0 ± 1.1	3.39 ± 0.15	0.8
F output	84.8 ± 0.3	89 ± 1	53.9 ± 0.4	3.00 ± 0.05	ND

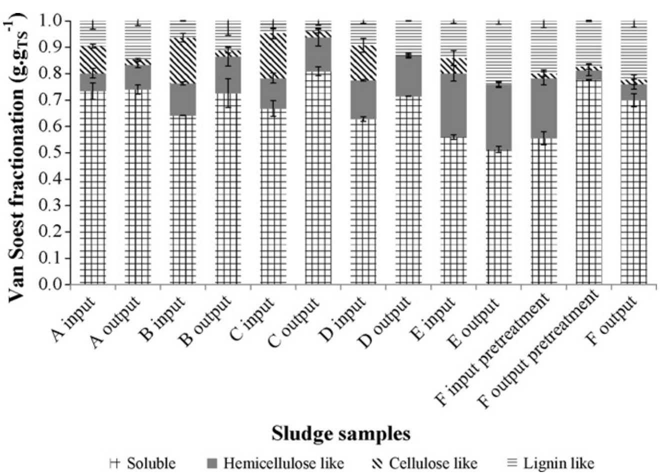


Fig. 1. Fractionation of sludge total solids by Van Soest method.

fractionation should make it possible to characterise the bioaccessibility of the matter, however, it provides little information on the actual nature of the matter.

Fig. 1 shows that the soluble fraction in neutral detergent was the largest fraction, representing around 63% of total solids. It was mainly composed of soluble matter, extracellular substances and inorganic matter which were soluble in the neutral detergent. The other fractions varied in a large range and represented around 37% of total solids.

3.1.2. *Output sludge and reduction of matter*

For the output sludge samples, results showed a reduction in total solids and the organic content decreased due to the mineralisation of matter during the digestion step. Proteins were the most important biochemical constituents of the organic matter, ranging from 0.43 to 0.63 $g_{BSA}\,g_{VS}{}^{-1}$, with a high value of 0.82 $g_{BSA}\,g_{VS}{}^{-1}$, due mainly to the anaerobic biomass present in the sludge effluent (Table 3). Indeed, the principle of anaerobic digestion is based on the growth of anaerobic biomass. Thus, outlet or digested sludge is composed of the recalcitrant matter in the waste activated sludge and of the anaerobic biomass, proteins being the major constituent of this latter.

Except for sludge D, COD/TOC ratios of digested sludge were higher than those for WAS before anaerobic digestion. This may be explained by the reduction (in the sense of oxidation/reduction) of organic matter during anaerobic digestion.

Having obtained the characterisation of input and output sludge, we were able to determine the mean removal of each measured component during the full-scale anaerobic digester process. Results are given in Table 4. Little removal was obtained for the proteins, some 34 ± 9% of reduction. This indicates that proteins can be considered as a poorly biodegradable compound. These results are in agreement with those of Miron et al. [41]. Because the proteins originate from the biological activity, they need a breakdown of the floc structure and of the membrane cells in order to be available to the anaerobic biomass. Thus, this phenomenon reduces the protein degradation. Moreover, a large concentration of proteins was measured in the effluent due to the biological activity during the anaerobic process, and this may also explain the low reduction of proteins. In contrast, a high reduction for sugars, lipids and VFAs was obtained with a mean reduction of 67 ± 12%, 66 ± 13% and 88 ± 11%, respectively. The carbohydrates present in the WAS are defined as polysaccharides that can contribute to forming a gel-matrix which acts as a cement for sludge floc [42]. These constituents form the first layer of the floc and are more readily available during anaerobic conversion. Thus, the type of constituent making up the sludge strongly influences its biodegradability, as already stated by Hartmann and Ahring [43].

Table 3
Biochemical composition of sludge samples.

