Advanced anaerobic processes to enhance waste activated sludge stabilization

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ABSTRACT

The requirement for enhanced stabilization processes to obtain a more stable, pathogen-free sludge for agricultural use is an increasing challenge to comply with in the waste hierarchy. With this in mind, the Routes European project ('Novel processing routes for effective sewage sludge management') is addressed to assess innovative solutions with the aim of maximizing sludge quality and biological stability. In order to increase anaerobic stabilization performances, the sequential anerobic/aerobic process and the thermophilic digestion process, with or without integration of the thermal hydrolysis pre-treatment, were investigated as regards the effect on sludge stabilization, dewaterability and digestion performances. Thermal pre-treatment improved anaerobic digestion in terms of volatile solids reduction and biogas production, but digestate dewaterability worsened. Fluorescence *in situ* hybridization (FISH) quantification showed an increase of methanogens consistent with the increase of biogas produced. The aerobic post-treatment after mesophilic digestion had a beneficial effect on dewaterability and stability of the digested sludge even if was with a reduction of the potential energy recovery.

Key words | anaerobic/aerobic sequential treatment, dewaterability, FISH analysis, pre-treatment, thermophilic anaerobic digestion

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INTRODUCTION

Developing sustainable, environmentally friendly sludge management is one of the big challenges of the coming years because of the fast increase in sludge production combined with the scarcity of disposal sites. Due to the presence of nutrients and organic matter in the treated sludge, agricultural use represents a natural outlet option as a supplement to other fertilizers. However, growing concern over the presence of contaminants and pathogens, together with the tightening of quality standards, limit this option. The development of agricultural recycling therefore depends mainly on possibilities to improve sludge quality and increase confidence in sludge safety. Among various treatment options, anaerobic digestion has been recognized as an appropriate technology to approach the problem of sludge reuse, not least because of its methane production, that can substantially decrease plant costs.

Anaerobic digesters are normally operated at either mesophilic (35 °C) or thermophilic (55–60 °C) temperatures. In general, mesophilic anaerobic digestion of sewage sludge is more widely used compared to the thermophilic method, mainly due to its lower energy requirements and the higher stability of the process; nevertheless, thermophilic digestion is more efficient in terms of organic matter removal, methane production and sanitation. Anaerobic digestion of organic matter occurs by the sequential cooperative action of a number of different bacterial trophic groups. These microorganisms cooperate sequentially in order to achieve degradation of different substrates (O'Flaherty *et al*. 2006). Thus, the performance of an anaerobic digestion process is primarily linked to the structure of the microbial community present in the system (Demirel & Scherer 2008).

However, the transformation efficiency of the sludge organic particulate matter into biogas is generally low. Renewed interest for anaerobic digestion rose from the possibility of a significant performance improvement by applying an appropriate sludge pre-treatment, chemical, mechanical or thermal, leading to the breakage of flocs and cell walls enhancing solids hydrolysis (Khanal *et al.*

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2007; Braguglia *et al.* 2011). At the same time, an aerobic post-treatment could potentially provide optimal biodegradability conditions for different volatile solids fractions, improving stabilization and reducing polymer demand (Parravicini *et al.* 2008; Tomei *et al.* 2011) for the anaerobically digested biosolids.

Stability is generally defined as the point at which the food (i.e., organic matter) for rapid microbial activity is no longer available. For this reason, the concept of stability is usually associated with that of odour and putrescibility, too. The volatile/total solids ratio and/or the percentage of volatile solids destroyed can be used as a stability index. Ratios below 0.60 and percentages above 40%, generally, give an indication of achieved stabilization.

The scope of this activity was to investigate sustainable options to enhance anaerobic digestion in order to improve biosolids quality in terms of stability and dewaterability, and maximizing energy recovery. The enhanced stabilization processes evaluated in this work were: (1) thermophilic anaerobic digestion (TAD); (2) thermal pre-treated TAD; and (3) sequential mesophilic anaerobic/aerobic digestion. In order to compare the results, the waste activated sludge (WAS) used as feed was the same for the entire experiment.

Moreover, structure and population dynamics of the anaerobic mixed cultures selected in the pre-treated thermophilic digestion processes were evaluated by fluorescence *in situ* hybridization (FISH) analysis.

METHODS

Sludge composition

WAS was sampled from municipal wastewater treatment plants. Total and volatile solids (TS and VS) were determined according to *Standard Methods* (APHA 1998). To analyse the soluble phase, the particulate sludge matter was removed by centrifugation (10 min at 5,000 rpm) and the resulting centrate was filtrated through 0.45 μm filters. Soluble chemical oxygen demand (sCOD), measured in duplicate, was determined by means of Spectroquant Merck Cell tests (EPA method 410.4).

