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Effect of pre-treatments on hydrolysis and methane production potentials of by-products from meat-processing industry

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ABSTRACT

In this study, the effect of five pre-treatments (thermal, ultrasound, acid, base and bacterial product) on hydrolysis and methane production potentials of four by-products from meat-processing industry was studied. The bacterial product Liquid Certizyme 5™ increased soluble chemical oxygen demand (CODsol) of digestive tract content and drumsieve waste the most as compared to untreated material (62 and 96%, respectively), while ultrasound was the most effective to increase CODsol with dissolved air flotation (DAF) sludge (88%) and grease trap sludge (188%). In batch experiments, thermal treatment increased methane production potential of drumsieve waste, acid of grease trap sludge and all pre-treatments of DAF sludge. However, with all other pre-treatments, methane production potential was decreased compared to untreated materials, apparently due to inhibition by hydrolysis products and/or possible re-crystallization of some compounds. Methane production potentials from the untreated materials were as follows: digestive tract content 400 ± 50 m^3 CH_4/t volatile solids $(VS)_{added}$, drumsieve waste 230 ± 20 m^3 CH_4/tVS_{added}, DAF sludge 340 ± 17 m^3 CH_4/tVS_{added} and grease trap sludge 900 ± 44 m^3 CH_4/tVS_{added}.

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1. Introduction

Stabilization of biodegradable materials prior to reuse or disposal is increasingly required due to tightening legislation. In the European Union (EU), landfilling of untreated organic waste is gradually being reduced and will be totally prohibited (1999/31/EC). This increases the amount of substrate available for different treatment processes, such as incineration, composting and anaerobic digestion. Of these, anaerobic digestion offers several advantages. The produced biogas (methane content 50–70%) can be used as electricity, heat and/or vehicle fuel, and the stabilized digestate as organic fertilizer and soil improver provided all legislation is followed. Anaerobic digestion also minimizes the amount of organic wastes and prevents greenhouse gas emissions the materials produce during uncontrolled degradation.

Another EU regulation (1774/2002/EC) steering the treatment of organic materials concerns health rules for treatment and disposal of animal by-products (ABP). It was prepared in the wake of widespread animal diseases, such as bovine spongiform encephalopathy (BSE) and foot-and-mouth-disease, and defines three different ABP categories with distinct requirements for treatment and disposal of the materials (Table 1). Though manure and digestive tract content are included in category 2, they can be digested without sterilization. It has, however, been proposed that if any other material of animal-origin is to be co-digested, hygienization has to be applied. So far (December 2007), materials passing 6 mm sieves and ending up in wastewater are considered outside ABP regulation. Thus, they can be freely digested despite being produced in slaughterhouses or meat-processing plants.

Some by-products from meat-processing industry, such as grease trap sludge, are lipid-rich, small particles and pasties, have little fibrous structure and high water content [1], which makes them suitable substrate for anaerobic digestion. On the other hand, digestive tract content consists of partly digested fodder with carbohydrates and lignin. Lipid-rich materials have high methane production potential [1–7], but their degradation products, long-chain fatty acids (LCFAs), can be inhibitive in high concentrations. LCFA inhibition was long believed to be irreversible [8], but recent studies have shown the contrary, though recovery takes a long time [9,10]. Also, high concentration of ammonia is inhibitive and may pose problems when digesting protein-rich materials [11].

Pre-treating organic materials prior to anaerobic digestion aims at enhanced hydrolysis and thus more complete degradation, as bacterial cells are only able to uptake small molecules. Several pre-treatments have been attempted with meat-processing industry residues and slaughterhouse wastewaters, including physical (e.g. particle size reduction and temperature treatment: [12,13]),

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Table 1
Categorization of animal by-products according to EU regulation (1774/2002/EC)

Category	1	2	3
Material	TSE-risk, unknown or possible risk for public health, hygienic risk	Risk for other illnesses than TSE, Screened-out material from anti- and post mortem controls	Materials from animals fit for human consumption, but not used for commercial reasons
Treatment and requirements for anaerobic digestion	Not suitable for digestion	Sterilization: 133 °C, 3 bar, 20 min, <50 mm	Hygienization: 70 °C, 60 min, <12 mm
Example from materials and fractions	Ruminant spinal cord, scull, brains and eyes of animals older than 12 months	Manure, digestive tract content, blood, perished animals, animals died in storage	Catering waste, meat-containing wastes from food industry and dirty residues

TSE, transmissible spongiform encephalopathy.

chemical (e.g. alkali addition: [5,12]) and biological (e.g. enzymes: [5,6,12,14,15]) methods.

Thermal pre-treatment (i.e. high temperatures) is a physical method to differentiate liquid organic material from solid organic material and to loosen the cell structure of the remaining solid particles. Temperatures below 100 °C have been found more effective in increasing biogas production from waste activated sludge, food industry wastewater and sewage sludge than higher temperatures [16–19]. Previous research with thermally pre-treated fatty materials is scarce, though Mendes et al. [14] reported thermally pre-treated lipids to be non-susceptible to flotation in digesters.

A more novel physical method, ultrasound, effectively increases soluble chemical oxygen demand (CODsol), volatile solids (VS) and methane yield, e.g. in sewage and waste activated sludge [20–25], municipal wastewaters [26] and industrial wastewaters [27,28]. Ultrasound pre-treatment evokes cavitation by bubble formation in the liquid phase [20]. Cavitational collapse of bubbles produces local heating and pressure at liquid/gas interface, turbulence, formation of radicals and high-rate shearing phenomena in the liquid phase [29]. Pre-treatment of excess sludge has been found more lytic using low frequencies, with 20–40 kHz reported to decrease particle size and increase CODsol [20,30], while higher frequencies (e.g. 3217 kHz [30]) have better radical formation ability and disinfection efficiency [31]. Ultrasound pre-treatments (35 °C, 20–31 kHz) are reported to achieve 20% [22], 40% [25,32] and 89% [21] increase in CODsol, while methane yield increased with raw sewage sludge by 13–18% [32] and with waste activated sludge almost by 50% [22]. To our knowledge, ultrasound pre-treatment has not been previously used with meat-processing by-products.

Chemical pre-treatments with acid or base have been reported to increase the ratio of CODsol to total COD, and to reduce VS [33] and lipid content in waste activated sludge [34]. Heo et al. [35] reported NaOH (45 meq/l, 4 h, 35 °C) to increase COD solubilization (31%) and biogas production (73%) of waste activated sludge. Massé et al. [5] noticed NaOH (5–40 meq, pH 13, 4 h) to be more efficient with proteins than with lipids when treating slaughterhouse wastewater. Similarly, acid pre-treatment (60 meq HCl, 30–120 min, 35 °C) has been reported to increase solubilization and reduce particle size of organic matter in septic tank sludge [36], but apparently it has never been used with meat-processing by-products.

During anaerobic degradation, acidogenic bacteria excrete hydrolytic enzymes which enable the degradation of particles into smaller compounds. Thus, biological pre-treatments using enzymes have been studied with different materials. With slaughterhouse wastewater, pancreatic lipase PL 250 (4–24 h) reduced the average particle size by 60% and increased lipid hydrolysis by 40% during 24 h [6,14]. While the use of pure enzymes has been studied, bacterial product producing hydrolytic enzymes at favorable conditions and used, e.g. for preventing formation of solid grease in removal tanks, have apparently not been studied for pre-treatment purposes.

