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ANAEROBIC DIGESTION DEGRADABILITY OF WASTE SLUDGE AFTER ULTRASOUND AND ENZYMIC HYDROLYSIS PRETREATMENT

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Abstract

Sludge disposal is one of the highest expenses in the management of a wastewater treatment plant. It is common practice to take advantage the anaerobic digestion of sludges to produce biogas, which is a good resource of fossil carbon-free methane, to balance at least partially the energy needs for the sludge treatment. It is possible to enhance the sludge degradability, and therefore the biogas production, by pretreating the sludge before carring out the digestion, in order to speed up the limiting stage of the hydrolysis by physically or chemically disrupting the solid matrix. At present, a remarkable reserach effort is spent to compare the yield of all the treatments that may be used.

The objective of this work is to present two pretreatments (ultrasounds and enzymic hydrolysis), and a combination of both, and to test their effectiveness at laboratory scale by implementing two rounds of biochemical methane potential tests at mesophilic conditions of a mixture of primary and secondary sludges.

Results of the comparison between untreated and treated samples, though scattered, indicate an increase in biogas production (approx. +20% in volume), an overall higher degradability (better COD conversion and higher rate of destroyed volatile solids) and an improvement in the process kinetics (approx. +10% of the hydrolysis constant). An hypothetical plant scale energy analysis is also computed to assess the energetic and economic feasibility of the pretreatments.

Moreover, the ultrasound and the enzymic hydrolysis treatments appear to have a very similar yield in biogas production, so that the choice of the most suitable treatment has to be taken considering other conditions, such as investment and management costs, plant conformation, reliability of the technology and others.

keywords: waste sludge, anaerobic digestion, biogas, pretreatment, ultrasound, enzymic hydrolysis.

Sommario

Lo smaltimento dei fanghi di supero è una delle spese più rilevanti nella gestione di un impianto di depurazione delle acque. È prassi comune implementare sistemi di digestione anaerobica dei fanghi per produrre biogas, una importante risorsa di metano che non contribuisce all'effetto serra, per poter bilanciare, almeno in parte, la richiesta di energia per il trattamento dei fanghi. È possibile aumentare la degradabilità dei fanghi, e di conseguenza la produzione di biogas, applicando pretrattamenti a monte della digestione, i quali distruggono la matrice solida del fango con lo scopo di accelerare l'idrolisi, che è la fase limitante di tutto il processo. Al momento è in corso un notevole lavoro di ricerca per confrontare il rendimento di tutti i trattamenti che è possibile utilizzare.

L'obiettivo di questa tesi è presentare due pretrattamenti possibili (ultrasuoni e idrolizi enzimica) e una applicazione congiunta di entrambi. Se ne testa inoltre l'efficacia implementando a scala di laboratorio due campagne di esperimenti per valutare il potenziale biochimico di metanizzazione in condizioni mesofile di fanghi misti primari e secondari.

I risultati del confronto tra fanghi non pretrattati e pretrattati indicano un incremento della produzione di biogas (circa +20% in volume), una complessiva migliore degradabilità (in termini di conversione di COD e di distruzione di solidi volatili) e un miglioramento della cinetica del processo (la costante di idrolisi aumenta di circa 10%). È stato inoltre calcolato un bilancio energetico di un ipotetico impianto in cui siano implementati i pretrattamenti considerati.

Infine, il trattamento a ultrasuoni e quello di idrolisi enzimica risultano avere una resa molto simile in quanto a produzione di biogas, e quindi la scelta del trattamento più adatto è da valutare considerando altri fattori, come costi di investimento e di gestione, la reale possibilità di implementazione in un impianto già esistente o l'affidabilità della tecnologia adoperata.

parole chiave: fanghi di depurazione, digestione anaerobica, biogas, pretrattamento, ultrasuoni, idrolisi enzimica.

Resumen

La eliminación de los lodos es un gasto considerable en la gestión de una estación depuradora de aguas residuales. Una práctica común de tratamiento de los lodos es la implementación de un sistema de digestión anaeróbica para la producción de biogas, un importante recurso de metano que no contribuye al efecto invernadero, para equilibrar lo más posible la necesidad de energía para los otros procesos de acondicionamiento de los lodos. Es posible incrementar la degradabilidad de los lodos, y entonces la producción de biogas, a través de la aplicación de un pretratamiento a cabecera del digestor anaeróbico, con el objetivo de destruir la componente sólida del lodo para acelerar la hidrólisis, que representa la fase limitante de todo el proceso. Actualmente un considerable trabajo de investigación se está realizando en todo el mundo para comparar el rendimiento de todos los pretratamientos que se pueden aplicar.

El objetivo de este proyecto fin de carrera es el estudio de dos posibles pretratamientos (ultrasonidos y hidrólisis encimática) y de una aplicación conjunta de ambos. Además, se compureba la eficacia de los tres a escala de laboratorio para evaluar el potencial bioquímico de metanización en condiciones mesofílicas de lodos mixtos primarios y secundarios.

Los resultados de la comparación entre lodos non tratados y tratados muestran un aumento en la producción de biogas (alrededor de 20% en volumen), una general mejor degradabilidad (en cuanto a conversión de DQO y destrucción de sólidos volátiles) y una mejora en la cinética del proceso (el coeficiente de hidrólisis aumenta aproximadamente de 10%). Un balance energético ha sido también calculado para un ipotética planta que implemente los pretratamientos considerados.

Finalmente, los tratamientos con ultrasonidos y de hidrólisis encimática parecen tener un rendimiento muy parecido en términos de producción de biogas. La opción de tratamiento más adecuado se ha entonces de elejir considerando otros factores, como los costes de inversión y de mantenimiento, la efectiva posibilidad de implementación en una planta existente, la fiabilidad de la tecnología utilizada u otros.

Chapter 1

Introduction

Sludge is the final product of the wastewater treatment process and it is a semi-solid residue composed of various organic and inorganic solids that the treatment achieved to separate from water. Wastewater treatment plants generate a large amount of sludge, roughly 1% of the volume of the influent to be treated, with a constant increase in the last decades. Concerning the European Union, in period 1992-2006 sludge production increased by 78%, from 5,5 to 9,8 million tons, especially because of the application of the 91/271/EEC Directive on Urban Waste Water Treatment.

Sludge management is the major issue of wastewater treatment plants: it can be assesed as 60% of the total plant capital cost or as approx. 500 € per ton of dry mass, and the laws for sludge disposal are becoming increasingly stringent. The European Commission encourages to exploit sludge as fertiliser in agriculture, but this option has been applied only for approx. 37% of the total amount of sludge produced[1], mainly because of the awareness regarding risks for environment and human health due to the possible presence of toxic compounds. The remaining portion is disposed off in landfills or incinerated. Sludge reduction has therefore become a major issue, in terms of economical and environmental sustainability.

There are two different, but non-alternative, approaches to sludge reduction:

1. the reduction of the amount of sludge produced, if sludge is considered waste, or

2. the recovery of materials or energy from sludge, if sludge is considered a resource.

Case #1 is considered when there are no economic advantages that make reuse of sludge an attractive investment. When this happens, the best option is to continue to reduce the amount of sludge produced, in terms of volume of wet sludge or, preferibly, of dry mass of sludge. Sludge reduction may be achieved through process of energy recovery from sludge, such as anaerobic digestion. In fact, the benefits associated with anaerobic digestion of sludge are huge, from the already mentioned mass reduction to odour removal, pathogens reduction, less energy use and, of course, energy recovery in the form of methane. Although anaerobic digestion has many positive characteristics, its developement and implementation has not been as quick as was expected, for many reasons. Anaerobic reactors are difficult to manage for the slow culturing of anaerobic microorganisms. Moreover, anaerobic digestion processes may not produce sludge to levels suitable for direct discharge, thus requiring post treatments. Therefore, methods for enhancing the activity of anaerobic bacteria and improving the efficiency of the anaerobic treatment are undergoing to a remarkable research effort. There are mainly two ways to deal with this issue:

- co-digestion, i.e. simultaneous anaerobic digestion of sludge and one or more different matrices with better biodegradability characteristics

- apply a pretreatment to the sludge: this will lead to the breakdown of organic matter, releasing the substrate that bacteria will digest, thus allowing a faster and better performing degradation

This thesis will focus on the second possibility, by analyzing the vast range of pretreatments that have been studied and applied so far, and by implementing two of them at laboratory scale to test their feasibility and effectiveness.

Co-digestion, instead, is particularly interesting in presence of organic residues which disposal can be challenging. For example, in Asturias (Spain) more cattle manure is produced than can be safely applied as fertilizer, therfore systems of co-digestion of the manure with other substances, such as glycerin, are being succesfully implemented [Castrillón et al., 2011]. This is because cattle manure has a high concentration of

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[1] Overall value for the 27 member states, referred to year 2002 (Denmark, France, Portugal and Belgium-Brussels region), 2003 (Ireland, Luxembourg, Belgium-Walloon region), 2005 (Austria, Finland, Estonia) or 2006 (remaining countries including Belgium-Flemish region)

methanogenic bacteria, and is therefore particularly suitable for this process. Cattle manure was used also in this study as inoculum, to start up the digestion.

It is important to point out that anaerobic digestion has an interesting outcome, not only as a method to improve the sludge stabilization and reduction, but also as a way to generate "clean" energy, because the methane produced is fossil carbon free, and therefore does not promote the greenhouse effect. Methane produced this way can be used to produce electricity, as a fuel for vehicles or as a heating source, lowering dependence and cost of other energy sources, that very often are not sustainable, in terms of environmental, economical and social impacts.