Sludge	Proteins ($g_{BSA}\,g_{VS}{}^{-1}$)	Carbohydrates ($g_{Glu}\,g_{VS}{}^{-}$)	Lipids ($g\,g_{VS}{}^{-1}$)	VFAs ($g\,g_{VS}{}^{-1}$)	Sum of Prot, Carb, Lpd, VFAs ($g\,g_{VS}{}^{-1}$)
A input	0.47 ± 0.02	0.23 ± 0.01	0.06	0.06 ± 0.00	0.82 ± 0.03
A output	0.82 ± 0.13	0.20 ± 0.04	0.02	0.00 ± 0.00	1.04 ± 0.17
B input	0.34 ± 0.02	0.28 ± 0.01	0.07	0.08 ± 0.00	0.77 ± 0.03
B output	0.55 ± 0.02	0.12 ± 0.01	0.04	0.00 ± 0.00	0.71 ± 0.03
C input	0.40 ± 0.01	0.30 ± 0.01	0.09	0.10 ± 0.00	0.89 ± 0.02
C output	0.51 ± 0.04	0.19 ± 0.03	0.07	0.03 ± 0.00	0.80 ± 0.07
D input	0.45 ± 0.07	0.23 ± 0.02	0.06	0.04 ± 0.00	0.78 ± 0.09
D output	0.58 ± 0.03	0.13 ± 0.02	0.05	0.02 ± 0.00	0.78 ± 0.05
E input	0.47 ± 0.01	0.17 ± 0.03	0.00	0.02 ± 0.00	0.66 ± 0.04
E output	0.43 ± 0.06	0.12 ± 0.02	0.01	0.00 ± 0.00	0.56 ± 0.08
F input pretreatment	0.47 ± 0.02	0.12 ± 0.01	0.01	0.02 ± 0.00	0.62 ± 0.03
F output pretreatment	0.56 ± 0.03	0.10 ± 0.01	0.04	0.03 ± 0.00	0.73 ± 0.04
F output	0.63 ± 0.02	0.08 ± 0.02	0.04	0.01 ± 0.00	0.76 ± 0.04

Table 4
Mean reduction (%) of measured constituents between input and output of the full-scale anaerobic process.

Components	TS	VS	Proteins	Sugars	Lipids	VFAs	Soluble Van Soest	Hemicellulose like	Cellulose like	Lignin like
Digester A	38	64	38	70	87	100	62	53	87	53
Digester B	41	56	30	81	70	100	54	40	100	18
Digester C	44	52	39	70	63	88	49	48	88	34
Digester D	10	37	20	64	52	70	40	ND[a]	89	ND[a]
Digester E	15	25	30	44	ND[a]	86	48	19	93	ND[a]
Digester F	41	51	45	63	58	84	67	4	28	27
Mean reduction (%)	31	48	34	65	66	88	53	33	81	33
Standard deviation (%)	14	14	9	12	13	11	10	21	26	15

[a] ND: not determined.

Concerning the Van Soest fractionation, the results show that the soluble and cellulose-"like" fractions were the most biodegradable, with a reduction of 53 ± 10% and 8 ± 26%, respectively. On the other hand, the hemicellulose- and lignin-"like" fractions were less biodegradable, with a reduction of 33 ± 21% and 33 ± 15%, respectively. The high values of standard deviations were induced by errors in measurement due to the main successive extractions.

The Van Soest method is based on consecutive extractions by solvents with increasing extractive power. The extracted fractions thus represent decreasing accessibility. In this way, the biodegradability of the first fraction (soluble fraction) can be assumed to be higher than that of the second (hemicellulose-"like") fraction which may be higher than the third (cellulose-"like") fraction biodegradability, and so on. However, the results show that the most readily biodegradable fractions were soluble and cellulose-"like". This could confirm that biodegradability depends on the accessibility of the sludge constituents and on their chemical nature.