In this study, the effect of different pre-treatments (thermal, ultrasound, acid, base addition and bacterial product) on solubilization of organic material, i.e. hydrolysis, and methane production potentials of different by-products from meat-processing plants was studied.

2. Materials and methods

2.1. By-products from meat-processing plants and inoculum

The studied materials were chosen according to their availability for treatment in Finland (Table 2) and were received from a slaughterhouse (Lappeenranta, Finland) and a meat-processing plant (Mikkeli, Finland) handling cows and pigs. Only digestive tract content was categorized in ABP regulation (category 2), while the other studied materials, i.e. drumsieve waste, dissolved air flotation (DAF) sludge from the slaughterhouse and grease trap sludge from the meat-processing plant, were not included in the regulation due to passing 6 mm sieves. Grease trap sludge was already pre-treated with a bacterial product (details given below) at the meat-processing plant to prevent it from solidifying in the trap. Inoculum was digested sewage sludge from a municipal wastewater treatment plant (Mikkeli, Finland).

2.2. Pre-treatments

Digestive tract content was firstly grinded with a kitchen blender to ensure particle size < 12 mm (ABP regulation), while the other materials did not need grinding. The applied pre-treatments were performed separately to each material prior to batch exper-

Table 2
Characteristics of the studied materials and inoculum

Material	pH	TS (%)	VS (%)	CODsol/VS	CODsol (g/l)	Total VFA (g/l)	VFA from CODsol (%)	NH$_4^+$–N (g/l)
Inoculum	7.1	3.2 ± 0.05	1.8 ± 0.03	–	–	–		0.070
Digestive tract	7.2	12 ± 0.5	11 ± 0.5	0.38	4.0 ± 0.03	2.6	65	0.13
Drumsieve waste	6.6	14 ± 2	14 ± 2	0.06	0.85 ± 0.03	0.20	24	0.19
DAF sludge	6.9	4.3 ± 0.03	3.5 ± 0.04	1.6	5.6 ± 0.2	1.6	29	0.50
Grease trap sludge	5.6	11 ± 0.8	11 ± 0.8	0.6	6.6 ± 0.1	3.4	52	0.48

Standard deviation given where applicable ($n = 3$).

Table 3
Pre-treatment conditions and the studied materials: DTC, digestive tract content; DSW, drumsieve waste; DAF, sludge from dissolved air flotation; GTS, grease trap sludge

Pre-treatment	Conditions	Studied materials
None	–	Inoculum, DTC, DSW, DAF, GTS
Thermal	70 °C, 60 min	DTC, DSW, DAF, GTS
Ultrasound	24 kHz, 5600 ± 300 kJ/kg TS	DTC, DSW, DAF, GTS
Base	2 M NaOH (6–14%), pH 12–12.2, 4 h	DTC, DSW, DAF, GTS
Acid	6 M HCl (2–8%), pH 2–2.5, 4 h	DTC, DSW, DAF, GTS
Bacterial product	Liquid Certizyme 5TM, 60 mg/l, 24 h	DTC, DSW, DAF[a]

[a] Grease trap sludge was treated with Liquid Certizyme 5TM already at the meat-processing plant.

iments (Table 3). Thermal pre-treatment was the hygienization required by ABP regulation (70 °C, 60 min), and it was performed in an incubator (Termaks TS 8056, Norway). Ultrasound (Hielscher UP200H ultrasonic processor, Germany) treatment was performed in 5 l plastic bowls with an energy input of 5600 ± 300 kJ/kg TS under temperature control at 25 °C. Base (2 M NaOH) and acid (6 M HCl) pre-treatments were carried out in nitrogen flushed, mixed (HS 501 digital, IKA Labortechnik, Germany) 500 ml glass vessels and neutralized with NaOH (2 and 0.1 M) or HCl (6 and 0.1 M) to pH 7.0 before batch experiments. pH was measured at various parts of the sample during the performance of the base and acid treatments. The samples were also simultaneously mixed. Pre-treatment with bacterial product was performed with Liquid Certizyme 5TM (Certified Laboratories, NCH Finland Ltd.), designed to prevent grease from solidifying in sewers and removal tanks and consisting of three different bacteria: *Bacillicus subtilis*, *Bacillicus licheniformis* and *Bacillus thuringiensis* (108 cfu/ml). The bacteria grow and produce protease, amylase and lipase enzymes when exposed to viable conditions. The manufacturer's dose recommendation, 300 cfu/500 ml, was followed using nitrogen-flushed, mixed (HS 501 digital, IKA Labortechnik, Germany) 1 l glass bottles for 24 h at 25 °C.

2.3. Batch experiments

Methane production potentials of all studied materials were determined in batch experiments in duplicate 2 l glass bottles incubated statically at 35 °C (43 bottles altogether; pre-treated DAF sludge with only one replica). The potentials were determined with and without pre-treatments and a set of bottles were prepared with inoculum alone with its methane production subtracted from the studied materials. Inoculum (750 g/batch) and studied materials were added into the bottles in a $VS_{substrate}/VS_{inoculum}$ ratio of 1. Distilled water was added to produce a liquid volume of 1.5 l. pH of each batch was adjusted to 7.0 with 2 M NaOH or 6 M HCl, and sodium bicarbonate ($NaHCO_3$, 3 g/l) was added as buffer. Headspaces of the bottles were flushed with nitrogen gas for 5 min, after which the bottles were sealed with rubber septa. The produced biogas was collected into aluminium gas-bags (Tesseraux Spezialverpackungen GmbH, Germany). Bottles were mixed manually before each gas measurement.

2.4. Analyses and calculations

Biogas volume was measured with water displacement and its methane content with gas chromatography (Agilent 6890N: PerkinElmer Elite-Alumina column 30 m × 0.53 mm, flame ionization detector 225 °C, oven 100 °C, inlet 225 °C, carrier gas helium 10 ml/min, split ratio 35:1, injection volume 100 μl). Biogas measurements were performed every day in the beginning of the study and one to three times per week when biogas production started to reduce. Volatile fatty acids (VFAs) were measured with a gas chromatogram (Agilent 6890N: Agilent HP-FFAP capillary column 30 m × 0.32 mm × 0.25 μm, flame ionization detector 225 °C, oven 100–160 °C 25 °C/min, inlet 230 °C, carrier gas helium 0.7 ml/min, split ratio 2.3:1, injection volume 1 μl). Total solids (TS) and VS were analyzed according to APHA [37]. CODsol was analyzed after filtration through Whatman GF/A glass microfibre-filters (Scheicher & Schuell, Germany) according to Finnish standard method [38], and pH was measured with WTW 340i pH-meter and electrode (Germany). Ammonium nitrogen was analyzed photometrically (HACH LANGE DR 2800 VIS photometer, Germany) using cuvette tests (HACH LANGE LCK302, 47–130 mg/l, Germany). Particle size distribution (PSD) was analyzed after batch experiment using high-end dispersion analyzer LUMiSizer® (L.U.M. GmbH, Germany), measuring average particle sizes as arithmetic average volume diameters (nm). To give an exact diameter of particles, the density of protein, carbohydrate and lipid particles should be known. However, as the studied materials were composites of these substrates, particle density was considered constant (1.0) and the results were reported with percentage comparison of particle sizes within each studied material (untreated vs. pre-treated). Specific methanogenic activity (SMA, ml CH_4/gVSd) was calculated between two measurement points giving the steepest slope of the cumulative methane production curve. CODsol/VS ratio was used in results to ignore the change in VS during the pre-treatments (possible evaporation and dilution of material).