The thesis is divided into five chapters:

Chapter 2 Introduction to the anaerobic digestion process and review of the scientific papers regarding the pretreatment possibilities of wastewater sludges, with particular focus on sonication and enzymic hydrolysis, the two methods actually applied in the experimental work

Chapter 3 Explanation of materials and methos employed in laboratory, the parameters considered and the instruments used

Chapter 4 Analysis of the experimental results

Chapter 5 Energy balance of the whole pretreatment + anaerobic digestion process, to understand its actually feasibility at plant-scale

Chapter 6 Conclusions and further future developments

Moreover, the thesis is completed by two appendices:

Appendix A Complete data sets of the laboratory measurements (analytical characterizations and biogas production and composition)

Appendix B Brief description of the two wastewater plants from which the sludges were collected

Chapter 2

The anaerobic digestion process

The anaerobic digestion is a process made up by a series of microbiological mechanisms that, in absence of oxygen, convert degradable organic matter to biogas (mainly methane and carbon dioxide).

The digestion process is divided into four stages and involves five different groups of bacteria. The first stage is the *hydrolysis* in which hydrolytic bacteria convert original compounds (carbohydrates, proteins, lipids) to monomers, amino acids and long chain fatty acids. Next stage is *acidogenesis*, in which organic acids formed in the hydrolysis are converted by acetogenic micro-organisms to short chain fatty acids, ethanol and methanol, with production of H_2 and CO_2. After that, acetotogenic bacteria, by means of *acetogenesis*, convert the products of the acidogenesis into acetic acid and formic acid, again with production of H_2 and CO_2. The final stage is the *methanogenesis*, in which methanogenic bacteria produce methane from acetic acid or by concurrent oxidation of H_2 and reduction of CO_2.

Anaerobic digestion yield and efficiency is strictly correlated with growth and acclimation of the bacterial species responsible for the process. In particular, temperature plays a fundamental role, as each bacterial population can survive only at specific temperature conditions. The choice of the best temperature to carry out the digestion is therefore very important to ensure the best possible kinetics and the bacterial growth. It is possible to identify three intervals of temperature:

- Psychrophilic conditions: 4-15°C

- Mesophilic conditions: 20-40°C, with optimal value around 35°C

- Termophilic conditions: 45-70°C, with optimal value around 55°C

At mesophilic conditions, the digestion process is more stable and less sensible to changes in the operating conditions. At termophilic conditions, high temperatures exert a sanitizing effect on pathogens. However, the optimal temperature conditions have to be chosen considering energetic issues too, so that mesophilic conditions are usually the best alternative.

Among the four phases that compose the digestion process, the hydrolysis is usually the rate-limiting step as most of the organic matter consists of particulate or colloidal material and therefore requires a first phase of disaggregation to macro-compounds, followed by the hydrolysis. The possibility to facilitate the hydrolysis phase and so to enhance the whole process is currently undergoing a good research effort. In particular, the field of pretreatments is attracting much interest, as it is an efficient way to release energy to the sludge from an external source, energy that is used to rupture the cell wall of the organic matter, thus releasing the intracellular matter into the aqueous phase, making it directly available to the hydrolytic bacteria.

Generically, pretreatments have a threefold purpose of increasing biogas production, decreasing the amount of sludge to be disposed of and increasing the sludge stability by increasing its dewaterability. Each pretreatment differentiate from the others in terms of results by its effectiveness in these three aspects: it is possible to choose the most appropriate pretreatment depending on the reasons for which it is necessary, in addition to obvious considerations about the investment and operating costs, the availability of space, the desired efficiency, etc.

2.1   Pretreatments

There is a very wide range of pretreatments that are being investigated and applied, and it is common to classify them according to the origin of the forces involved (mechanical, chemical or biological) so that there are physical, chemical and biological pretreatments, plus another class considering the combination of two or more single pretreatments.

Pretreatments available in literature are briefly described below, with particular focus on those specific for sludge treating. The majority already have full-scale plant applications.
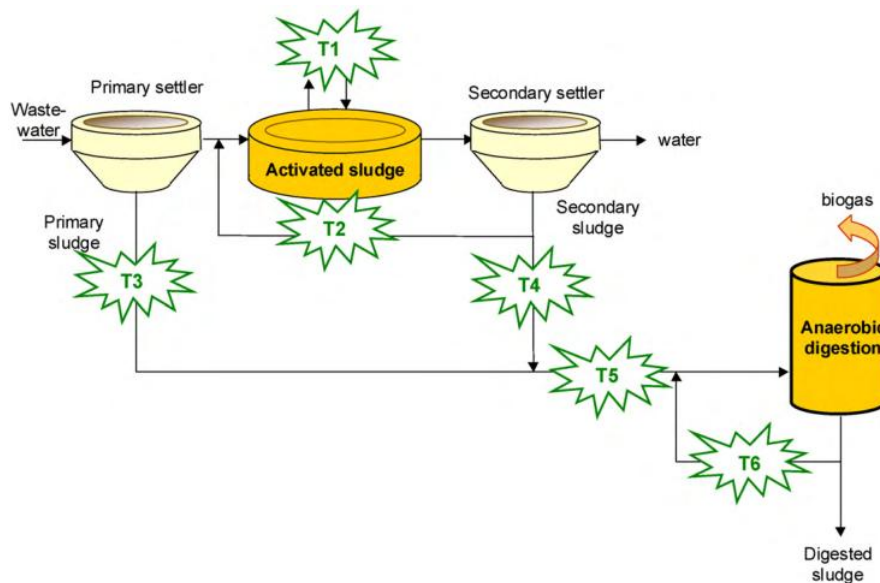
Figure 2.1: Potential location for sludge treatments in a conventional wastewater treatment plant. T1: Cotreatment on activated sludge process. T2: Cotreatment on the activated sludge recirculation loop. T3: Pretreatment of primary sludge before anaerobic digestion. T4: Pretreatment of waste activated sludge before anaerobic digestion. T5: Pretreatment of mixed sludge before anaerobic digestion. T6: Cotreatment on the anaerobic digester recirculation loop [Carrère et al., 2010]. Pretreatments described in this chapter are type T4 and/or T5.

2.1.1 Physical pretreatments

Thermal hydrolysis

Thermal pretreatment aims to improve the hydrolysis rate by destroying sludge cell walls with high temperatures. A temperature range of 160-180°C is considered to be the best, combined with treatment times between 30 and 60 minutes and pressure ranging from 600 to 2500 kPa [Carrère et al., 2010]. Higher temperatures lead to higher disintegration rates but reduce the biodegradability of the sludge, as formation of non-biodegradable compounds is observed. At lower temperatures (experiments at 70 and 121°C) the increase in biogas production is limited to +20-30%, whilst at 160-180°C it reached a +40-100% increase (but results are very scattered). The increase of methane production has been linked to sludge COD solubilisation by linear correlations [Bougrier et al., 2008]. This leads to an increase of biodegradability by 40-60%, meaning an increase of the hydrolysis rates and a consequent reduction of the hydraulic retention time in the digester up to 2,9 days. Moreover, a higher dewaterability is observed (approx. +30%) allowing higher efficiencies in the water removal final stage of sludge treatment. Another important effect of thermal hydrolysis is the sanitation achieved by high temperature. It is also important to point out that there is no extra energy need, as energy requirements can be covered by excess biogas production and the energy balance is generally positive.

Negative aspects of thermal hydrolysis concern implementation and plant operation, as corrosion and fouling of mechanical parts is a big issue and bad odours are produced. Moreover, digested sludge undergone thermal hydrolysis has a higher organic content (up to $+13,2\% \, g_{CODs}/g_{VS}$) [Pérez-Elvira et al., 2008].

However, thermal hydrolysis is a well established treatment and is used in many plants, mainly in northern Europe. The two more widely used technologies are the Norwegian CAMBI® and the VEOLIA BIOTHELYS®.

In Italy the biggest wastewater plant with a thermal hydrolysis equipment is Monza-S. Rocco (650000 p.e.). The biggest one of the world is probably that of Dublin, Ireland (1200000 p.e.).

Ultrasonication

This pretreatment is described in greater detail in chapter 2.2, as it is one of the pretreatments applied in the research part of this thesis (chapter 3 onwards).

Radiation driven pretreatment

This class is composed by all kind of pretreatments performed by microwaves, electron beams or gamma-rays radiations. It is a kind of pretreatment that has not been implemented yet in full-scale plants, but it is undergoing a good research effort as radiations can travel through materials generating considerable changes to material structure and properties, and it is already a well established technique to sanitize medical and laboratory instruments.

Radiation show to be particularly effective when treating cellulose-based materials, as energy tends to be absorbed into the molecules generating short- and long-term radicals that start chain reactions causing the degradation of the material. In particular, radical species located in the cellulose crystalline region undergo a progressive decay, resulting into an even deeper and faster degradation [Chunping et al., 2008].

Microwaves, electron beams and gamma-rays radiation pretreatments, though based on the same concept, have very different implementations and effectiveness, depending mostly on radiation dose and penetration power.

Microwaves cause a rapid heating of sludge because of molecular rotation. Cells structure is therefore destroyed, releasing water. The two main effects are the increment in the sludge dewaterability and the sanitation. Laboratory tests showed that biogas production enhancement is linearly correlated to the specific energy supplied and in inverse proportion to the sludge water content, suggesting that a previous dehydration should be carried out in order to achieve higher efficiencies.

Electron beam accelerators are complex devices that accelerate electrically charged particles in a specific direction, allowing materials to undergo to a so-called absorbed radiation dose, measured in Grays (Gy = J/kg). Effectiveness of this technology was proved by several studies. In particular, [Park et al., 2009] proved that electron beams are able to increase soluble COD concentrations when applied with high absorbed radiation doses and thin sludge samples: the best result (7 kGy radiation dose and 0,5 cm thick sludge samples) achieved a 22% increase in biogas production.

By contrast, gamma-rays radiations are not yet considered as an attractive option because of their potential dangerousness and difficult operativeness.