In the case of sludge F treatment, a thermal pretreatment step was included. This process enhances biogas production from anaerobic degradation while at the same time reducing the sludge treatment plant size and, thus, the investment costs [44]. The positive impact of this thermal pretreatment is shown by the increase in anaerobic biodegradability as determined by BMP tests, rising from 46 ± 2% to 55 ± 1% (Table 5). The applied heat broke down sludge floc cohesion and facilitated the solubilisation of organic matter. The organic matter thus became was more accessible to the anaerobic biomass, leading to better conversion of the matter into methane. Fig. 1 confirms this result. The applied heat solubilised the hemicellulose-"like" compounds into soluble constituents which were readily biodegradable. The other both fractions were not, or only slightly, impacted by the thermal pretreatment. This was also observed for manure samples [45]. The improvement in the biodegradability of the manure by thermal or thermo-alkali pretreatments was linked to a decrease of the hemicellulose-"like" fraction and an increase of the soluble in the neutral detergent fraction.

However, it was not possible to compare mean reductions obtained from each anaerobic process. In fact, the operating conditions of the full-scale anaerobic digesters were different and some abnormal performances were observed. Digesters D and E had low reduction coefficients which seemed to have been underestimated.

Table 5
Methane potential and biodegradability of input sludge samples.

Sludge	BMP ($mL_{CH4}\,g_{VS}^{-1}$)	BMP ($mL_{CH4}\,g_{COD}^{-1}$)	Biodegradability
A input	335 ± 7	190 ± 4	0.54 ± 0.01
B input	345 ± 12	230 ± 8	0.66 ± 0.02
C input	250 ± 16	183 ± 12	0.52 ± 0.04
D input	427 ± 10	263 ± 6	0.75 ± 0.02
E input	206 ± 14	123 ± 8	0.35 ± 0.02
F input pretreatment	268 ± 11	161 ± 6	0.46 ± 0.02
F output pretreatment	322 ± 7	193 ± 4	0.55 ± 0.01

These problems could be due to the sampling or variations in the operating conditions such as a decrease in the SRT before the sampling of input and output sludge samples. In conclusion of this part, a rigorous comparison of the biodegradability of the different sludge samples could not be carried out on the basis of measurements of the removal of sludge constituents. It was thus necessary to have recourse to a standard measurement of anaerobic degradability which was obtained by BMP tests.

3.2. Prediction of WAS anaerobic biodegradability

The BMP tests were carried out with input sludge samples as substrates, under thermophilic condition and over 24 days. The results are listed in Table 5 and show good repeatability in each case.

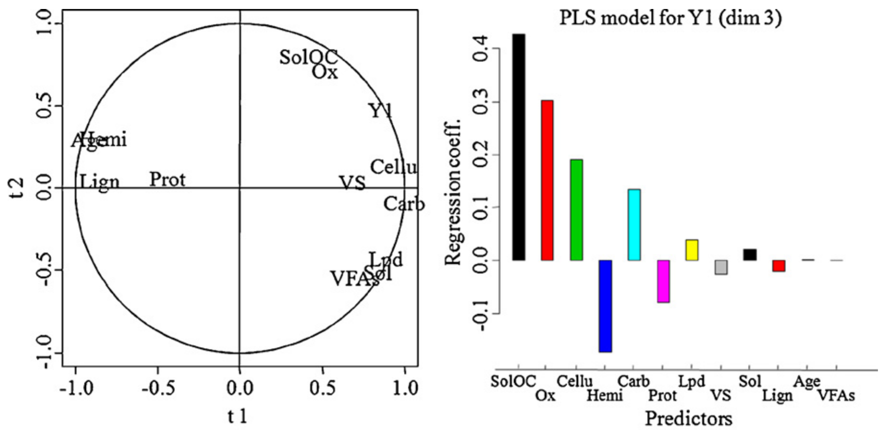
One of the objectives of this study was to find a method of characterisation enabling us to explain the different levels of biodegradability among WAS samples as well as the improvement in biodegradability by thermal pretreatment. The strategy used consisted in looking for relationships between sludge characterisation and anaerobic biodegradability using a regression method to develop a simple tool for prediction. Moreover, the model application needed to be suitable and fast in order to minimise time-consuming laboratory work. A PLS analysis was thus carried out to analyse the influence of sludge sample characterisation on the anaerobic biodegradability and to find pertinent biodegradability indicators. Six sludge samples were taken into account (samples A–F). Sludge sample F after thermal pretreatment was not taken into account for constructing the model but was used to validate it. The dependent variable (Y block) was thus the biodegradability of six sludge samples measured in triplicate (18 points).