3. Results

3.1. Effect of pre-treatments on solubilization

Five different pre-treatments (thermal, ultrasound, base, acid and bacterial product) were used in order to hydrolyze by-products from meat-processing industry. All the studied pre-treatments solubilized part of the organic material (Table 4). The most effective pre-treatments for digestive tract content were base and bacterial product, increasing CODsol/VS by 74 and 64%, respectively, while with drumsieve waste, all pre-treatments increased CODsol/VS by over 600% as compared to CODsol/VS of the untreated material. The most effective pre-treatment was bacterial product with 920% increase in CODsol/VS. VFA comprised of 76–88% of CODsol in digestive tract content after the pre-treatments as compared to 65% in the untreated material. With drumsieve waste the respective percentage was 24–47% compared to 24% in untreated. Total VFA of digestive tract content and drumsieve waste increased with CODsol, depending on the effectiveness of pre-treatments (Table 4). VFA of both materials were mostly acetic and propionic acids (data not shown).

Ultrasound was the most effective pre-treatment with the lipid-rich materials, DAF sludge and grease trap sludge. Ultrasound and also bacterial product increased CODsol/VS of DAF sludge by 76%, while with grease trap sludge, ultrasound and thermal treatments increased it by 121 and 98%, respectively, compared to the CODsol/VS of untreated material (Table 4). However, base resulted in reduced CODsol/VS (−5%) with grease trap sludge. Total VFA of DAF sludge increased by 55 ± 17% during the pre-treatments, but it did not follow the increase of CODsol and the bacterial product increased it more (102%) than ultrasound (44%). CODsol of the pre-treated DAF sludge comprised of 22–38% total VFA as compared to 29% of untreated material. VFA was mostly acetic and propionic acids (data not shown). With grease trap sludge, VFA decreased 4 ± 1.9% during the pre-treatments and contained longer fatty acid chains, such as valeric and caproic acid, than the other studied materials (data not shown). Moreover, ammonium nitrogen con-

Table 4

TS, VS, CODsol/VS ratio, CODsol and NH_4^+–N of pre-treated materials and the change (%) in VS, CODsol and NH_4^+–N compared to untreated materials

Material	Treatment	TS (%)	VS (%)	CODsol/VS	CODsol (g/l)	Total VFA (g/l)	VFA from CODsol (%)	NH_4^+–N (g/l)	VS (%)	CODsol (%)	NH_4^+–N (%)
Digestive tract content	Thermal	15 ± 0.2	14 ± 0.2	–[a]	–[a]	–[a]	–[a]	–[a]	29	–[a]	–[a]
	Ultrasound	11 ± 0.05	10 ± 0.02	0.51	5.1 ± 0.2	4.02	79	0.23	−3.8	27	72
	Base	11 ± 0.2	9.0 ± 0.20	0.67	6.1 ± 0.1	5.06	83	0.21	−13	51	58
	Acid	11 ± 0.05	9.5 ± 0.09	0.48	4.6 ± 0.2	3.5	76	0.22	−9.2	14	62
	Bacterial product	12 ± 0.01	10 ± 0.02	0.63	6.5 ± 0.03	5.7	88	0.31	−0.7	62	136
Drumsieve waste	Thermal	18 ± 0.07	17 ± 0.08	–[a]	–[a]	–[a]	–[a]	–[a]	22	–[a]	–[a]
	Ultrasound	12 ± 0.70	11 ± 0.7	0.4	4.3 ± 0.1	2.0	47	0.52	−20	407	175
	Base	12 ± 1.8	10 ± 1.2	0.57	5.9 ± 0.07	1.9	32	0.13	−24	591	−32
	Acid	11 ± 0.04	10 ± 1.5	0.46	4.8 ± 0.1	1.2	29	0.18	−22	464	−3.2
	Bacterial product	15 ± 0.04	14 ± 0.04	0.64	9.0 ± 0.1	2.2	24	0.070	4.3	963	59
DAF sludge	Thermal	5.6 ± 0.10	4.5 ± 0.06	1.9	8.7 ± 0.03	2.7	31	0.56	30	55	12
	Ultrasound	4.6 ± 0.04	3.7 ± 0.4	2.9	11 ± 0.07	2.4	22	1.0	6.6	88	100
	Base	4.4 ± 0.10	3.0 ± 0.09	2.5	7.6 ± 0.3	2.6	34	0.29	−14	35	−42
	Acid	4.2 ± 0.01	2.8 ± 0.1	2.0	5.7 ± 0.1	2.1	37	0.43	−18	0.6	−14
	Bacterial product	4.2 ± 1.0	3.0 ± 1	2.9	8.7 ± 0.1	3.3	38	3.8	−14	54	660
Grease trap sludge	Thermal	16 ± 0.3	15 ± 0.3	1.2	18 ± 0.2	3.2	18	0.78	40	178	63
	Ultrasound	15 ± 0.2	14 ± 0.1	1.3	19 ± 0.2	3.5	18	0.53	30	188	11
	Base	12 ± 0.9	10 ± 0.9	0.57	5.8 ± 0.4	3.0	52	0.20	−6.7	−13	−58
	Acid	10 ± 0.03	9.3 ± 0.02	0.75	7.0 ± 0.2	3.2	46	0.42	−16	5.5	−12

[a] CODsol of thermally treated digestive tract content and drumsieve waste could not be measured due to little filterable liquid in the samples.

centration was mostly increased with all studied materials during thermal, ultrasound and bacterial product treatments, while the chemical treatments decreased it with all but digestive tract content.

3.2. Methane production potentials

Methane production potentials of untreated and pre-treated materials were determined in batch experiments at 35 °C. Methane production started immediately from digestive tract content, drumsieve waste and DAF sludge, but that from grease trap sludge showed a lag phase of 2–13 days (Fig. 1). Thermal pre-treatment shortened the lag into approximately 2 days as compared to the lag of the untreated material (17 days), while the other pre-treatments prolonged it by 6–11 days. Especially DAF sludge exhibited ideal methane production in the sense that it started immediately and the extractable methane was produced quickly during 10–15 days with both untreated and pre-treated fractions. With drumsieve waste and digestive tract content, methane production started also immediately, but continued slightly longer with all fractions than 10–15 days but thermally treated drumsieve waste (12 days).