Collision plate

In this treatment, sludge is hurled to a collision plate by a high pressure pump (30-50 bar) with speeds of 30-100 m/s. The sludge jet that collides with the plate breaks the bacterial cell membrane, thus providing a better solubilization and accelerating the availability of the degradable matter. This process has only been applied at laboratory scale and provided a hydraulic retention time decrease from 14 to 6 days, without affecting anaerobic digestion performance [Carrère et al., 2010].

High pressure homogenizer

Sludge pressure is increased up to 900 bar, then sludge goes through an homogenization valve under strong depressurization [Carrère et al., 2010]. At full-scale implementation, a fraction of digested sludge was treated at 150 bar and re-introduced into the digester, leading to 30% increase of biogas production and 23% reduction of sludge volume. However, a decrease in sludge dewaterability was reported.

The main disadvantages related to this pretreatment are the high energy needs and problems of obstruction and erosion of the instruments. However, high pressure homogenization technique has various plant-scale implementations, the most important being

- the CROWN PROCESS (BIOGEST), that operates at 12 bar.

- the CELLRUPTOR or RAPID NON-EQUILIBRIUM DECOMPRESSION RND PROCESS (ECOSOLIDS), which is based on the introduction of a soluble gas in the sludge stream that diffuses rapidly across the cell walls and generates extremely high shear rates causing irreversible cell disruption and releasing the interstitial water. An increase in biogas production from $0,3 - 0,6$ to $0,48 - 0,816\,m^3/kg_{VS}$ was registered.

- the MICROSLUDGE PROCESS (PARADIGM ENVIRONMENTAL TECHNOLOGIE). First step is the weakening of cell walls by adding chemicals to set pH to 11 or 2. Then, a high pressure (830 bar) homogenizer provides the cell disruption. This process was applied in the Los Angeles wastewater plant, where a 7% increment in degradation was registered.

Lysis centrifuge

The lysis centrifuge operates directly on the sludge stream in a dewatering centrifuge (2200-3200 rpm). A lysate stream and a thickened sludge stream are thus produced, that can be re-suspended with the liquid

stream. There are several full-scale implementations in Czech Republic and Germany, where an increase in biogas production of 15-26% was registered [Carrère et al., 2010]. Better results (+86%) were achieved by adding aerobic lysate to thickened sludge at 6% solid concentration [Elliott and Mahmood, 2007].

Concerning operational problems, this technique appears to give a good balance between advantages and disadvantages, as energy needs are very little, it does not need any chemical additive and there is no formation of bad smells. On the other hand, there is a considerable wear of materials and a not so high decomposition rate.

Stirred ball mills

Stirred ball mills pretreatment employs a cylindrical grinding chamber almost entirely filled with glass spheres that rotate under the action of a high speed stirrer (1000 rpm). In this way, disintegration of sludge solid matter is achieved, with release of intracellular matter. To allow continuous operation, the spheres are held by centrifugal force and by a bottom sieve, so that the sludge can flow through the grinding chamber.

Effectiveness of this treatment relies mainly on the choice of spheres dimension and stirrer speed. Many studies show that better results are achieved by reducing the spheres diameters up to 0,2-2,8 mm. This range of diameters prevents problems of losing the spheres with the flow and allow a complete disintegration of the cells.

The main problem of this technique is the heating produced by the impacts between the spheres and the friction with the chamber walls: it is therefore necessary a cooling system to dissipate it, such as a cooling jacket. [Chisti and Moo-Young, 1986] experimented an increase of more than 10°C when changing tip speed from 5 to 10 m/s.

Achievements of this treatment are [Winter and Müller, 2002]

- 26% increase of degradation rate in digestor

- as a consequence of the disintegration, the fraction of small particles increases, with consequent reduction of the resistance to filtration

- lower solid content after dewatering

By contrast, the main problem is the very high energy demand.

The most important stirred ball mills currently used in wastewater plants are NETZSCH MOLINEX KE5®, NETZSCH LME 50K®, DRAISWERKE COSMO 25® and the DYNE®.

2.1.2 Chemical pretreatments

Alkali treatment

The alkali treatment is a chemical treatment used to hydrolyze and decompose lipids, carbohydrates and proteins into smaller soluble substances such as volatile fatty acids, polysaccharides and amino acids.

The alkali treatment is based on disruption of cellular matter by increasing pH with, in order of efficacy, NaOH, KOH, $Mg(OH)_2$ or $Ca(OH)_2$. In this way, proteins are forced to loose their natural form, lipids saponify and RNA hydrolyzes. However, too high concentrations of Na^+ or K^+ may cause subsequent inhibition of anaerobic conditions.

Another mechanism concurrent to solubilization is the ionization of hydroxyl ions ($^-OH \rightarrow {}^-O^-$) that cause distruction of extracelluar polymeric substance (EPS, i.e. proteins, polysaccharides, lipids and nucleic acids, that hold together sludge particles to form flakes). Cells cannot survive in such a high pH environment, and therefore their internal matter is released, and the solubilization process is complete.

It is shown that sludge solubilization and anaerobic biodegradability increase with the alkali dose and temperature [Valo et al., 2004]. Also the choice of the hydroxide to use is important, as it is shown that NaOH achieves better solubilization efficiency than $Mg(OH)_2$ or $Ca(OH)_2$. By contrast, NaOH is more expensive compared to $Mg(OH)_2$ or $Ca(OH)_2$. A good cost-effectiveness balance could be given by using the KOH, although only few experiments were done so far.

The two most important researches about alkali (NaOH) treatment applied to sludge are [Lin et al., 1997] and [Li et al., 2008], which results can be summarized as follows:

- soluble COD increases up to 40%, with an optimum NaOH concentration of 0,05 mol/L, equivalent to 0,16 g/g dry solids.

- better dewatering of treated sludge at high NaOH concentrations. Instead, at low NaOH concentrations dewatering decreases, and is better to switch to $Ca(OH)_2$, as Ca^{2+} ions improve re-flocculation

- soluble COD removal increment around 50% during anaerobic digestion

- volatile solid removal increment with increasing NaOH concentrations and for higher sludge solid concentration values

- very high increment in biogas production (up to 287%) for sludge with high solid concentrations

- biogas methane content can rise up to 80% with increasing NaOH concentrations

- pH stabilization

- alkalinity increments with increasing NaOH concentrations, during the anaerobic digestion

Alkali pretreatment is also very commonly combined with ultrasonication (see chapter 2.1.4).

Acid hydrolysis

The acid pretreatment is carried out by adding sulfuric acid to the sludge with subsequent heating in a reactor. The three most important parameters to be considered are reaction time, pH and temperature. [Neyens et al., 2003] proved that the best result is achieved at 155°C, 2,5 bar and 60 minutes. Regarding pH, it was proved that best results were obtained for pH = 2, but this causes the formation of sulphates during the digestion up to a concentration of 40 g/L, and it is therefore necessary their removal with $Ca(OH)_2$. It is possible to avoid this problem by working at pH = 3. Temperature was further investigated in the same study, and it was proved that solid removal is directly proportional to temperature. However for temperature higher than 120°C the solid removal improvement decreases, and temperatures higher than 155°C are considered not worthy.

Overall, results can be summarized as follows:

- dry solids (DS) content increases at lower pH: 74,8% at pH = 1, 70,6% at pH = 3, compared to 22,5% of an untreated sample

- sludge volume after the treatment is reduced up to 25% at pH = 3, compared to an untreated sample

- heavy metals such as Cu and Hg and phosphates are released into the liquid phase and can be removed later: heavy metals can be found within the complex organic molecules that are destroyed during the hydrolysis, releasing metals and soluble salts.

Acid hydrolysis is a widely spread pretreatment, applied in many full-scale plants. It is very commonly combined with the thermal treatment (see chapter 2.1.4).

Ozonation

Ozonation is the most widely used chemical treatment. Ozone (O_3) molecules attack cell walls releasing soluble COD and react with organic compounds of the cell membrane (polysaccharides, proteins and lipids) producing lower weight compounds, that can be more easily degraded. Ozone can be industrially produced with a well established technique, based on ionization of air or of pure oxygen flow by means of a high potential difference. Moreover, ozonation technique is also widely applied in water purification processes because of its strong sanitizing properties.

The main parameters influencing the ozonation process are the water content of the sludge, the particle dimension and the ozone concentration. In particular, concerning water content, is has been shown that 30% is the optimum value, and corresponds to the fiber saturation point.

Several studies [Carrère et al., 2010; Elliott and Mahmood, 2007] report that the following results can be achieved by ozonation (refer also to fig. 2.2):

- COD increment of 11% or 16% with ozone rates of 0,03 g_{O_3}/g_{TSS} and 0,06 g_{O_3}/g_{TSS}, respectively, applied to the anaerobic digester sludge recirculation

- volatile solid reduction up to 59%, with a 0,05 g_{O_3}/g_{TSS} treatment, in an anaerobic digestion process. It is possible to reach 80% sludge reduction implementing a recirculation system in the anaerobic reactor with a dose of 0,045 g_{O_3}/g_{TSS}

- it was also proved that a dose of 0,06 g_{O_3}/g_{TSS} increment anaerobic digestion: biogas production can raise up to +30-40%. This was confirmed by other studies, at different ozonation rates (fig. 2.3). It is important to point out that optimum value appears to be 0,1 g_{O_3}/g_{COD}, while 0,2 g_{O_3}/g_{COD} dose has an even lower yield than 0,05 g_{O_3}/g_{COD}.

The main disadvantages of the ozone pretreatment are the increase of COD in the digested sludge and the high costs involved, as it is necessary to supply a high amount of ozone to reach a good efficiency. However there are many advantages, mainly related to the oxidation of pollutants and the improvement of sedimentation and dewaterability of the treated sludge. Another big advantage is the great availability of ozone generators, such as the SORBIOS, the OZONIA OZAT CFS-1 or the SIAD MIXFLO3.