The effectiveness of the disintegration pre-treatments was evaluated by measuring the 'disintegration degree' (DD_{COD} %), namely the ratio of the sCOD increase due to pre-treatment with the maximum possible sCOD increase (Braguglia *et al.* 2006).

Total soluble nitrogen was determined in triplicate by photometric determination using the nitrogen (EN ISO 25663) cell test by Spectroquant (Merck); ammonia nitrogen was determined according to method 4500-NH_3 C of *Standard Methods* (APHA 1998).

Specific surface charge

The charge density determinations were performed by the particle charge detector PCD02 (Mütek GmbH, Herrsching) operating the principle of the so-called 'streaming current detector'. Sludge samples were centrifuged at 5,000 rpm for 10 min, the centrate was then filtered through a 1.2 μm filter and titrated in the PCD to determine the quantity of charge related to the colloidal particles, performing two replicates per sample. Sludge samples carried a net negative surface charge, mainly due to ionization of functional groups of the polymeric substances. In order to compensate for the different amounts of dry solids contained in the sludge, surface charge was 'normalized' with respect to total solids content.

Capillary suction time

Sludge filterability was estimated using a capillary suction apparatus (CST) supplied by Triton Electronics Ltd, UK. A stainless-steel tube with an inner radius of 0.535 cm and filter paper Whatman No. 17 were used. Each sludge was analysed five times and the results averaged, before being standardized to the total solids concentration (Vesilind 1988).

Microscope analyses

Sludge aliquots were diluted with distilled water and analysed by means of the contrast-phase microscope Zeiss Axioskop with ×10 of resolution.

Ultrasound pre-treatment (acoustic cavitation)

The disintegration was performed with an ultrasonic processor UP400S (dr. Hielscher, Germany) operating at 255 W and 24 kHz. Sonication energy input was varied between 2,200 and 8,800 kJ kg^{-1} dry solid on 500 mL of waste activated sludge placed in a 1 L beaker with the probe located at 3 cm above the beaker's bottom.

Hybrid pre-treatment (hydrodynamic cavitation/chemical disintegration)

Mechanical disintegration of 25 L samples of activated sludge was carried out using the hydrodynamic cavitation process. The experimental set-up consisted of a 12 bar pressure pump, rating 1.1 kW, output 500 l h^{-1}, which recirculated sludge from a container through a 1.2 mm nozzle. To force 25 L of sludge through the nozzle took 3 min. Disintegration was carried out for 15, 30, 60 and 90 min. For chemical disintegration by alkalization, from 1 to 6 mL of NaOH 2 M per mL of sludge was used. Sodium hydroxide was added to samples of activated sludge to maintain a given pH value (8, 9, 10 and 11) for 30 min.

Thermal pre-treatment

Thermal pre-treatment was carried out using a bench scale autoclave Laboklav 25b, with a total capacity of 25 L and able to work at $T_{max} = 135\,^{\circ}C$ and $p_{max} = 312$ kPa. The tests were conducted with times from 5 to 20 min on 300 mL of gravity-thickened WAS.

TAD of untreated and pre-treated sludge

Digestion of sludge was carried out using two glass anaerobic digesters operated in semi-continuous mode under the same conditions. One reactor, as control unit, was fed with untreated WAS, and the second one with the same sludge but after thermal pre-treatment ($T = 135\,^{\circ}C$, $p = 312$ kPa, $t = 20$ min). Both jacketed reactors (7 L) were completely mixed and maintained at the constant temperature of 55 °C.

The produced biogas was collected by water displacement in a biogas collection unit. The gas meter consisted of a volumetric cell for gas–liquid displacement, a sensor device for liquid level detection, and an electronic control circuit for data processing and display. Biogas composition was measured using a GC PerkinElmer Autosystem, equipped with TCD (thermal conductivity detector).

In test #1 the reactors were operated at a hydraulic retention time (HRT) of 8 days with a corresponding organic loading rate (OLR) of 1.7 gVS L^{-1} d^{-1}. In test #2 the organic load was decreased to 1.0 gVS L^{-1} d^{-1} (HRT = 15 days). The disintegration degree of the pre-treated feed was kept constant (13–14%).

Data were collected in steady-state conditions, that is, after reaching constant specific biogas production.