Methane production potentials of the studied materials were 400 ± 50 m^3 CH_4/tVS_{added} for digestive tract content, 230 ± 20 m^3 CH_4/tVS_{added} for drumsieve waste, 340 ± 17 m^3 CH_4/tVS_{added} for DAF sludge and 900 ± 44 m^3 CH_4/tVS_{added} for grease trap sludge (Table 5). All pre-treatments decreased the methane production potential of digestive tract content, while with drumsieve waste; thermal pre-treatment resulted in significantly increased methane production (340 ± 7 m^3 CH_4/tVS_{added}). With DAF sludge, all pre-treatments improved the methane production potential, with base being the most efficient (390 m^3 CH_4/tVS_{added}). With grease trap sludge, acid increased the methane production potential to 1010 ± 50 m^3 CH_4/tVS_{added}, while the other pre-treatments either did not change the potential (ultrasound and base) or decreased it slightly (thermal).

SMA of the untreated materials was the highest with grease trap sludge (60 ml CH_4/gVSd) and the lowest with drumsieve waste (27 ml CH_4/gVSd; Table 5). With digestive tract content, all pre-treatments decreased SMA on average by 58 ± 9%. Ultrasound treatment of DAF sludge increased SMA by 19%, while the other treatments reduced it. With all pre-treated drumsieve waste and grease trap sludge, respective average increases in SMA were 15 ± 6% and 14 ± 3%, compared to the untreated materials. The exceptions were bacterial product and drumsieve waste and thermally treated grease trap sludge, the SMA of which decreased by 17 and 22%, respectively.

Pre-treated materials had higher CODsol and CODsol/VS ratio than untreated materials at the beginning of batch experiments, with the exception of base treated grease trap sludge (Tables 2 and 4). During the batch experiment, their CODsol was also removed by a higher percentage, on average 93 ± 2.7%, than that of the untreated materials (average 80 ± 14%). Total VS removal was quite similar with the other materials (90 ± 1% with digestive tact content, 92 ± 1% with drumsieve waste and 93 ± 1.3% with grease trap sludge) except with DAF sludge having thus the lowest biodegradability (68 ± 5.8%). Compared to original concentrations, ammonium nitrogen in digestive tract content and drumsieve waste increased on average by 69 ± 19% and 78 ± 37%, respectively, during the batch experiment, while it decreased in the batches with DAF sludge and grease trap sludge by 49 ± 14% and 26 ± 18%, respectively. The only exception was base treated grease trap sludge with an increase of 78%.

At the end of the batch experiments, digestive tract content treated with the bacterial product had a 58% smaller residual particle size, while all pre-treatments of drumsieve waste resulted in

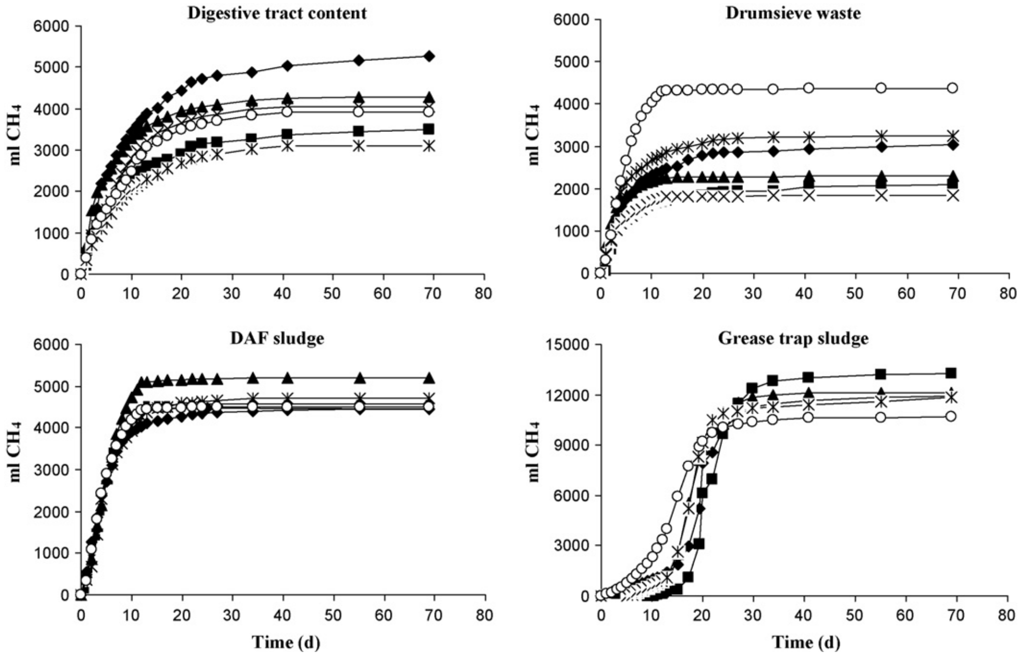

Fig. 1. Cumulative methane production of untreated and pre-treated materials in batch experiments. Untreated fractions (♦), temperature treated (○), ultrasound treated (*), acid treated (■), base treated (▲) and enzyme treated (×). Note the different scale in *y*-axis with grease trap sludge.

an 11 ± 8% smaller particle size than the untreated wastes (Table 5). Average particle size of DAF sludge was 250 ± 50% higher after all pre-treatments while that of grease trap sludge was 17% smaller after thermal and ultrasound treatments than the original materials. Average particle size of all studied materials was the largest after the chemical pre-treatments, except for digestive tract content, which was the largest after physical (ultrasound and thermal) and chemical (acid and base) pre-treatments compared to the untreated fractions.

PSD range was the largest in all chemically treated batches. Particles of chemically treated drumsieve waste, DAF sludge and grease trap sludge were 11, 37 and 22%, respectively, larger compared to the average particle sizes of otherwise treated and untreated fractions. With chemically treated digestive tract content and drumsieve waste, the largest PSD increase (36 and 14%), was found in the group of larger particles (40% of particles in measured distribution), while with chemically treated DAF sludge and grease trap sludge, the increase (36%) was the most notable in the group of smaller particles (40% of particles in measured distribution) compared to particle size differences of batches with untreated and otherwise pre-treated materials.

4. Discussion

4.1. Effect of pre-treatments

With digestive tract content and drumsieve waste, the most suitable treatments were bacterial product and base treatments with a CODsol/VS increase of 64–919% as compared to untreated materials. With the bacterial product, the facultative bacteria *B. licheniformis*, capable of producing protease, amylase and cellulose, most likely effectively hydrolyzed proteins and carbohydrates to VFA, especially with digestive tract content. Moreover, NaOH has been reported more efficient in hydrolyzing proteins and carbohydrates than lipids [34], which could explain the higher solubilization of digestive tract content and drumsieve waste during base treatment compared to that of lipid-rich DAF sludge and grease trap sludge. With DAF sludge and grease trap sludge, the most suitable treatment was ultrasound with a CODsol/VS increase of 76 and 121%, respectively. Moreover, with DAF sludge, the bacterial product was as effective as ultrasound.

After the pre-treatments, total VFA of digestive tract content and drumsieve waste correlated with CODsol (respective correlations 0.99 and 0.87), but total VFA of DAF sludge did not increase linearly with the CODsol (e.g. base treatment was more effective than ultrasound and enzyme treatment increased VFA 102% than other treatments 46 ± 13%). The reason could be that CODsol of DAF sludge and grease trap sludge contained more LCFA than VFA, as supported by notable amounts of valeric and caproic acids (the largest analyzed VFA) in grease trap sludge. Total VFA of digestive tract content and drumsieve waste increased the most after the bacterial and base treatments. With drumsieve waste, ultrasound treatment also produced VFA effectively (+900%), while bacterial treatment was the most effective with DAF sludge (+102% VFA). Ultrasound treatment increased the VFA of grease trap sludge by 3.4%, but otherwise VFA was degraded during pre-treatments probably due to high LCFA and relatively low VFA content.