3.2.1. PLS analysis with the main analysed parameters

The first PLS model was designed with all the analysed parameters, i.e. 12 explicative variables: the organic matter content (VS expressed in $g_{VS}\,g_{TS}^{-1}$), the soluble organic carbon (SolOC) (expressed in $g_C\,g_{vssoluble}^{-1}$), the biochemical fractions defined by proteins (Prot expressed in $g_{BSA}\,g_{VS}^{-1}$), carbohydrates (Carb expressed in $g_{Glu}\,g_{VS}^{-1}$), lipids (Lpd expressed in $g\,g_{VS}^{-1}$), VFAs (expressed in $g\,g_{VS}^{-1}$), the Van Soest fractions defined by soluble (Sol), hemicellulose (Hemi), cellulose (Cellu), lignin (Lign) (expressed in $g\,g_{TS}^{-1}$), the sludge age (age expressed in days) and the COD/TOC ratio (Ox).

Fig. 2 presents the correlation circle and the regression coefficients (centred variables) for the PLS model 1. The lowest PRESS (0.22) was obtained for a model dimension equal to 3.

The correlation circle (Fig. 2) shows a correlation between the hemicellulose-"like" and lignin-"like" fractions from the Van Soest method and the sludge age. According to Metcalf and Eddy [46] sludge is a reflection of the characteristics of input wastewater and of the operating conditions of the aerobic process. Thus, the retention time in the aerobic process (or sludge age) can have a major impact on the composition of the organic matter and on



Model equation: PLS model 1

$$BD = 0.311 - 0.001VS - 0.219Prot + 0.283Carb + 0.181Lpd + 0.051Ox + 0.396SolOC$$
$$+ 2.10^{-5}Age + 0.054Sol - 0.352Hemi + 0.434Cellu - 0.060Lign - 0.003VFAs$$

Fig. 2. Correlation circle and regression coefficient representation for the model of dimension 3 with all 12 parameters.

sludge floc cohesion. This result was confirmed by the characterisation of input sludge samples. Indeed, the Van Soest method (Fig. 1) showed that sludge samples E and F (F input pretreatment) from an extended aeration process contained more lignin-, cellulose- and hemicellulose-"like" fractions than other sludge samples. The soluble fraction was also smaller, representing only 56% of the solid matter as against some 67% for the sludge with a high load. Moreover, the matter identified by biochemical characterisation (proteins, carbohydrates, lipids, VFA) represented a fraction of the organic matter that was smaller than in the case of sludges with a high load (Table 3). Thus, the matter in sludge samples originating from an extended aeration process was more embedded in the floc matrix and thus more difficult to extract. Proteins and carbohydrates in a lower extend were less accessible for their quantification.

The purpose of the model was to predict sludge biodegradability with a limited number of parameters. Consequently, a second PLS model was applied based on only the most relevant parameters obtained in the previous model: SolOC, Ox, Cellu, Hemi and Carb. Fig. 3 presents the regression coefficients (centred variables) for a model dimension equal to 2. The mean squared prediction error (PRESS) of PLS model 2 was better than the PLS model 1, with a value of 0.03 compared to 0.22. A lower prediction quality could have been expected by decreasing the number of explicative variables. However, a few parameters used in the first PLS analysis were not relevant and decreased the prediction quality. This may have been due to the VS and VFA parameters which were found to have a negative though not very significant impact, whereas a large fraction of organic matter and a high VFA concentration are generally assumed to have a positive impact on biodegradability.

For PLS models 1 and 2, the most influential parameters are the soluble organic carbon concentration and the COD/TOC ratio. The soluble organic carbon concentration, which represents the organic matter readily available to the anaerobic biomass, appears in the correlation with a positive coefficient. Thus, the biodegradability will be higher for sludge that contains a large fraction of soluble matter. Moreover, this has already been observed by Climent et al. [47], Carrère et al. [20] and Ferrer et al. [48] who showed that the solubilisation of sludge improves its biodegradability. The COD/TOC ratio (Ox) has a positive coefficient in the linear correlations. This is in agreement with the definition of this parameter. A high COD/TOC ratio indicates a lower oxidised state of organic carbon which is more readily available as an energy resource.