The biggest implementation of ozonation process in Italy is in the wastewater plant of Caronno Pertusella (100000 p.e.).
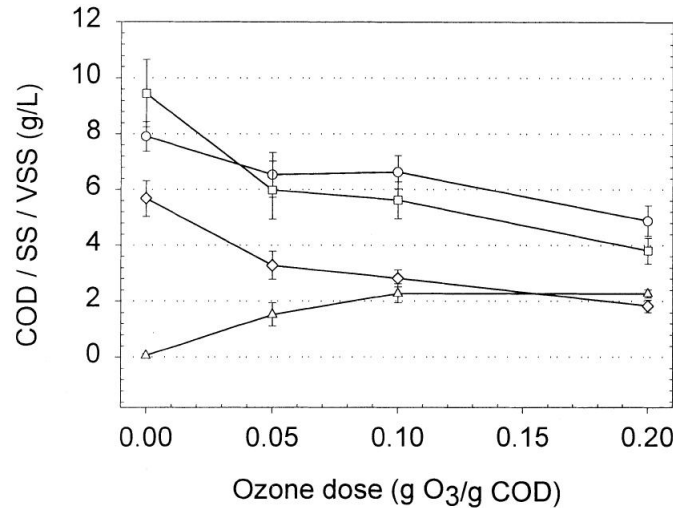
Figure 2.2: The effect of ozone on the sludge particles (circles: total COD; triangles: soluble COD; squares: SS; diamonds: VSS) [Weemaes et al., 2000]
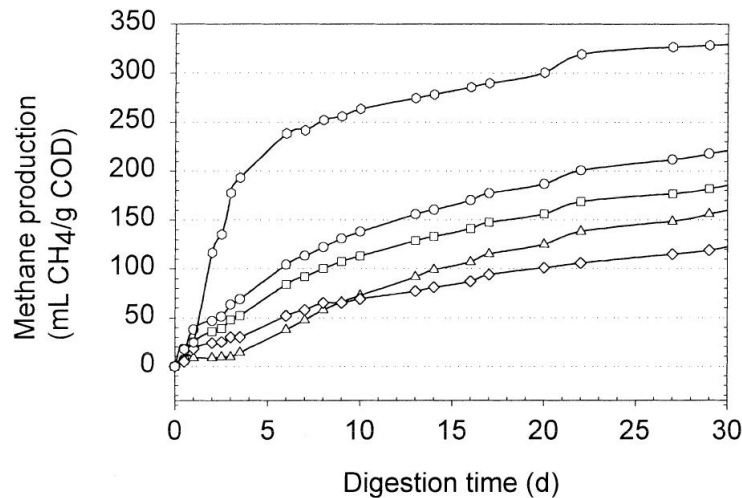
Figure 2.3: Methane production of the ozonized sludge (triangles: 0,2 g_{O_3}/g_{COD}; diamonds: no O_3; squares: 0,05 g_{O_3}/g_{COD}; circles: 0,1 g_{O_3}/g_{COD}; hexagons: acetate) [Weemaes et al., 2000]

Wet oxidation

Wet oxidation is a pretreatment in which oxygen or air is used as oxidant. Oxidation is normally performed for 10-15 minutes (up to 30 in special cases), temperatures between 170 and 200°C and pressure of 10-12 bar. It is also possible to make the process exothermic in order to reduce the energy to be supplied, by working at temperatures above 170°C.

To evaluate the effectiveness on methane production, [Taherzadeh and Karimi, 2008] carried out laboratory tests at 185-220°C and oxygen pressure of 10-12 bar for 15 minutes. Methane production increased by 35-70%. Other tests under the same conditions [Elliott and Mahmood, 2007] reported an increment of 50-60%. However data are few and very scattered.

Wet oxidation, despite being effective, was implemented only at laboratory scale and is still too expensive to be actually used. Moreover, it gives problem of odours and corrosion of the instruments and temperature

control is difficult because of the hardly supervisable reactions.

In Italy, the plant of Rovereto (TN) implements this technlogy (200000 p.e.).

2.1.3 Biological pretreatments

Enzymatic hydrolysis

This pretreatment has not to be confused with the similar one described in chapter 2.3 that is one of the pretreatments applied in the research part of this thesis (chapter 3 onwards).

The enzymatic pretreatment is based on the effect of hydrolytic enzymes (known as hydrolase), as they are able to quicken the hydrolysis process and therefore the whole digestion process. In order to increase the efficiency, more hydrolytic enzymes can be externally added to the sludge. Enzymes fit for this purpose are extracellular ectoenzymes and esoenzymes, which main difference is that ectoenzymes are located on the cell walls while esoenzymes are in the sludge water or absorbed by extracellular polymeric substances (EPS) into the sludge matrix.

EPS can be categorized into two parts, loosely bound (LB) and tightly bound (TB) fractions. Enzyme distribution in the sludge can be expressed in terms of localization in the TB or LB fractions or in the sludge water. Assay tests showed that the protease activity was localized mainly on the sludge, while α-amylase and α-glucosidase activities were largely bound with LB-EPS, and few protease, α-amylase, or α-glucosidase activities were associated with the TB-EPS fraction [Yu et al., 2007].

Another study [Yang et al., 2010] showed that α-amylase enzymes have higher efficiencies, compared to the protease ones: with a 6% enzymatic concentration, VSS decrease of 54,24% and 39,70% in reactors where, respectively, α-amylase or protease enzymes where added.

However the hydrolytic activity was higher by operating with a mixture of enzymes: due to the heterogeneous sludge composition, different enzymes are necessary to hydrolyze different substrates. The same study compared the action of amylase-only treatment with another in which a mix of enzymes was used. It was demonstrated that the best result corresponds to a mixture of amylase and protease in a 3-to-1 proportion.

As usual in biochemical processes, temperature is an important parameter: the higher it is, the more effective the process will be. A temperature of 50°C appears to give the best performance, while temperatures higher than 50°C cause the inactivation of enzymes. pH has a great influence in the system too, as optimal values are around pH = 7, but protease enzymes need a very low pH (around 2).

On the overall, adding amylase and protease enzymes to the sludge let to the following results (see also fig. 2.4):

- ratio soluble/total COD incremented

- volatile suspended solids decreased by 42%, 56,32% and 68,43% for protease, amylase and mixed-enzyme treatment (compared with 10% for the control test), that corresponds to a Michaelis-Menten model: for an increasing amount of enzymes, at low concentrations, VSS removal increased linearly. For higher concentrations, VSS removal remains constant

- biogas production is said to increase, but results were not published
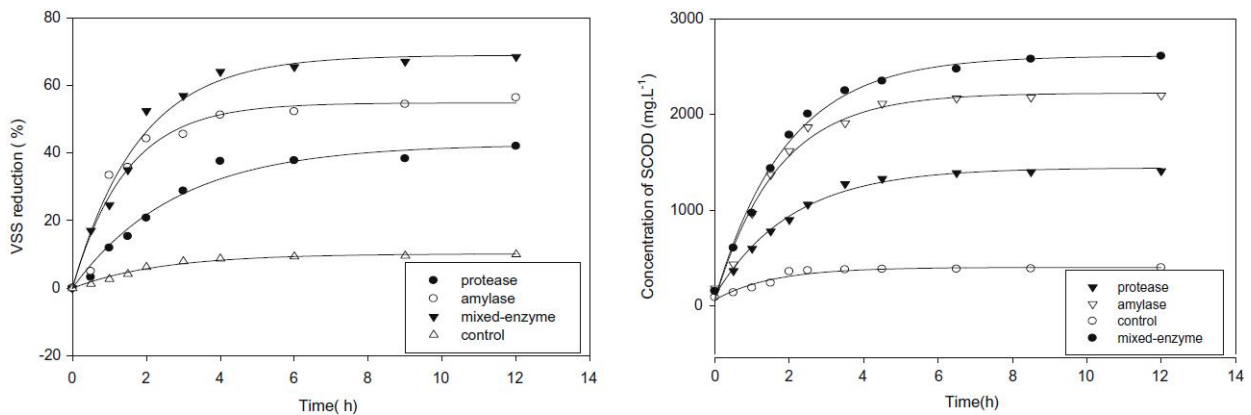
Figure 2.4: Variation of VSS reduction and SCOD production in different enzymatic hydrolysis experiments (dosage of enzymes = 6%; protease:amylase = 1:3; hydrolysis temperature = 50°C) [Yang et al., 2010].

Another study [Mendes et al., 2006] compared the action of sludge pretreated with lipase (the enzyme that catalyzes the hydrolysis of lipids) with an untreated sample. It was shown that proteins hydrolysis settled

around 30% and was not influenced by the treatment time, whereas lipids hydrolysis increased for higher treatment times (28,6% with a 4 hours treatment, 40,0% after 24 hours). After this, BMP tests were carried out, and the digested sludge composition was analyzed, giving the following results:

- sugars removal was complete in all the pretreated samples and in one of the two unpretreated samples, showing that this is the first and simpler stage in the hydrolysis

- proteins removal settled around 88-89% in both pretreated and unpretreated samples

- lipids removal best result was achieved in the 12-hours pretreated sample (80,3%), with overall substantial difference between treated and untreated samples

- lipids hydrolysis appears to be the limiting stage for the whole digestion process, concerning biogas production and organic matter removal: the best result was achieved for the 12-hours pretreated sample (78,2% COD removal and 445 mL of biogas produced).

Full scale applications of enzymatic hydrolysis are very few, and this method is still at research stage. It is expected to have good development in the next years as it is easy to implement, it has no supplementary energy costs and no chemicals additives are necessary. By contrast, enzymes are expensive to purchase or produce and there is a considerable bad odour generation. The only Italian plant where an enzymatic hydrolysis process is implemented is probably Brescia (30000 p.e.).