Sequential anaerobic/aerobic digestion process

Laboratory scale reactors of 7.4 L were operated in series, with WAS fed to the anaerobic reactor once per day and an equivalent volume of digested sludge extracted from the anaerobic reactor and fed to the following aerobic reactor. Both reactors were equipped with mechanical stirrers.

The first reactor was equipped with a thermostatic jacket and a control device keeping the temperature at 37 ± 0.5 °C. The working volume was 7 L and HRT was controlled at 15 days. The second reactor, operated under aerobic conditions, had a working volume of 4.5 L, and air was supplied by a compressor able to maintain the concentration of dissolved oxygen at levels of ~3 mg/L. The reactor was operated at room temperature with an HRT of 12 days.

Biogas production and methane content were determined following the same procedure described for the thermophilic digestion process.

Microbial population analysis

For microbial population analysis, each reactor was periodically sampled during start-up and steady-state operating conditions. FISH analysis was performed on paraformaldehyde/ethanol-fixed biomass samples according to the procedure described in Amann et al. (1995). The following probes were used: EUB338mix for total bacteria and ARCH915 for total archaea (Loy et al. 2007). Samples were examined by epifluorescence microscopy (Olympus BX51). All the hybridizations with specific probes were carried out in combination with DAPI staining to estimate the portion of cells targeted by group-specific probes out of the total cells. Quantification of positive cells was done on images taken from the samples with a digital camera (Olympus XM-10), using the ImageJ software package (version1.37v, Wayne Rasband, National Institute of Health, Bethesda, MD, USA, available in the public domain at http://rsb.info.nih.gov/ij/index.html).

RESULTS AND DISCUSSION

Cavitation and thermal hydrolysis pre-treatment

The release of organic matter expressed as an increase in sCOD value is considered the key parameter to follow the sludge biomass disintegration needed to improve the hydrolysis rate in the successive digestion process. In this section, the effect of three different pre-treatments, namely ultrasound and hydrodynamic disintegration (Table 1), and thermal hydrolysis (Table 2) are presented. As regards the acoustic cavitation, low frequency ultrasound pre-treatment affected sludge properties significantly: in fact, by progressively increasing the energy up to 8,800 kJ/kgTS, the solubilization increased linearly, reaching a disintegration degree of around 9%. Besides the sCOD increase, the release of colloidal particles also occurred, causing filterability worsening. In fact, after 8 min of sonication, CST increased by nearly 100 times (up to 40 sec L/gTS), and specific surface charge by more than 25 times (up to 2,559 mC/gTS), with respect to the untreated sludge. This phenomenon confirms the disintegration of sludge leading to a significant decrease in particle dimensions. As a consequence, the specific surface, and thus the charge density, increased. Disintegration was confirmed by microscope analysis, highlighting that by applying energy up to 10,000 kJ/kgTS, the sludge floc structure was completely broken and high cell dispersion in the liquid phase was observed.

In addition, hydrodynamic cavitation exerted floc disintegration and solubilization. In fact, after just 10 min of pre-treatment the disintegration degree was around 12%, rising with increasing time up to 30% after 45 min of pre-treatment.

During the cavitation tests, both acoustic or hydrodynamic, TS and VS content remained almost unvaried, evidencing that no mineralization or evaporation phenomena take place in these conditions.

Based on several factors, including high power consumption and additional chemical sludge decomposition, the hydrodynamic disintegration time of 30 min was chosen for further investigation. Hydrodynamic disintegration was coupled with alkaline addition to assess the improvement due to the chemical decomposition principally in terms of the solubilization of organic matter (Figure 1).

The pronounced hydrolysis effects resulting from the process of pre-alkalization may be attributed to the 'softening' of cell walls of bacteria, dissolving non-cellular polymers at the same time.

In addition, the effect of thermal pre-treatment on WAS was investigated in order to verify efficiency as regards organic solubilization and colloid release in relation to dewaterability parameters as specific surface charge and filterability (Table 2).