We tested a simplified regression (PLS model 3) with both these parameters. As expected, the PRESS was higher and equal to 0.42 for a model dimension of 1. Thus, these two macroscopic parameters are important but not sufficient alone to predict sludge biodegradability. Other important parameters are the cellulose-"like" fraction and carbohydrate concentration with positive coefficients and the hemicellulose-"like" fraction with a negative coefficient. However, these two parameters belong to two different characterisation methods which were used to identify the quantity and the quality of organic matter: Van Soest fractionation and biochemical analysis. However, the model application has to be quickly applied and pertinent in order to minimise demanding and time-consuming laboratory work. Thus, it is necessary to determine the most relevant analytic methods for predicting methane potential.

Further PLS models were applied based on two parameter groups: the first group was constituted of characterisation obtained by the Van Soest method and the second grouped the data on biochemical composition. The most influential parameters which belong to macroscopic analysis (SolOC and Ox) were retained in both models. Thus, six variables in the first case (PLS model 4) and five variables in the second case (PLS model 5) were used to carry out PLS analysis. The sludge age was left out since it correlated with the parameters of both groups.

3.2.2. PLS analysis with the Van Soest characterisation method

The results of PLS model 4 from the Van Soest characterisation led to a mean squared prediction error (PRESS) equal to 0.05 with the dimension 2 (Fig. 4). The hemicellulose-"like" fraction had a negative impact on the biodegradability. This is in agreement with the results of Hattori and Mukai [28] and Parnaudeau et al. [30]. Indeed, these authors found a negative correlation between hemicellulose- and lignin-"like" concentrations of sewage sludge and their carbon decomposition rates in soil. The signs of regression coefficients (Fig. 4A) are in agreement with the mean reduction of these fractions (Table 4). Indeed soluble and cellulose-"like" fractions appeared with positive coefficients and showed the highest reductions whereas the hemicellulose- and lignin-"like" fractions appeared with negative coefficients. The soluble fraction represents a large quantity of matter which is readily extracted by the method and thus readily available for the biomass. However, despite the fact that the cellulose-"like" fraction needs a high-powered extracting detergent (acid detergent), this fraction seems to have been well degraded. This could mean that the anaerobic biomass had

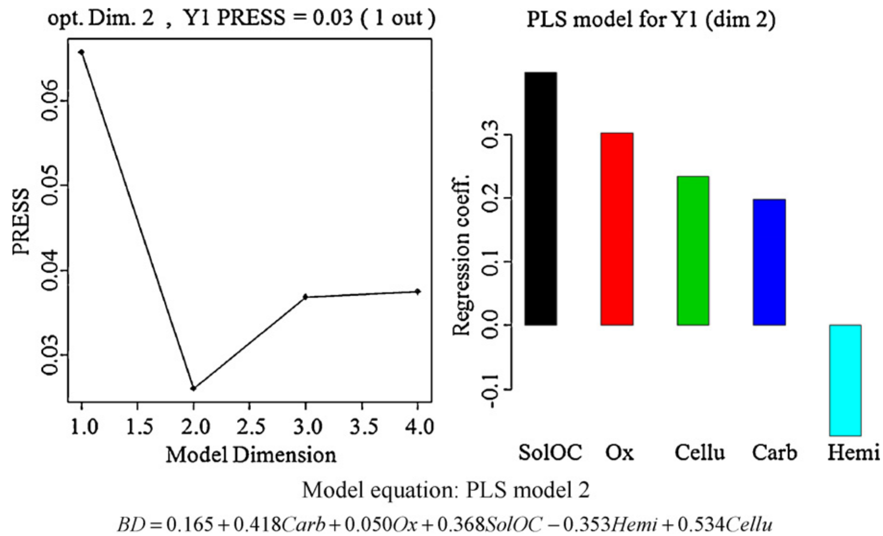


Fig. 3. Mean square prediction error (PRESS) and regression coefficient representation for the dimension 2 model, with the most important parameters.

enough enzymatic material to degrade this matter contained in the cellulose-"like" fraction.