VS of digestive tract content and drumsieve waste were mostly reduced during the pre-treatments (1–24%), except for the bacterial product, which did not significantly change the VS of either. With chemical treatments, reduced VS can be explained with dilution due to addition of the treatment chemical and the following neutralization. During ultrasound treatment, the VS of digestive tract content did not change notably, but the adhesion water from the particles was apparently released increasing the liquid and CODsol concentration [20]. Conversely, thermal treatments increased VS due to evaporation of water.

Concentration of ammonium nitrogen in digestive tract content increased during all pre-treatments, while with all other materials, ultrasound and bacterial product increased it and chemical treatments decreased it. Accordingly, Chiu et al. [21] and Bougrier et al. [39] reported that ultrasound pre-treatment of waste activated sludge did not lead to nitrogen mineralisation or volatilisation, but to 41% increase in soluble organic nitrogen. Moreover, bacterial product was known to excrete proteases resulting in degraded proteins. However, as opposed to the results of the present study, base treatment (40 meq NaOH/l) was reported to increase soluble nitrogen in waste activated sludge by 34% [21]. The present base treatment may have caused ammonium loss as evaporation, as at high pH, it is converted to gaseous ammonia [40,41]. Chemical pre-treatments may also have caused molecular nitrogen (N_2) production and ammonium removal as reported with septic tank sludge [36]. Moreover, acid (HCl) treatment may have removed soluble ammonium due to possible salt formation of chloride aminies (ammonium chloride; NH_4Cl with nitric acid, ammonium nitrate) [42]. Thus, ultrasound and bacterial product seemed more promising alternatives for production of fertilizers due to maintaining the nitrogen in solution.

Table 5
Methane yield per tonnes VS and wet weight added, specific methanogenic activity of the inoculum and the characteristics of the digested residues

Material	Treatment	CH_4 prod. (m^3 CH_4/tVS$_{added}$)	CH_4 prod. (m^3 CH_4/t w w$_{added}$)	SMA (ml CH_4/gVSd)	TS (%)	VS (%)	CODsol (g/l)	N_4^+–HN (g/l)	PSD (%)	pH
Digestive tract content	Untreated	400	42	25	2.0 ± 0.04	1.03 ± 0.04	0.58 ± 0.6	0.10	–	7.3
	Thermal	310	42	22	2.0 ± 0.03	1.04 ± 0.02	0.24 ± 0.4	0.16	31	7.3
	Ultrasound	250	25	15	2.1 ± 0.05	1.06 ± 0.07	0.25 ± 0.03	0.13	30	7.4
	Base	320	30	33	1.9 ± 0.01	1.0 ± 0.01	0.38 ± 0.02	0.13	24	7.5
	Acid	270	25	18	2.0 ± 0.01	1.0 ± 0.01	0.53 ± 0.04	0.11	13	7.3
	Bacterial product	320	33	18	1.9 ± 0.03	1.02 ± 0.04	0.31 ± 0.03	0.13	−70	7.2
Drumsieve waste	Untreated	230	30	18	1.9 ± 0.1	1.0 ± 0.08	0.46 ± 0.08	0.11	–	7.4
	Thermal	340	56	27	2.2 ± 0.01	1.2 ± 0.02	0.43 ± 0.003	0.13	20	7.4
	Ultrasound	210	23	22	2.1 ± 0.14	1.1 ± 0.12	0.41 ± 0.07	0.12	−12	7.4
	Base	170	18	25	2.0 ± 0.07	0.80 ± 0.50	0.45 ± 0.01	0.13	−0.5	7.3
	Acid	160	16	31	1.9 ± 0.05	1.05 ± 0.02	0.54 ± 0.01	0.09	−4.6	7.5
	Bacterial product	140	20	15	2.0 ± 0.08	1.1 ± 0.07	0.43 ± 0.01	0.14	−27	7.5
DAF sludge	Untreated	340	12	13	1.9 ± 0.09	0.95 ± 0.04	0.53 ± 0.02	0.08	–	7.5
	Thermal	360	16	23	2.1 ± 0.03	1.0 ± 0.02	0.53 ± 0.02	0.09	240	7.4
	Ultrasound	370	14	37	2.1 ± 0.08	1.1 ± 0.03	0.52 ± 0.04	0.10	260	7.5
	Base	390	12	31	2.3 ± 0.05	1.1 ± 0.05	0.59 ± 0.003	0.07	310	7.5
	Acid	350	10	37	2.4 ± 0.02	1.1 ± 0.04	0.65 ± 0.02	0.08	310	7.5
	Bacterial product	350	11	33	2.3 ± 0.03	1.1 ± 0.04	0.65 ± 0.01	0.08	125	7.6
Grease trap sludge	Untreated	900	99	60	1.7 ± 0.03	0.80 ± 0.02	0.5 ± 0.003	0.10	–	7.6
	Thermal	840	129	47	1.7 ± 0.05	0.80 ± 0.04	0.42 ± 0.04	0.12	−17	7.4
	Ultrasound	890	126	70	1.7 ± 0.02	0.80 ± 0.01	0.46 ± 0.01	0.12	−16	7.4
	Base	900	92	68	1.6 ± 0.20	0.80 ± 0.2	0.55 ± 0.02	0.12	19	7.3
	Acid	1010	93	73	1.7 ± 0.04	0.80 ± 0.03	0.53 ± 0.02	0.11	8.0	7.5

Particle size distribution (PSD) per average weight was compared to untreated materials. Standard deviation given where applicable.

4.2. Comparison of pre-treatment efficiency

Thermal pre-treatment was performed as the required hygienization in ABP-regulation (70 °C, 60 min; 1774/2002/EC), but the improved hydrolyzing effect may have enhanced with longer duration. For example Skidias et al. [18] and Climent et al. [19] used 70 °C for 7–72 h as a pre-treatment of primary and secondary sludge, of which 7 h resulted in 43% more VFA and 36–42% higher biogas production. The most significant drawback of thermal treatment is its energy-intensity, wherefore low temperature and short time are preferred. Still, energy can efficiently be used by achieving the hygienization temperature with the produced biogas and by using heat exchangers.

Ultrasound was found the most versatile pre-treatment, as it effectively solubilized all the studied materials (32–536% increase in CODsol/VS and 27–408% increase in CODsol compared to untreated materials). This is comparable or higher than the 40 and 89% increase in CODsol when sonicating (20 kHz) raw sewage sludge [32] and waste activated sludge [21,24]. The studied physical (ultrasound and thermal) pre-treatments showed good potential for pre-treating meat-processing industry by-products rapidly, and they have already been reported as the most potential pre-treatments for sewage sludge [43].