2.1.4   Combined pretreatments

Thermochemical treatment

Many studies proved the effectiveness of the joint application of alkali treatment and thermal hydrolysis.

Comparing the different hydroxides that can be used for the alkali part of the treatment, $Ca(OH)_2$ e $Mg(OH)_2$ gave the best yield [Neyens et al., 2003].

Another research [Tanaka et al., 1997] compares three different pretreatments, all of them performed at their best conditions of temperature, time and concentration: the alkali treatment with a dose of 0,6 g_{NaOH}/g_{VSS}, the thermal one at a temperature of 180°C and the combined thermochemical one at a temperature of 130°C for 5 minutes, with a dose of 0,3 g_{NaOH}/g_{VSS}. The thermochemical gave the best results, as shown in fig. 2.5.
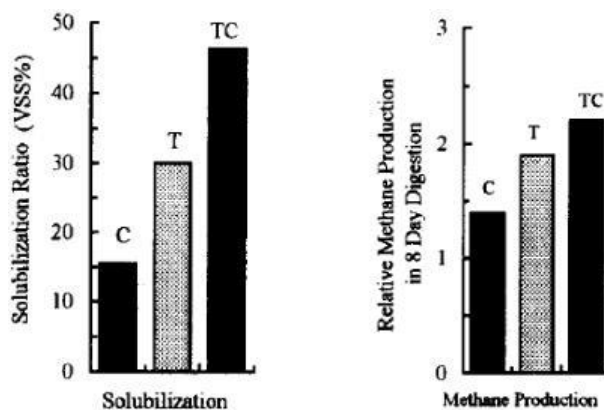
Figure 2.5: Chemical (C), thermal (T) and thermochemical (TC) pretreatments comparison [Tanaka et al., 1997]

Another possibility is to use KOH. [Valo et al., 2004] compared the effect of alkali, thermal and thermochemical pretreatments in two different conditions (1,68 g_{KOH}/L and pH =10; 3,65 g_{KOH}/L and pH = 12) and two different temperatures (130 and 170°C). Results are reported in table 2.1 and show that thermochemical pretreatment had by far the best yield.

In the same research the influence of oxidant addition (H_2O_2) to the thermochemical pretreatment was studied. At 130°C, 150 mmol/L of H_2O_2 increased soluble COD from 30,6% to 35,0%. At 90°C, the same amount of H_2O_2 leads to the same result achieved at 130°C with no H_2O_2 added. Instead, at 90°C, by doubling the oxidant amount (300 mmol/L), soluble COD increases by only 23,6%. This results are important because, at industrial scale, it is preferred to operate at high temperatures rather than with chemicals, because chemical supplies are usually expensive and they increase the net production of sludges to dispose.

Table 2.1: Chemical, thermal and thermochemical pretreatments: COD variation comparison. *Adapted from* [Valo et al., 2004]

		soluble/total COD
alkali	pH = 10	+9,3%
	pH = 12	+30,7%
thermal	130°C	+30,6%
	170°C	+59,5%
thermochemical	130°C	+63,1%
(pH = 12)	170°C	+83%

Microwave+alkali treatment

The alkali and the microwave pretreatments can be effectively combined, taking advantage of the property of the alkali to improve the dewaterability, that is the main problem of the microwave treatment.

[Doğan and Sanin, 2009] studied the alkali NaOH pretreatment combined with microwave irradiation (160°C). Their combination was assessed in terms of COD solubilization, turbidity and capillary suction time (CST). Results are reported in table 2.2. It was shown that by combining the two pretreatments, the ratio of soluble/total COD increased from 0,005 (untreated) to 0,18, 0,27, 0,34 and 0,7 with combined pretreatment (respectively for pH = 10, 11, 12 and 12,5). In addition, BMP tests were performed for pretreated samples with pH = 10, pH = 12, MW only, MW + pH = 10 and MW + pH = 12. The best result was achieved by the combined microwave + pH = 12 pretreatment, with a 18,9% increase compared to the untreated sludge. Moreover, the combined pretreatment proved to be an efficient method to minimize sludge production, as its dewaterability was 22% higher than the ine of the untreated sludge.

Table 2.2: Initial and final VSS and final COD, CST and turbidity [Doğan and Sanin, 2009]

	VSS initial (mg/L)	VSS after digestion (mg/L)	% VSS Reduction	soluble COD (mg/L)	CST (s)	Turbidity (NTU)
Untreated	5190	3010	42,00	262,5	138	484
pH = 10	4690	2960	42,97	240	157	525
pH = 12	4540	2955	43,06	272,5	151	538
MW only	4440	2525	51,35	292	129	566
MW + pH = 10	4330	2830	45,47	329	142	675
MW + pH = 12	4260	2685	48,27	322	135	707

Sonication+alkali pretreatment

[Jin et al., 2009] study proved that the combination of sonication and alkali pretreatment is more efficient than sonication or alkali alone, particularly when considering soluble COD concentration. In particular, they concluded that NaOH gives better results than Ca(OH)$_2$, and that total efficiency follows the order: simultaneous treatment > alkaline treatment followed by ultrasonic treatment > ultrasonic treatment followed by alkaline treatment.

For the simultaneous pretreatment, a dose of NaOH (0,1 g/g_{SS}), a short pretreatment duration with NaOH (30 minutes) and low ultrasound specific energy (3750 kJ/kg_{SS}) appeared to be enough to rise COD concentration (figure 2.6). Furthermore, optimal parameters were assessed for the same simultaneous pretreatment, and resulted to be 0,04 mol$_{NaOH}$/L, treatment duration time of 30 min, and sonication energy of 7500-9000 kJ/kg_{SS} (figure 2.7). With these values, efficiency of degradation of organic matter increased from 38,0% to 50,7%, which is significantly higher than that with only ultrasound (42,5%) or with only NaOH (43,5%).

2.2 Ultrasonication

The ultrasound is cyclic sound pressure (compression and expansion) with a frequency equal or greater than 20 kHz, i.e. greater than the upper limit of human hearing.
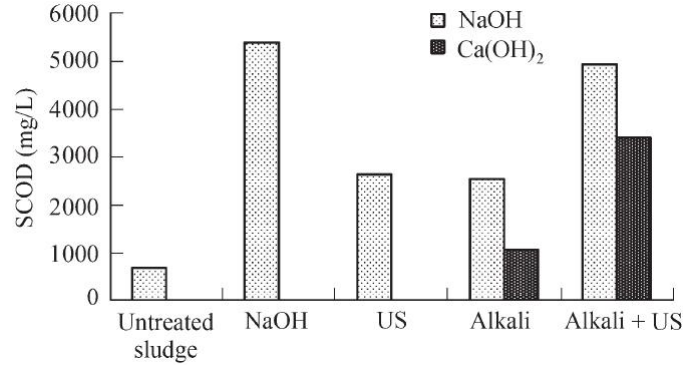
Figure 2.6: Effect of combined alkaline and ultrasonic (US) treatment on sludge solubilization [Jin et al., 2009]
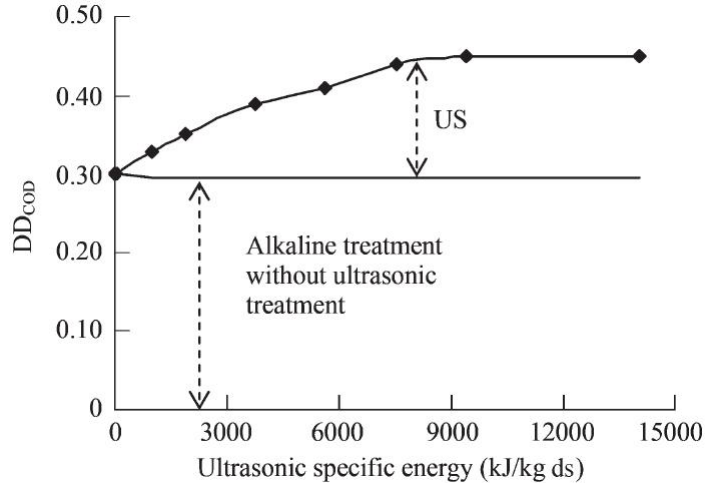
Figure 2.7: Variation of the degree of sludge disintegration (see eq. 2.1, page 27) with sonication [Jin et al., 2009]

The basic goal of ultrasound technique is to destroy bacterial cell walls and to let intracellular matter available for subsequent degradation to CH_4 and CO_2 in anaerobic digestion. When the ultrasound wave propagates, the compression cycles exert a positive pressure on the liquid by pushing the molecules together and the rarefaction cycle exerts a negative pressure by pulling the molecules from one another. Because of this excessively large negative pressure, cavitation bubbles are formed in the rarefaction regions. These microbubbles grow in successive cycles until they reach an unstable dimension that make them collapse violently producing shock waves (temperature around 5000°C and pressure of 500 atm at a lifetime of few microseconds). This process by which the bubbles form, grow and undergo violent collapse is known as *cavitation* [Pilli et al., 2010].

Ultrasounds can be generated by two mechanisms: magnetostictive and piezoelectric. In magnetostrictive technique, the electrical energy is converted to mechanical energy (vibration) with a magnetic coil attached to vibrating piece like nickel and Terfenol-D. In the piezoelectric technique, the electrical energy is converted to high frequency electric energy with piezoelectric crystals attached to the vibrating piece (called sonotrode). A converter converts the electrical or mechanical energy to sound waves and the booster is a mechanical amplifier that increases the vibration (amplitude) generated by the converter.

2.2.1 Sludge disintegration

The most common way to express the applied power/energy supplied for the sludge disintegration is the specific energy, which can be calculated as

$$E_{spec} = \frac{P \cdot t_s}{V \cdot TS} \ \left[\frac{kJ}{kg}\right]$$

where $P\,[kW]$ is the power of the ultrasonic homogenizer, $t\,[s]$ the sonication time, $V\,[L]$ the sludge treated volume and $TS\,[kg/L]$ the sludge total solid content.