The efficiency in disintegrating organic matter was highlighted by the increase of sCOD: after just 5 min a

Table 1 | Cavitation tests on WAS

	Untreated	Pre-treated		
Acoustic cavitation (ultrasound)				
Sonication time (min)		2	4	8
Sonication specific energy (kJ/kgTS)		2,200	4,400	8,800
DD_{COD} (%)		2.9%	6.2%	8.5%
TS (g/L)	27.7	27.3	27.3	27.2
VS/TS (%)	68	68.5	68.5	69
sCOD (mg/L)	< 15	553	1,175	1,598
CST (sec.L/gTS)	0.4	17.5	24.8	39
Particle charge density (mC/gTS)	99	1,185	1,552	2,559
Hydrodynamic cavitation				
Pre-treatment time (min)	0	15	30	45
DD_{COD} (%)	–	11%	23%	32%
TS (g/L)	17.4	17.2	17.3	17.2
sCOD (mg/L)	532	1,210	1,790	2,380

Table 2 | Thermal hydrolysis tests on WAS

	Untreated	Pre-treated		
Pre-treatment time (min)	–	5	10	20
Specific energy (kJ/kgTS)	–	6,300	12,500	25,000
DD_{COD} (%)	–	18.6	20.1	21.8
TS (g/L)	18.8	19.0	18.8	19.7
VS/TS (%)	72	75	75	74
sCOD (mg/L)	40	3,860	3,990	4,340
CST (sec.L/gTS)	0.7	48	59	66
Particle charge density (mC/gTS)	124	3,111	3,840	4,241

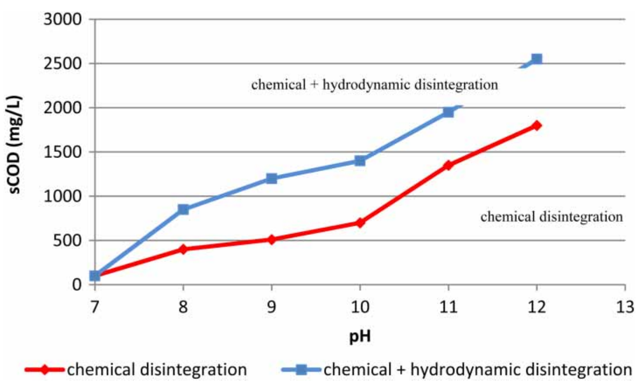

Figure 1 | Release of soluble COD after chemical disintegration alone, and coupled with hydrodynamic cavitation.

disintegration degree of around 19% was achieved. By enhancing the pre-treatment time up to 20 min the increase was less intense, achieving a disintegration degree of 22%. The increase of the specific charge was due to the increased amount of colloidal and polymeric material in solution, confirming floc disintegration. Moreover, a strict correlation between particle charge density and CST was observed, indicating that the increase of colloidal particles in solution due to pre-treatment negatively affects the sludge filterability.

Untreated WAS was characterized by the typical morphology with filamentous bacteria protruding from the surface of compact flocs. No significant effect on floc dimensions and material dispersion was observed after 20 min of thermal pre-treatment, but the significant increase of sCOD up to 4,300 mg/L highlighted that, despite no relevant physical disintegration, particulate solubilization had already occurred. High temperatures do not present the mechanical effect of floc breakage, typical of the shear forces generated by cavitation pre-treatments, but allow the floc matrix to destructurate, dissolving the entrapped organics.

Taking into account the aim of this work, namely to investigate sustainable options to enhance anaerobic digestion in order to improve biosolids quality, thermal hydrolysis was chosen as the pre-treatment due to: (1) the sludge solubilizing efficiency, (2) the hygienization potential, and (3) the heat recovery needed for the successive thermophilic digestion process.

TAD process

The thermophilic digestion of thermal pre-treated sludge during test #1 was characterized by higher VS destruction (42 *vs* 45%), higher cumulative biogas production and comparable methane content of the biogas (~ 70%) with respect to the digestion of untreated sludge. The specific biogas production on the VS fed to the digesters was comparable for both untreated and pre-treated sludge (Figure 2).

The pre-treated digested sludge turned out, however, to be richer in sCOD with respect to the untreated sludge (1.1 g/L *vs* 0.7 g/L) and showed worse dewaterability (60% higher CST value).

Furthermore, digestion test #2, carried out at lower OLR, highlighted that with thermal pre-treatment significant gains in VS removal (from 43 to 46%) and biogas production (Figure 2) occurred. After digestion, the sludge in the reactor fed with pre-treated sludge proved to be richer in soluble organic matter with respect to control (1 g/L instead of

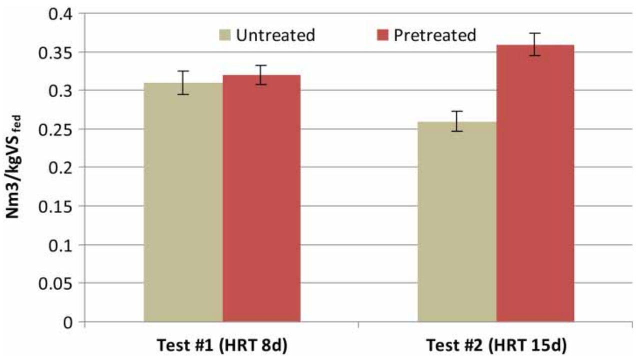

Figure 2 | Specific biogas production ($Nm^3/kgVS_{fed}$) of untreated and thermal pre-treated sludge.