Chemical pre-treatments were effective in solubilizing carbohydrate and protein-rich digestive tract content and drumsieve waste, while the solubilizing effect on the greasy materials was not as significant, as also reported by Karsson [34]. Still, the effect of decreasing particle size (as explained during following discussion on methane production potentials) may be beneficial to anaerobic digestion and even better than direct solubilization to CODsol, especially with easily inhibitive greasy materials [10,19,44]. Still, the loss of CODsol during base treatment of grease trap sludge should be taken into account, as it was most likely explained by grease disintegrating to glycerol and LCFA, then reacting with NaOH and forming unboiled soap. This is further supported by the fact that during the batch experiment, VS of base treated grease trap sludge decreased by 80%, the reported digestion value of soaps [45].

The bacterial product increased CODsol of all studied materials up to 10 times, comparable or higher than the solubilizing efficiency of pure enzymes (Lipase G-1000, CODsol increase 6–27% [14]; Pancreatic lipase PL 250, CODsol increase 40% [5]). Moreover, little if any VS were lost except with DAF sludge (−14%). Also, the dose of the bacterial product was small compared to, e.g. pure bacterial lipase LG-1000, which is efficient in doses >1000 mg/l, and it did not work as an extra substrate as pure enzymes in the study of Cirne et al. [10], who noticed bacterial pre-treatments to increase the production of methane as an extra substrate. The applied treatment time (24 h) may have been too long for DAF sludge and grease trap sludge causing VS loss, and the treatment time should be optimised case-specifically. All-in-all, the bacterial product proved very feasible for pre-treating different materials for anaerobic digestion and is more economical than pure enzymes.

4.3. Methane production potentials

Untreated digestive tract content ($400\,m^3\,CH_4/tVS_{added}$, $42\,m^3\,CH_4/t\,w\,w_{added}$) produced 27 ± 7% more methane than the pre-treated fractions with no positively effect by any of the studied pre-treatments. This is in agreement with the results of Angelidaki and Ahring [46] and Lehtomäki et al. [47], who pre-treated carbohydrate-containing materials (manure and energy crops, respectively) with different pre-treatments and gained little if any increase in methane production. This might due to reduced VS during the pre-treatments [47] and/or re-crystallization of cellulose after the pre-treatments (acid, base and ultrasound), shrinking the surface area for hydrolytic bacteria in the batches [48]. Particle size in pre-treated digestive tract content was 22 ± 9.6% larger in the end of the batch experiment than that of the untreated material (excluding treatment with bacterial product) further supporting the possible re-crystallization. The SMA of base treated digestive tract content was 32% bigger than that of the original material, but the methane production was still notably lower ($320\,m^3\,CH_4/tVS_{added}$). This could be due to the same possible phenomenon of hydrolyzation of bigger particles into smaller ones by chemical pre-treatment which momentarily speeds up the methane production process. Still, the argued re-crystallization possibly decreased the biodegradability of the material and thus the methane yield.

Another possibility for the low methane production potentials is the high VFA in pre-treated digestive tract content. Climent et al. [19] reported VFA concentration of 3.9–4.9 g/l in thermally treated (70 °C) sewage sludge possible inhibit methane production. Kalle and Menon [55] reported methane production of acetate to decrease 52% when VFA concentration 2.2 g/l was added to the digester. Based on these results, the high VFA concentration of some pre-treated materials may have been the reason for lower methane production compared to untreated materials.

Drumsieve waste ($230\,m^3\,CH_4/tVS_{added}$ and $31\,m^3\,CH_4/t\,w\,w_{added}$) produced 48% more methane after thermal treatment probably due to increased VS (22%), release of soluble organic compounds and degradation of particles. Hydrolysis at 25 °C produces more branched VFA, such as isovalerate and isobutyrate than hydrolysis at 35 °C, and branched VFA are more inhibitive to aceticlastic methanogens [55]. This could partly explain the 44% higher methane production (ml) of thermally treated drumsieve waste.

Also, Vlyssides and Karlis [17] pre-treated waste activated sludge from food industry wastewater treatment at 50–90 °C and reported effective solubilization and 10% faster digestion. Similarly, Climent et al. [19] found temperature pre-treatment (70 °C, 9 h) to be the most effective in increasing soluble organic content (43%) and biogas production (50%) from sewage sludge. Also, the SMA of inoculum was increased from 18 to $27\,ml\,CH_4/gVSd$. However, despite increased SMA (except for bacterial product) the other studied pre-treatments did not increase methane production from drumsieve waste. This might be due to inhibition by intermediate degradation products, caused by effective solubilization and subsequent excess amount of soluble organic material for the methanogens [10,14,49,50]. CODsol of drumsieve waste was increased from 0.85 to 4.3–9 g/l during the pre-treatments.

Methane production potential of DAF sludge ($340\,m^3\,CH_4/tVS_{added}$ and $12\,m^3\,CH_4/t\,w\,w_{added}$) was increased by all studied pre-treatments, as was the SMA of inoculum. Base treatment increased methane production potential the most, despite the notably lower CODsol/VS than with bacterial product and ultrasound treatments. Similarly, acid treatment increased CODsol/VS of DAF sludge the least of all pre-treatments, and still, methane production potential was improved the most. As particle size and structure are important factors in hydrolysis and accessibility of hydrolytic enzymes [51], acid and base may have cut bigger particles into smaller ones, helping microbial hydrolysis during the batch experiment. Massé et al. [5] reported that alkaline pre-treatment reduced especially the amount of particles larger than 500 μm and reduced the particle size of slaughterhouse wastewater by 73 ± 7% of the initial average particle size. It is unfortunate that the presently applied PSD method did not allow reliable measurement of pre-treated samples prior to batch experiment to verify this. Still, due to the chemical treatments (base and acid) and their hydrolysis of bigger particles, 21 ± 9% more CODsol was left in the digested materials after the batch experiment and VS removal was 1–6% lower compared to the batches with otherwise treated materials.

Grease trap sludge had high methane production potential ($900\,m^3\,CH_4/tVS_{added}$ and $99\,m^3\,CH_4/t\,w\,w_{added}$), but the methane production started after a lag phase of 2–17 days most likely due to rapid build-up of LCFA and/or VFA and subsequent inhibition of further degradation [4,10,19,49,44,52]. CODsol of both untreated and pre-treated grease trap sludge was high (6.6–19 g/l), but the produced CODsol was of different quality as, e.g. thermal treatment shortened the lag by 3 days and ultrasound prolonged it by 8 days as compared to untreated material, as also noticed by Cirne et al. [10]. Unfortunately, VFA analysis showed no further clarification, as both thermal and ultrasound treatments resulted in 18% VFA of CODsol. In fact, VFA was not significantly changed during the pre-treatment suggesting formation of LCFA. Biomass-associated LCFA has been reported inhibiting at 4.0–6.3 g/l [9,53,54].

While the other pre-treatments did not significantly change (ultrasound and base) or slightly decreased (thermal) the methane production potential of grease trap sludge, acid increased it to $1010\,m^3\,CH_4/tVS_{added}$. This may be due to the earlier discussed hydrolysis of bigger particles, as CODsol of the acid pre-treated fraction was only increased by 5.5%. Thermal treatment, on the other hand, decreased the inoculum SMA from 60 to $47\,ml\,CH_4/gVSd$, while the other pre-treatments increased it. This could due to evaporation of water and resulting higher concentration of inhibiting LCFA. Moreover, thermal treatment may have changed the characteristics of the otherwise highly biodegradable material to somehow more slowly degradable though the reason for it was not evident in the present results.