The expected disintegration mechanisms during sonication are:

- Hydro-mechanical shear forces

- Oxidising effect of radical species ·OH, ·H,·O and ·N produced under the ultrasound radiation

- Thermal decomposition of volatile hydrophobic substances in the sludge

- Increase of temperature

The high temperature produced during the bubble implosion decomposes water into extremely reactive hydrogen atoms (H^+) and hydroxyl radicals (·OH) and in the cooling phase these radicals will recombine to form hydrogen peroxide and molecular hydrogen. Therefore, sludge disintegration is expected to occur in two ways: by hydro-mechanical shear forces and by the oxidising effect of ·OH. However, it was shown [Wang et al., 1999] that the oxidation effect of the hydroxide radical on sludge is negligible, therefore the disintegration of the sludge occurs mainlu by hydro-mechanical shear forces produced by cavitation bubbles.

The sonication process affects physical, chemical and biological sludge properties: consequently, the degree of sludge disintegration has to be evaluated based on the changes in physical (particle size distribution, turbidity, settleability, mass composition and microscopic examination), chemical (increase in soluble COD, protein concentration, polysaccharide content of the supernatant, nitrate nitrogen and release of NH_3) and biological (heterotrophic count and specific oxygen uptake rate) properties. These aspects were assessed in many studies, whose results are briefly summarized below.

Particle size With increase in sonication time, particle size reduces gradually. For example, the particle size reduces from 165 μm to 135 μm and 85 μm with a sonication time of 0,49 min and 1,6 min respectively. The effect of sonication on particle size is usually compared using the uniformity coefficient (dp_{60}/dp_{10}) and the dp_{10}: ultrasonication has been reported to increase uniformity coefficient of sludge by five-fold and decreases in particle size gradually with increase in specific energy [Pilli et al., 2010].

Dewaterability Ultrasonication has both positive and negative effects on sludge dewaterability. With an increase in sonication time, dewaterability of sluge decreases gradually because of the increase in the amount of small particles formed after sonication and the larger surface area for holding water. However, anerobic digestion of sludge has a positive effect on dewaterability, so it is possible to say that dewaterability of the digested sludge increases with sonication [Pilli et al., 2010].

Turbidity and settleability It was shown that sludge turbidity and settleability have opposite response to sonication, as settleability improves and turbidity decreases when the specific energy supplied is less than 1000 kJ/kg$_{TS}$, and vice versa, when energy is greater than 5000 kJ/kg$_{TS}$, settleability decreases and turbidity increases, due to complete breakdown of flocs and increase of EPS concentration [Feng et al., 2009].

COD The soluble COD of sludge increases due to solubilization of solid phase matter and increase in the concentration of organic matter and EPS in the aqueous phase. Therefore, almost all the researchers have applied soluble COD as a parameter to evaluate the sludge disintegration efficiency. However, comparison of the results is difficult because of many factors that affect the sludge disintegration, such as the sludge type, total solid content, power supply, frequency, temperature, treatment duration, etc.

Ultrasonication has no influence on the total COD of the sludge, so that the ratio soluble/total COD represents the release of the organic matter from solid to liquid state after ultrasonication.

Many studies analyzed the effectiveness of ultrasonication in terms of COD solubilization. It was shown that to achieve a solubilization rate of 75-80% a minimum of 90 minutes of ultrasonication pretreatment was required, and that at least 30-40 minutes of ultrasonication are necessary to get 50% solubilization. By contrast, it was reported that after 96 seconds of ultrasonication the disintegration rate was more than 30%, suggesting that sonication time and solubilization rate are not linearly correlated [Pilli et al., 2010].Soluble COD concentration is sometimes used to compute the so-called degree of disintegration, a comparison the soluble COD increase by sonication and the maximum possible soluble COD increase obtained by alkaline hydrolysis ($CODs_{NaOH}$):

$$DD_{COD} = \frac{CODs - CODs_0}{CODs_{NaOH} - CODs_0} \tag{2.1}$$

Also, the effect of total solids concentration on the degree of disintegration was evaluated [Nels et al., 2000]. Increasing the total solid concentration, the solubilization of COD increased leading to an optimum beyond which solubilization decreased due to attenuation effects. This fact shows that higher solids in the liquid produce more cavitation sites and more hydro-mechanical shear forces due to implosion of more

bubbles, and beyond the optimum concentration the homogeneous distribution of the acoustic waves is disrupted by absorption effects.

Nitrogen and ammonia Ultrasonication increases organic nitrogen and ammonia concentration in sludge samples, therefore NH_3 assessment can also be used to evaluate the degree of disintegration. [Bougrier et al., 2005] report that at a specific energy of 15000 kJ/kg$_{TS}$, the organic nitrogen solubilization is 40%, and the maximum solubilization occurs at a specific energy input of 10000 kJ/kg$_{TS}$. Moreover, with the increase of specific energy input and total solid content, the release of ammonia nitrogen increases.

The total nitrogen solubilization increased linearly with the increase in specific energy above 3600 kJ/kg$_{TS}$ and a solubilization of 19,6% was achieved at specific energy input of 108000kJ/kg$_{TS}$.

[Feng et al, 2009] observed that nitrate nitrogen concentration increased at ultrasonication energies higher than 5000 kJ/kg$_{TS}$, while the increase of nitrate nitrogen concentration was smaller than ammonium nitrogen at similar conditions due to generation of hydroxyl radicals through acoustic cavitation.

2.2.2 Sludge degradability and methane production

Many studies were done to assess the methane generation from sludges pretreated with ultrasonication and it is possible to conclude that sonication increases biogas production rate at lower hydraulic retention time.

[Wang et al., 1999] proved that the percentage of methane increases gradually with increase in sonication time: the methane amount increased by 12%, 31%, 64% and 69% corresponding to the sonication time of 10, 20, 30 and 40 minutes respectively. Therefore the optimum pretreatment time for enhancing the anaerobic digestion efficiency should be approximately 30 minutes.

The effect of specific energy input on biogas production is evaluated by [Bougrier et al., 2005]. The biogas production increased with increase in specific energy input, from 20,5 mL (untreated sample) to 23, 25,6, 25,7, 31,2 and 32,8 mL at the specific energy of 660, 1350, 2700, 6950 and 14547 kJ/kg$_{TS}$ respectively. However, at higher energies the biogas was almost the same, with an increase of only 5,1% when doubling the specific energy from approx. 7000 to 14500 kJ/kg$_{TS}$.

The effect of the food/inoculum ratio (F/I) on anaerobic digestion of the sonicated sludge is evaluated by [Braguglia et al., 2006]. The biogas production rate increases with increasing the F/I ratio, and a maximum biogas gain of 25% was observed when F/I = 0,5. The same study stated that the ultrasonication pretreatment considerably enhanced the hydrolysis rate constant from 0,06-0,17 day^{-1} to 0,13-0,23 day^{-1}.

2.2.3 Energetic analysis

Energetic considerations about the ultrasonication pretreatment are very scattered and sometimes even unconsistent, as the energy needs of a sonication system and the energy production achievable with the biogas produced during the anaerobic digestion depend massively on how the pretreatment is implemented, sonotrode efficiency, heat losses and of course, the origin of the sludge, its solid content, the treatment duration, etc. Moreover, [Pérez-Elvira et al., 2010; Pérez-Elvira et al., 2009] state that the electrical and cavitation efficiency of a sonication laboratory equipment are too low compared to full-scale ultrasound devices, and therefore it is nonsense to present energy balances from laboratory-scale tests, as they are useless from a practical poit of view.

However, a laboratory scale heat balance can be useful to get an idea. For example, [Braguglia et al., 2008] report that biogas produced with sonicated sludge can supply the energy requirements only at low organic load rates (0,7 g$_{VS}$/L/d), whereas for the untreated sludge supplemental fuel is always required. For the test at high OLR (1,4 g$_{VS}$/L/d), biogas produced with sonicated sludge can supply more than 90% of the required heat, while for the untreated sludge this proportion lowers to 72-78%. The heat balance is resumed in table 2.3. It is therefore possibile to conclude that the heat balance is almost positive when using sonicated sludge, and this is especially true considering that plant-scale sonication equipments have much higher efficiencies.

Table 2.3: Heat balance for three laboratory digestion tests at three different organic load rates (OLR, g$_{VS}$/L/d) and degrees of disintegration (DD$_{COD}$) [Braguglia et al., 2008]

	OLR = 0,7		OLR = 1,4		OLR = 1,4	
	untreated	DD = 8%	untreated	DD = 4%	untreated	DD = 8%
Heat demand	614	614	908	908	490	490
Heat produced from biogas	472	640	713	900	353	456
Balance	-142	+26	-195	-8	-137	-34

Table 2.4: Operating conditions of the four tests [Braguglia et al., 2011]

test #	1	2	3	4
Duration [d]	67	41	63	76
OLR [g_{VS}/L/d]	0,7	1,4	1,4	2,8
HRT [d]	20	10	10	10
Sonication energy [kJ/kg$_{TS}$]	5000	5000	2500	1250
DD [%]	9	9	4	2,2
Solubilized COD loading rate [mg/L/d]	85	170	76	83

The same authors analyzed the problem in another article [Braguglia et al., 2011]. Of the four BMP tests they run (table 2.4), they concluded that the biogas produced with untreated and sonicated sludge could be sufficient to supply the energy requirement in the only case of the test carried out with thickened sludge.