3.2.3. PLS analysis with biochemical characterisation

The results from the biochemical characterisation are presented in Fig. 4. PLS model 5 was applied based on five explicative variables, namely: Prot, Carb, Lpd, Ox and SolOC. The best mean squared prediction error (PRESS = 0.12) was obtained when the dimension of the model was equal to 2. The results showed a positive correlation of carbohydrate and lipid concentrations and a negative correlation of protein concentrations. This observation is in agreement with the results of Miron et al. [41] who found that carbohydrates are more easily hydrolysed or decomposed than proteins.

3.2.4. PLS model validation

The thermally treated WAS, sample F, was used to validate the models. Thus, the PLS models were used to predict the biodegradability of this sludge sample. The results are presented in Table 6. The best biodegradability estimate was obtained for PLS model 5 based on biochemical characterisation, with a calculated value of 61% as against an experimental value of 55%, representing an error of 11%. For the other PLS models, the calculated values were overestimated by more than 22%. In particular, the PLS model based on the Van Soest characterisation did not succeed to estimate the biodegradability of the pretreated sludge sample in spite of its very low PRESS value.

The biodegradability of three other samples was estimated from PLS model 5. These samples were: one WAS from a high-load process with a sludge age of 0.36 days, the same WAS which was partially digested in thermophilic condition (sludge retention time of 8 days in the digester) and a crystalline cellulose sample. The biodegradability calculated from the model compared to the measured biodegradability, including the four points of validation, is presented in Fig. 5. A good regression coefficient, equal to 0.826, was obtained.

The biochemical characterisation method, associated with two macroscopic parameters (SolOC and Ox), seems to have provided pertinent parameters since a good prediction of sludge samples biodegradability was obtained. These characterisations are related to an initial characterisation of the quality and quantity of organic matter. In agreement with Schievano et al. [27], the qualitative and quantitative aspects of the organic matter were found to be important in predicting anaerobic degradation. They linked the

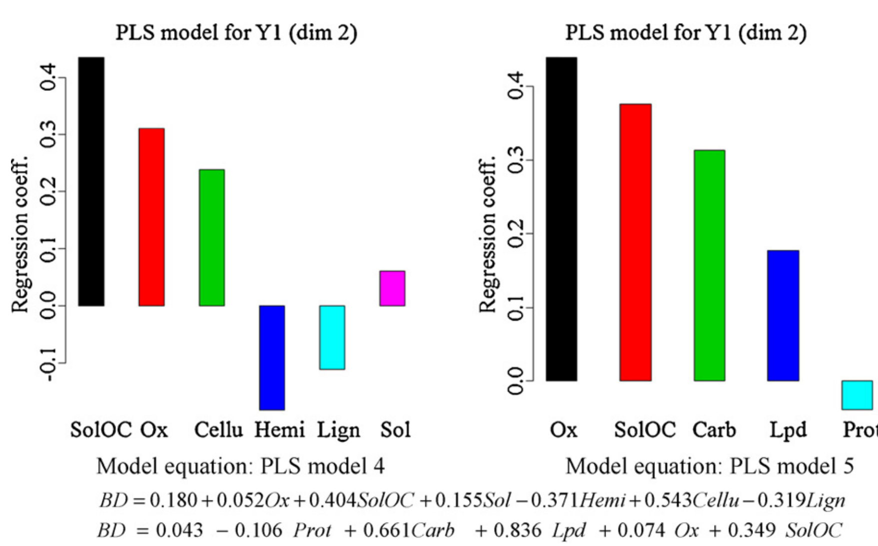


Fig. 4. Regression coefficient representations for PLS model 4 with the parameters of the Van Soest characterisation (A) and for PLS model 5 with the parameters of the biochemical characterisation (B) and SolOC and Ox.