0.6 g/L) and showed slightly worse filterability, with a normalized CST of 35 sec L g^{-1}TS against 30 sec L g^{-1}TS for the untreated digested sludge).

Cumulative biogas production always increased in the reactors fed with pre-treated sludge in comparison to the reference reactor; the biogas gain in tests #1 and #2 was 12 and 23%, respectively.

Soluble nitrogen and ammonia were monitored in order to control process stability and the potential risks of inhibition, because pilot scale results revealed that inhibition occurred at an ammonia concentration of 1,200 mg/L (Kayhanian 1999).

Our results showed that both thermal pre-treatment and anaerobic digestion led to an increase of soluble nitrogen, mainly composed of ammonium ions. In test #1, both untreated and pre-treated digested sludge showed a soluble nitrogen load of about 1 g/L, consisting almost entirely of ammonia (more than 90%). In test #2, the final nitrogen content of the digestate was slightly lower (0.9 g/L) due to the higher residence time in the digesters.

The biomolecular characterization of the microbial mixed communities selected during the reactor operation was performed by FISH analysis for the identification/quantification of bacteria and archaea out of the total biomass. Bacterial populations decreased over time, ranging from an initial abundance of ≈50% to a final abundance of ≈15% out of total cells; on the other hand, the archaeal population increased with time from about 16 to 65% out of total cells, highlighting that the methanogenic activity took place progressively and became the primary metabolic pathway into both reactors.

The archaeal population followed an increasing trend in both reactors. In the reactor fed with pre-treated sludge, despite the low initial relative abundance, likely due to high sCOD of the feed, the archaeal population had already reached high concentrations at the end of the first test, remaining stable with the increase of retention time. Methanogens showed higher abundance throughout the digestion of pre-treated sludge with respect to the control reactor (50 and 30% respectively), consistent with biogas production trends.

Sequential anaerobic/aerobic digestion process

The effect of an aerobic post-treatment on mesophilic anaerobic digested waste sludge was evaluated by means of a sequential process. The intermediate 15 day HRT value for the anaerobic digester was chosen because of good preliminary performances with a reduced reactor volume.

Concerning the aerobic digester, previous studies on sequential anaerobic/aerobic digestion showed that HRT values of 3–6 days in the aerobic stage were enough to show a significant improvement in VS degradation (Kumar *et al*. 2006), but these low HRT values are not suitable for nitrification, so a more conservative value of 12 days was considered in this study.

After a short start-up period of 15 days, the performance of the system varied as a consequence of the variability of the fed sludge, but the improvement of the digestion performance, gained with the additional aerobic stage, was maintained for the entire operation period.

Experimental results showed a satisfactory performance of the sequential digestion process with anaerobic VS removal efficiency of 47% and additional VS removal (+25%) in the post-aerobic stage. Concerning nitrogen fate in the proposed stabilization option, a marked nitrification efficiency (>80%) was observed. In addition, an improvement of sludge filterability was achieved through the post-aerobic stage as shown by the achieved CST reduction of 25%.

CONCLUSIONS

The investigated advanced stabilization processes, carried out with the same feed, namely real WAS, showed higher volatile solids removals with respect to the conventional mesophilic digestion process and the stability index of 40% was achieved either by adding an aerobic post-treatment or by increasing the process temperature up to thermophilic conditions (with or without thermal pre-treatment). The addition of an aerobic post-treatment provided the possibility of considerably improving sludge stabilization and filterability, but no gain in biogas production occurred, because the additional solids removal was in aerobic conditions. By increasing the temperature of the anaerobic digestion process to thermophilic conditions, process improvement regarding solids degradation and conversion rate into biogas was observed. However, the higher digestion temperature seemed to have a detrimental effect on the dewaterability. At the same time, thermal pre-treatment before thermophilic digestion generated more energy than it consumed, and quantitative FISH analysis showed an increase of methanogens consistent with the increase of biogas produced.

Finally, in order to choose the appropriate technology for implementation at a given plant, all the goals of anaerobic digestion should be evaluated according to a plant's needs and disposal routes and costs.

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