The thermal balance was evaluated considering the total amount of sludge fed to the digester and the cumulative biogas production at the end of the tests. The heat demand included the heat necessay to change the temperature of the influent sludge to the digestion temperature ($\Delta T = 20$°C) and the heat losses from digester:

- heat for sludge temperature increase $[kcal] = Q_{feed}[L/d] \cdot 20[°C] \cdot days[d]$

- heat losses, assuming that the digester temperature underwent a decrease of 0,56°C (=1°F) per day: $[kcal] = V_{digester}[L] \cdot 0,56[°C/d] \cdot days[d]$

The heat produced from the biogas has been accounted for considering an average presence of methane of 65% and the inferior calorific power of 8500 kcal/Nm^3, i.e.:

$$Heat\,produced[kcal] = V_{biogas}[Nm^3] \cdot 0,65[Nm^3_{CH4}/Nm^3_{biogas}] \cdot 8500[kcal/Nm^3]$$

Computing the heat balance this way, as said above, gave positive result only in the case of test #4 (table 2.5). For the other tests carried out at medium-low OLR, both for untreated and sonicated sludge, a supplementary fuel was always required, but the biogas produced with sonicated sludge can supply 80% of the required heat against the 60% for the untreated sludge. Moreover, the balance was evaluated cautiously assuming that there was 65% CH$_4$ in the biogas produced from both untreated and sonicated sludge digestion, though generally sonication treatment improves the methane percentage in the biogas.

Table 2.5: Heat balances [kcal/kg$_{VS}$] for semi-continuous digestion tests [Braguglia et al., 2011]

	test #1		test #2		test #3		test #4	
	untreated	sonicated	untreated	sonicated	untreated	sonicated	untreated	sonicated
Heat demand	2304	2304	1903	1903	1824	1824	874	874
Heat produced from biogas	1421	1991	1131	1488	1151	1452	1380	1517
Balance	-883	-313	-772	-415	-673	-372	506	643

After the heat balance, also an energetic balance was calculated.

Supplied energy was calculated as energy per volume unit as follows

$$\frac{E}{V} = \frac{P \cdot t \cdot \eta}{V} = \frac{300[W] \cdot t[min] \cdot 0,85}{60[min/h] \cdot 0,5[L]} = 8,5 \cdot t \left[\frac{Wh}{L}\right]$$

where P is the ultrasound power, V is the volume of each sample that was sonicated for $t = 2$ or 4 minutes and η is the energy transmitted.

Test #4 was performed on thickened sludge, so that it is necessary to take into account the energy used during thickening too:

$$\frac{average\ centrifugation\ energy}{sludge\ concentration} = 0,6 \left[\frac{kWh}{m^3_{sludge}}\right] /20 \left[\frac{kg_{VS}}{m^3_{sludge}}\right] = 0,03 \left[\frac{kWh}{kg_{VS}}\right]$$

The normalized energy production was calculated as

$$E_P \left[\frac{kcal}{kg_{VS}}\right] = V_{biogas} \left[\frac{Nm^3}{kg_{VS}}\right] \cdot \%CH_4 \cdot ICP \left[\frac{kcal}{Nm^3}\right] \cdot \eta_{el} = V_{biogas} \left[\frac{Nm^3}{kg_{VS}}\right] \cdot 0,65 \left[\frac{Nm^3_{CH4}}{Nm^3_{biogas}}\right] \cdot 8500 \left[\frac{kcal}{Nm^3}\right] \cdot 0,35$$
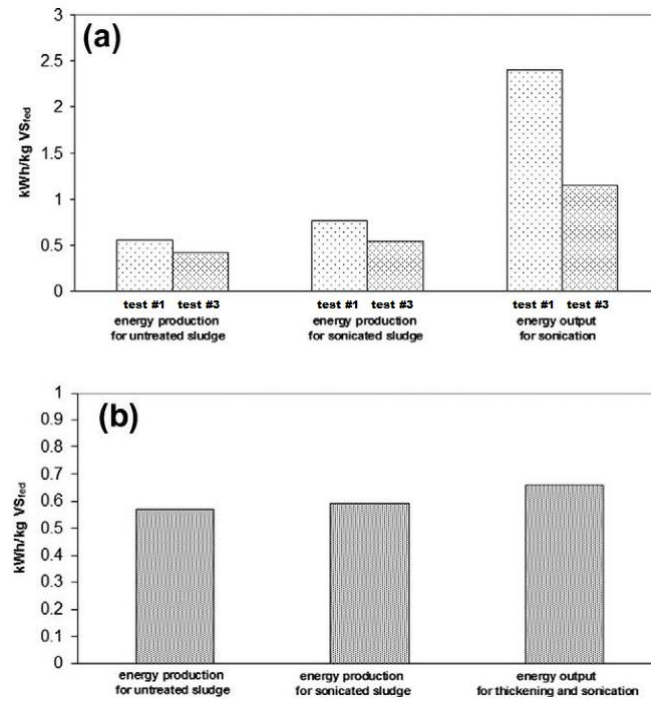
Figure 2.8: (a) Energy balance for the digestion of secondary sludge of test #1 and #3 and (b) of thickened sludge in test #4 [Braguglia et al., 2011]

where V_{biogas} is the specific biogas production with respect to the VS_{fed}, ICP is the inferior calorific power of methane and η_{el} is the electrical efficiency.

Results are represented in figure 2.8. The energy gained from the biogas produced by the sonicated sludge in tests #1 and 3 were around 0,76 and 0,54 kWh/kg$_{VS}$ respectively. The surplus of energy produced during digestion of sonicated sludge in these tests covered only approx. 10% of the energy requirement for sonication. However, it is important to note that the above data were obtained with a quite diluted sludge at an average concentration of 23 g/L. By contrast, in the energy balance for test #4 performed with thickened sludge maintaining however the same soluble COD load as in tests #1 and #3, just a slightly negative (-0,07 kWh/kg$_{VS}$) net energy balance was obtained, taking into account also the energy spent for thickening. Therefore, thickening appears to be a fundamental step to obtain good performance in sludge digestion. [Braguglia et al., 2011] conclude that digesting sonicated thickened sludge with a disintegration degree of 6% and an HRT of 10 days would provide a 40% gain in biogas, that would compensate the energy consumed by sonication. It is also important to remember that full-scale devices consume less energy than the laboratory-scale devices used in this research.

In [Barber, 2005] a full-scale energy balance is computed, and it is therefore possible to reach some useful conclusions, as it is assed considering the efficiencies of plant-scale devices, with particular reference to the Mannheim (Germany) wastewater treatment plant in Germany (725000 p.e.), where a part-stream ultrasound equipment is installed.

Fig. 2.9 represents the system that was considered, that is composed of a digester operating under the following conditions:

- flowrate of 200 m^3/d at 5% DS of which 75% are VS; sludge temperature of 15˚C

- ambient air temperature of 15˚C

- hydraulic retention time of 20 days

- digester volume of 1200 m^3, well insulated with a heat-transfer coefficient[1] of $2,5\,W/m^2/K$, operating temperature of 35˚C

It is proved that under these conditions the digester provides sufficient energy to be autothermic. With and without sonication, 331 and 254 kW are produced respectively. Sludge heating requires 187 kW and

---

[1] The overall heat-transfer coefficient $[W/m^2/K]$ is the ratio between the heat flux per unit area and the temperature difference. Its typical values are between 2 and 3 for well insulated digesters and 3 to 4 for poorly insulated digesters.

additional heating due to ambient air lossess adds up to 60 kW, with no difference between sonicated and non sonicated sludge. This leaves a surplus of 7 kW and 84 kW before and after ultrasound treatment respectively. The overall energy balance shows that 1kW of ultrasound energy used generates about 7 kW of electrical energy after losses. The energy balance of the sonication treatment appears therefore to be positive.
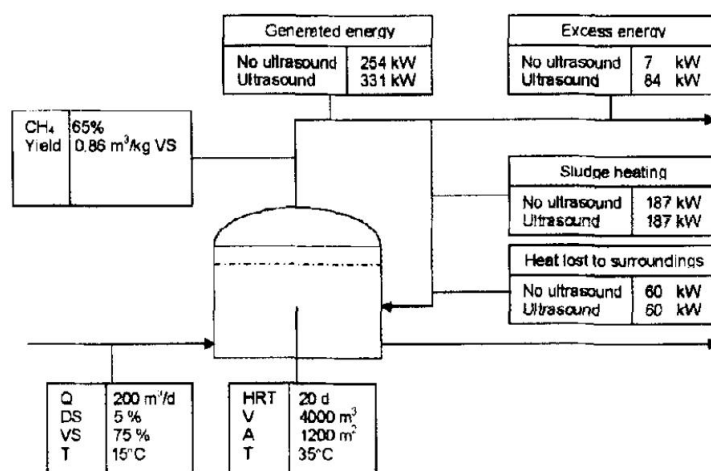
Figure 2.9: Typical energy balance over a digester with or without ultrasound treatment of influent [Barber, 2005]

The same study investigated the influence of ultrasound on the energy balance by changing the conditions. In a previous work of the same author, it was demonstrated that thickening the digester influent to 7% DS leads to a three-fold increase in energy production, compared with a sludge contaning only 3% DS. This was confirmed in other studies (such as the [Braguglia et al., 2011] described above) and even in the very Mannheim plant, where a full scale plant digesting an influent of 10% DS has experienced an increase in biogas production of 35%, from 458 to 581 L/kg$_{VS}$ following the incorporation of ultrasound treatment. The energy production at the site is even sufficient to provide the energy requirements for a subsequent drying plant, in addition to heating the digesters and site buildings. It is therefore possible to conclude that the energy balance improves greatly as the sludge is thickened.

As seen, energetic considerations about the ultrasonication pretreatment are somehow confused and limited to some specific cases corresponding to the several studies performed. It is however possible to give some conclusions regarding the economic and energetic viability of the ultrasound treatment.