Table 6
PLS model for predicting biodegradability of sludge sample F pretreatment output.

PLS models	Explicative variables	PRESS (model dimension)	R^2	Model validation[a] (prediction error)
1	SolOC, Ox, Cellu, Hemi, Carb, Prot, Lpd, VS, Sol, Lign, Age, VFAs	0.22 (3)	0.982	0.69 (25%)
2	SolOC, Ox, Cellu, Carb, Hemi	0.03 (2)	0.987	0.67 (22%)
3	Ox, SolOC	0.42 (1)	0.608	0.76 (38%)
4	SolOC, Ox, Cellu, Hemi, Lign, Sol	0.05 (2)	0.979	0.74 (35%)
5	Ox, SolOC, Carb, Lpd, Prot	0.12 (2)	0.957	0.61 (11%)

[a] Model validation = estimation of biodegradability of sludge sample F output pretreatment (experimental biodegradability = 0.55).

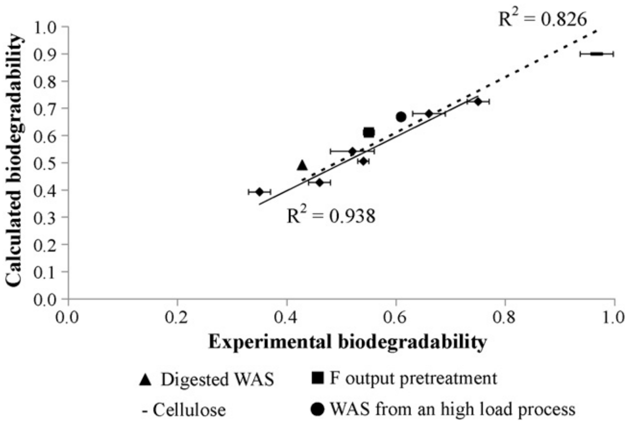


Fig. 5. Calculated and experimental biodegradability for calibration and validation of PLS model 5 based on the biochemical characterisation and SolOC and Ox (model calibration: ♦, —, R^2 = 0.938; model validation: ■ ▲ ●, –, R^2 = 0.826).

quality of organic matter to the oxygen demand during 20 h of respirometry assay (OD20), and to soluble fraction according to Van Soest fractionation (CS). These two parameters represent the degree of oxidation and the readily available matter. In our study, these aspects were related to the Ox and the SolOC parameters. Moreover, the biochemical fractions enabled an accurate characterisation of the organic matter content by identifying the more or less degradable compounds whereas Schievano et al. [27] identified only the quantity of matter by COT and VS.

The sludge composition is thus a relevant parameter in determining its biodegradability. Biochemical characterisation and the measurement of macroscopic parameters, as presented in this work, leads to a faster laboratory work. Moreover, the method recovered enough data to define both the availability and degradability of matter and enables us to predict the measured anaerobic biodegradability of our WAS samples. Moreover, considering that the unbiodegradable organic matter of WAS, as determined from aerobic conditions, remains unbiodegradable under anaerobic conditions [19], these results can also be applied to the aerobic digestion of sludge.

4. Conclusion

The impact of the composition of the organic matter on anaerobic biodegradability was evaluated by linking these two parameters using regression analysis.

The macroscopic (soluble organic carbon and COD/TOC ratio) and biochemical (carbohydrates, proteins and lipid concentrations) characterisation provided information on the bioavailability and the nature of the sludge sample compounds. Moreover, these parameters seem to be the most pertinent parameters in assessing the available matter and predicting the anaerobic biodegradability of WAS. These measurements, which are needed to apply the model, are relatively quick to obtain and thus offer a big advantage for routine applications. They could be considered as indicators of biodegradability.

Finally, Van Soest fractionation was used to identify the bioaccessibility of the organic matter but it was not a good tool for biodegradability predicting. This can be explained by the fact that the method was not suited to the sludges organic matter. It would more effective to establish a method with successive extraction steps, better adapted to the sludge samples.

Acknowledgements

The authors wish to thank Dr. Jean-François Durand sincerely for providing his PLS procedure.

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