As separate anaerobic digestion of the studied materials is unlikely, studies on co-digestion of the materials together and with some easily available materials, such as sewage sludge, are needed. Co-digestion is known to ease inhibition through dilution of inhibitive compounds and to bring synergistic effects on the digestion process, resulting in improved degradation and methane production [56]. Especially LCFA inhibition has been noticed to decrease with co-digestion of lipids and carbohydrates and proteins [57]. Accordingly, it may be expected that the pre-treatments improve methane production of the co-digested materials more than with present separate treatments.

5. Conclusions

The studied pre-treatments (thermal, ultrasound, base, acid and bacterial product) effectively hydrolyzed the studied organic by-products from meat-processing industry (digestive tract content, drumsieve waste, DAF sludge and grease trap sludge) to soluble organic compounds. Bacterial product and base were the most efficient pre-treatments with digestive tract content and drumsieve waste, while with DAF sludge, ultrasound and bacterial product and ultrasound and thermal treatment with grease trap sludge released CODsol the most.

Methane production potentials of the untreated materials were the following: digestive tract content $400 \pm 50\,\mathrm{m}^3\,\mathrm{CH}_4/\mathrm{tVS}_{\mathrm{added}}$, drumsieve waste $230 \pm 20\,\mathrm{m}^3\,\mathrm{CH}_4/\mathrm{tVS}_{\mathrm{added}}$, DAF sludge $340 \pm 17\,\mathrm{m}^3\,\mathrm{CH}_4/\mathrm{tVS}_{\mathrm{added}}$ and grease trap sludge $900 \pm 44\,\mathrm{m}^3\,\mathrm{CH}_4/\mathrm{tVS}_{\mathrm{added}}$. None of the pre-treatments improved the methane production potential of digestive tract content either due to VS loss, high VFA concentration or re-crystallization of the carbohydrate content, resulting in larger particles and increased recalcitrance of the material. Drumsieve was easily hydrolyzed but only thermal treatment resulted in increased methane production potential, while that of DAF sludge was improved by all studied pre-treatments. With grease trap sludge, only acid increased the methane production potential.

Thermal pre-treatment concentrated the studied materials through evaporation of water, while the chemical pre-treatments (base and acid) cut the larger particles into smaller, more easily hydrolyzed ones without directly solubilizing the material to CODsol. Ultrasound proved the most versatile pre-treatment, as it was effective with all the studied materials. Moreover, the bacterial product was especially suitable for carbohydrate-containing materials with low dose requirement and lower costs than pure enzymes.

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References

[1] K. Rosenwinkel, H. Meyer, Anaerobic treatment of slaughterhouse residues in municipal digesters, Water Sci. Technol. 40 (1) (1999) 101–111.

[2] J. Martinez, L. Borzacconi, M. Mallo, M. Galisteo, M. Vinas, Treatment of slaughterhouse wastewater, Water Sci. Technol. 3 (12) (1995) 99–104.

[3] D. Batstone, J. Keller, R. Newell, M. Newland, Modelling anaerobic degradation of complex wastewater. II: Parameter estimation and validation using slaughterhouse effluent, Bioresource Technol. 75 (2000) 75–85.

[4] G. Vidal, A. Carvalho, R. Méndez, J. Lema, Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters, Bioresource Technol. 74 (2000) 231–239.

[5] L. Massé, K. Kennedy, S. Chou, Testing of alkaline and enzymatic hydrolysis pretreatments for fat particles in slaughterhouse wastewater, Bioresource Technol. 77 (2001) 145–155.

[6] L. Massé, D. Massé, K. Kennedy, Effect of hydrolysis pre-treatment on fat degradation during anaerobic digestion of slaughterhouse wastewater, Process Biochem. 38 (2003) 1365–1372.

[7] M. Edström, Å. Nordberg, L. Thyselius, Anaerobic treatment of animal byproducts from slaughterhouses at laboratory and pilot scale, Appl. Biochem. Biotech. 109 (2003) 127–138.

[8] A. Rinzema, M. Boone, K. van Knippenberg, G. Lettinga, Bactericidal effect of long chain fatty acids in anaerobic digestion, Water Environ. Res. 66 (1994) 40–49.

[9] M. Pereira, Z. Sousa, M. Mota, M. Alves, Mineralization of LCFA associated with anaerobic sludge: kinetics, enhancement of methanogenic activity and effect of VFA, Biotechnol. Bioeng. 88 (2004) 502–511.

[10] D. Cirne, X. Paloumet, L. Björnsson, M. Alves, B. Mattiasson, Anaerobic digestion of lipid-rich waste—effects of lipid concentration, Renew. Energy 32 (2007) 965–975.

[11] K. Hansen, I. Angelidaki, B. Ahring, Anaerobic digestion of swine manure: inhibition by ammonia, Water Res. 32 (1998) 5–12.

[12] P. Dalev, Utilisation of waste feathers from poultry slaughter for production of a protein concentrate, Bioresource Technol. 48 (1994) 265–267.

[13] Z. Wang, C. Banks, Evaluation of a two stage anaerobic digester for the treatment of mixed abattoir wastes, Process Biochem. 38 (2003) 1267–1273.

[14] A. Mendes, E. Pereira, H. de Castro, Effect of the enzymatic hydrolysis pretreatment of lipids-rich wastewater on the anaerobic biodigestion, Biochem. Eng. J. 32 (3) (2006) 185–190.

[15] A. Valladão, D. Freire, M. Cammarota, Enzymatic pre-hydrolysis applied to the anaerobic treatment of effluents from poultry slaughterhouses, Int. Biodeter. Biodegr. 60 (2007) 219–225.

[16] H. Gavala, U. Yenal, I. Skiadas, P. Westermann, B. Ahring, Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevate temperature, Water Res. 37 (2003) 4561–4572.

[17] A. Vlyssides, P. Karlis, Thermal-alkaline solubilization of waste activated sludge as a pre-treatment stage for anaerobic digestion, Bioresource Technol. 91 (2004) 201–206.

[18] L. Skiadas, H. Gavala, J. Lu, B. Ahring, Thermal pretreatment of primary and secondary sludge at 70 °C prior to anaerobic digestion, Water Sci. Technol. 52 (1/2) (2005) 161–166.

[19] M. Climent, I. Ferrer, M. Mar Baeza, A. Artola, F. Vázquez, X. Font, Effects of thermal and mechanical pretreatments of secondary sludge on biogas production under thermophilic conditions, Chem. Eng. J. 133 (2007) 335–342.

[20] A. Tiehm, K. Nickel, U. Neis, The use of ultrasound to accelerate the anaerobic digestion of sewage sludge, Water Sci. Technol. 36 (11) (1997) 121–128.

[21] Y. Chiu, C. Chang, J. Lin, S. Huang, Alkaline and ultrasonic pretreatment of sludge before anaerobic digestion, Water Sci. Technol. 36 (11) (1997) 155–162.

[22] C. Bougrier, C. Albasi, J. Delgenès, H. Carrère, Effect of ultrasonic, thermal and ozone pre-treatments on waste activated sludge solubilisation and anaerobic biodegradability, Chem. Eng. Process 45 (2006) 711–718.

[23] C. Braguglia, G. Mininni, M. Tomei, E. Rolle, Effect of feed/inoculum ratio on anaerobic digestion of sonicated sludge, Water Sci. Technol. 54 (5) (2006) 77–84.

[24] J. Van Leeuwen, B. Akin, S. Khanal, S. Sung, D. Grewell, J. van Leeuwen, Ultrasound pre-treatment of waste activated sludge, Water Sci. Technol. 6 (Suppl. 6) (2006) 35–42.

[25] K. Nickel, U. Neis, Ultrasonic disintegration of biosolids for improved biodegradation, Ultrason. Sonochem. 14 (2007) 450–455.

[26] A. Antoniadis, I. Poulios, E. Nikolakaki, D. Mantzavinos, Sonochemical disinfection of municipal wastewater, J. Hazard. Mater. 146 (2007) 492–495.

[27] E. Gonze, N. Commenges, Y. Gonthier, A. Bernis, High frequency ultrasound as a pre- or a post-oxidation for paper mill wastewaters and landfill leachate treatment, Chem. Eng. J. 92 (2003) 215–225.

[28] A. Silva, A. Nouli, A. Carmo-Apolinário, N. Xekoukoulotakis, D. Mantzavinos, Sonophotocatalytic/H_2O_2 degradation of phenolic compounds in agro-industrial effluents, Catal. Today 124 (2007) 232–239.

[29] E. Gonze, L. Fourel, Y. Gonthier, P. Boldo, A. Bernis, Wastewater pre-treatment with ultrasonic irradiation to reduce toxicity, Chem. Eng. J. 73 (2) (1999) 93–100.

[30] A. Tiehm, K. Nickel, M. Zellhorn, U. Neis, Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization, Water Res. 35 (2001) 2003–2009.

[31] T. Blume, U. Neis, Improved wastewater disinfection by ultrasonic pre-treatment, Ultrason. Sonochem. 11 (5) (2004) 333–336.

[32] T. Benabdallah El-Hadj, J. Dosta, R. Márquez-Serrano, J. Mata-Álvarez, Effect of ultrasound pretreatment in mesophilic and thermophilic anaerobic digestion with emphasis on naphthalene and pyrene removal, Water Res. 41 (2007) 87–94.

[33] J. Lin, C. Chang, S. Chang, Enhancement of anaerobic digestion of waste activated sludge by alkaline solubilisation, Bioresource Technol. 62 (1997) 85–90.

[34] I. Karlsson, Carbon source for denitrification from pre-precipitated sludge, in: H. Hahn, R. Klute (Eds.), Chemical Water and Wastewater Treatment, Springer, Berlin, 1990, pp. 521–529.

[35] N. Heo, S. Park, H. Kang, Solubilization of waste activated sludge by alkaline pre-treatment and biochemical methane potential (BMP) test for anaerobic co-digestion of municipal organic waste, Water Sci. Technol. 48 (8) (2003) 211–219.

[36] C. Lin, Y. Lee, Effect of thermal and chemical pretreatments on anaerobic ammonium removal in treating septage using the UASB system, Bioresource Technol. 83 (2002) 259–261.

[37] APHA, Standard Methods for Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington DC, USA, 1998.

[38] SFS, 5504 Determination of chemical oxygen demand ($\mathrm{COD}_{\mathrm{Cr}}$) in water with the closed tube metho, oxidation with dichromate, Finnish Standards Association, Helsinki, 1988.

[39] C. Bougrier, H. Carrère, J. Delgenès, Solubilisation of waste-activated sludge by ultrasonic treatment, Chem. Eng. J. 106 (2) (2005) 163–169.

[40] A. van Velsen, G. Lettinga, D. den Ottelander, Anaerobic digestion of piggery waste; influence of temperature, J. Agric. Sci. 27 (1979) 255–267.

[41] R. Fessenden, J. Fessenden, Organic Chemistry, 6th ed., Brooks/Cole Publishing Company, Montana, 1998.

[42] W. Robinson, J. Odom, H. Holtzclaw Jr., General Chemistry with Quantitative Analysis, 10th ed., Houghton Mifflin, Boston, 1997.

[43] A. Elliot, T. Mahmood, Pretreatment technologies for advancing anaerobic digestion of pulp and paper biotreatment residues, Water Res. 41 (2007) 4273–4286.

[44] E. Salminen, J. Rintala, Anaerobic digestion of organic solid poultry slaughterhouse waste—a review, Bioresource Technol. 83 (2002) 13–26.

[45] D. Prats, M. Rodrguez, P. Varo, A. Moreno, J. Ferrer, J. Berna, Biodegradation of soap in anaerobic digesters and on sludge amended soils, Water Res. 33 (1999) 105–108.

[46] I. Angelidaki, B. Ahring, Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure, Water Sci. Technol. 41 (3) (2001) 189–194.

[47] A. Lehtomäki, T. Viinikainen, O. Ronkainen, R. Alen, J. Rintala, Effects of pre-treatments on methane production potential of energy crops and crop residues, in: Proceedings of the 10th World Congress of Anaerobic Digestion, vol. 2, Montreal, 2004, pp. 1016–1021.
[48] L. Fan, M. Gharpuray, Y. Lee, Evaluation of pre-treatments for enzymatic conversion of agricultural residues, Biotech. Bioenery Symp. 11 (3) (1981) 29–45.
[49] E. Salminen, J. Rintala, L. Lokshina, V. Vavilin, Anaerobic batch degradation of solid poultry slaughterhouse waste, Water Sci. Technol. 41 (3) (2000) 33–41.
[50] S. Lafitte-Trouqué, C. Forster, The use of ultrasound and γ-irradiation as pre-treatments for the anaerobic digestion of waste activated sludge at mesophilic and thermophilic temperatures, Bioresource Technol. 84 (2002) 113–118.
[51] W. Sanders, Anaerobic hydrolysis during digestion of complex substrates, PhD Thesis, Wageningen University, Wageningen, 2000.
[52] I. Koster, A. Cramer, Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids, Appl. Environ. Microbiol. 53 (1987) 403–409.
[53] M. Pereira, O. Pires, M. Mota, M. Alves, Anaerobic biodegradation of oleic and palmitic acids: evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge, Biotechnol. Bioeng. 92 (2005) 15–23.
[54] C. Hwu, S. Tseng, C. Yuan, Z. Kulik, G. Lettinga, Biosorption of long-chain fatty acids in UASB treatment process, Water Res. 32 (5) (1998) 1571–1579.
[55] G. Kalle, K. Menon, Inhibition of methanogenesis and its reversal during biogas formation from cattle manure, J. Biosci. 6 (3) (1984) 315–324.
[56] J. Mata-Alvarez, S. Mace, P. Llabrés, Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives, Bioresource Technol. 74 (2000) 3–16.
[57] Y. Kuang, Enhancing anaerobic degradation of lipids in wastewater by addition of co-substrate, PhD thesis, School of Environmental Science, Murdoch University, Melville, 2002.