It was shown that sonication gives important benefits to the sludge treatment process, such as

- increment in the hydrolysis rate of 25-50%

- possibility to increase the organic load rate by 20-50% whilst keeping hydraulic retention times constant, or to keep organig load rates constant and decrease hydraulic retention times by approx. 30%

- 25-50% increase in specific biogas yield, but this value depends greatly on sludge conditions

Concerning management costs, the most important expense is that of replacing the sonotrode as a result of cavitation damage, that happens every 1-2 years. Therefore designs which minimize sonotrode replacement have more economical whole-life costs. Electricity costs are usually lower, unless the price for electricity is high. Full scale plants with ultrasound technology have average payback periods of between 2-4 years [Barber, 2005].

Vendors estimate that installing an ultrasound system in a 130000 m^3/d plant would cost around €1,3-1,6 million, depending also on the subsidiary equipment, such as holding tanks, that may be required.

2.2.4   Full-scale implementations

Ultrasonication has been succesfully implemented in many wastewater facilities. In Italy, the biggest one is Cesena plant (120000 p.e.), while the biggest one in the world is probably that of Poznań, Poland (750000 p.e.).

There are currently two approaches on how to employ ultrasound, based on the quantity of the stream to be sonicated: full-stream and part-stream sonication. The full-stream approach involves treating the entire

stream with ultrasound, whereas a part-stream approach involves treating only a fraction of the sludge stream. At present, all of the suppliers of ultrasound devices use part-stream approach due to lower running cost. Moreover, it is common opinion that part-stream sonication has higher yields in terms of biogas production, suggesting that biogas production rises to a threshold value and then remains constant, even when a greater fraction of the stream is sonicated. However, some authors [Pérez-Elvira et al., 2010] found that there is a linear relationship between the percentage of sonicated stream and biogas productivity, and also some vendors are readapting their devices to work in full-stream conditions.

At present, plant-scale sonication is usually implemented by four industries. Historically, the first two are Waves and Dirk-IWE Tec, that are the two spin-off companies of the research groups about ultrasounds application in environmental engineering of the University of Hamburg-Harburg and the Fraunhofer Institute in Dresden, respectively. A third company, Sonico, was established by a consortium of U.K. and U.S. water agencies, led by Atkins, an engineering firm based in the U.K.. Finally, VTA is an Austrian company that installed its sonication devices in a number of wastewater plants throughout Austria, Germany, Czech Republic, Italy, Poland and Switzerland.

IWE Tec Ultrasound System®

The IWE Tec system (fig. 2.10) operates at sonication times of 30 to 60 seconds. Sonication components are manifactured by the German Hielscher, that is problably the biggest producer of ultrasound devices. IWE Tec considers the amplitude to be a key parameter in achieving the required sludge disintegration and therefore set the probes to run at 90 to 95% of maximum amplitude. The system is usually designed to run between 50 to 75% of the maximum power, to provide a buffer and prevent the units cutting out due to power overloads.

Figure 2.10: IWE Tec Ultrasound System®

The California Energy Commission in 2005 ranked the IWE equipment as "unreliable" because, during a test period, it was down 69 percent of the time. Also because of this, IWE made some advances to improve its system, that however still lack of demonstration testing:

- increase in the maximum amplitude from 25 μm to 50 μm

- increase in probe power, from 4 to potentially 16 kW. Probes over 4 kW have a new water cooling system

- the probe design has been changed from single cast piece to a two-piece probe to allow the lower portion, which has the most wear, to be replaced more frequently, while the upper portion can be replaced less frequently.

These are potentially significant changes regarding the cost-effectiveness of ultrasound application.

Data from IWE show that the older design, using 2 kW probes at the lower amplitude range typically provided improvements in anaerobic digestion as follows:

- Increase in volatile solids destruction of 20-25%

- increase in gas production of 25-30%

- improved dewaterability of 0-5%

Actual results vary depending on digestion performance without ultrasound, digester retention times and the proportion of secondary solids in the digester feed.

It is important to say that information about the IWE Tec systems are very fragmentary and scarcely updated, and it is even possible that the company closed the activity in the past few years.

Sonico

Sonico was established in 2002 to commercialize the ultrasound system sonix®. Sonix® main characteristic is to be composed of individual radial horns that are shaped like a ring. Horns are mounted in series in a reactor that typically contains three or five horns, as shown in figure 2.11.

The reactor is designed with flanges at both ends which connect to a 6 in. (approx. 15 cm) diameter pipe. The radial horn design and reactor design are covered by patents. The research for this system was carried out using 2 kW and 3 kW horns. Design of the ultrasound system was improved producing higher powered horns at 6 kW.

The Sonico approach to ultrasound application to wastewater sludges is based on treating a fraction of the entire secondary sludge stream. Research carried out developing this system showed that the maximum gas production is achieved by maximizing the fraction of sonicated sludge.
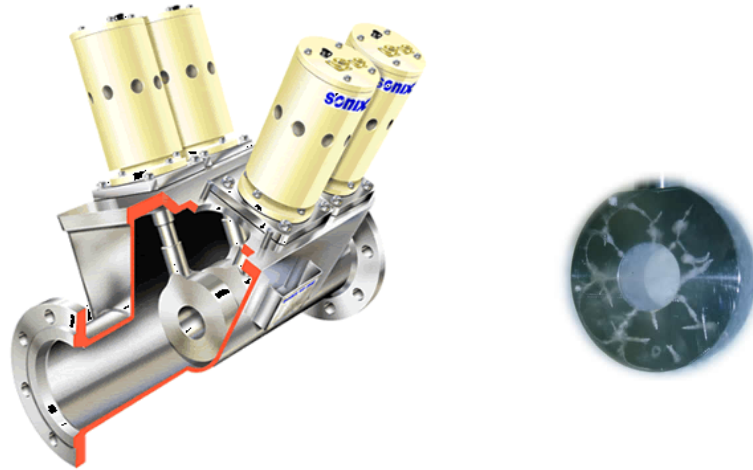
Figure 2.11: Sonix® V5 with detail of the cavitating ultrasound horn

The Sonico system operates at sonication times of around 2 seconds. The system is designed to typically run at 70 to 75% of the maximum amplitude, which provides buffering for changing loads. Some of the recent advances made by Sonico to improve the cost-effectiveness of the sonix system are:

- increase in the maximum amplitude from 12 to 16 μm

- increase in power, from 3 to 6 kWh

- improvements in the transducer cooling system

Data from Sonico ultrasound systems show that the older design, using 3 kW probes at the lower amplitude range, typically provided improvements in anaerobic digestion as follows:

- increase in volatile solids destruction of 30-50%

- increase in gas production of 30-50%

- improved dewaterability of 0-2,5%

- better digested stability by destruction of the filamentous organisms that often bloom in the digestion plants, causing their foaming and sometimes even temporary plant stop

The first full-scale sonix® plant was commissioned in Kävlinge, Sweden in December 2002, while the world largest sonix-ultrasound plant is the Mangere Wastewater Treatment Plant, New Zeland (2005).

Sonix system is likely to be the most successful plant-scale sonication system. The vendor claims that more sludge is treated by sonix than by any other ultrasound provider in the world.

UltraWaves Ultrasound System

Ultrawaves Wasser & Umwelttechnologien GmbH is a spin-off company of the University of Hamburg-Harburg that commercializes a sonication scheme called Waves®, using an ultrasound system provided by a German manufacturer, Sonotrode.
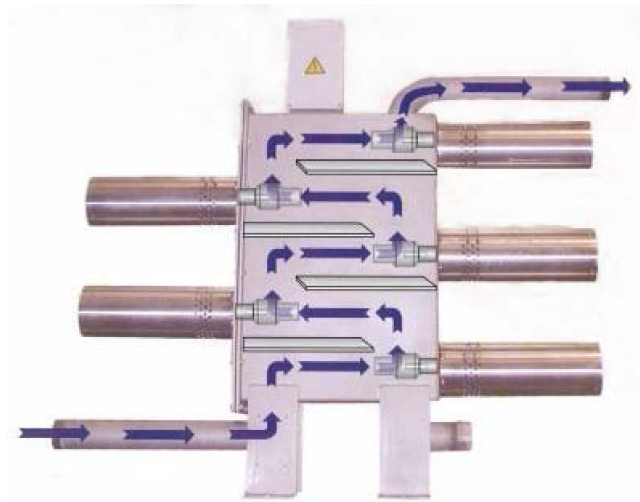
Figure 2.12: Flow path through the Waves® ultrasound unit

Ultrawaves has found its system to be most costeffective when treating 30 to 50% of the secondary sludge flow. It does not attribute the improvement in digester performance to enzyme activity, only to cell lysis.

The Ultrawaves system operates at sonication times of around 2 s. Ultrawaves considers the ultrasound density and dose to be the key parameters in achieving the required sludge disintegration, and they found that the intensity was highest with the shorter probes. The maximum amplitude on the Ultrawaves system is 40 μm, although the operating set point is fixed at 25 μm. Ultrawaves has found that for their system, operating at higher amplitudes reduces the life of the equipment.

Data from Ultrawaves ultrasound systems using its current 2 kW probes typically provided improvements in anaerobic digestion as follows

- decrease in the digestion time up to 60%

- increase in gas production of 10-50%

- increase in volatile solids destruction corresponding to gas production.

The average specific energy dose of the ultrasound units is generally 0,04-0,05 kWh/kg DS (or 159 kJ/kg$_{DS}$). Actual results vary depending on digestion performance without ultrasound, digester retention times and the proportion of secondary solids in the digester feed.

Ultrawaves installed the Waves® system in many wastewater plants, mainly in Germany.

VTA Technologie GmbH

The Reverse Flow Disintegration Unit®, developed and patented by VTA, achieves the disintegration effect by means of ultrasound at 25 kHz frequency. A part of the surplus sludge continuously flows top down through the disintegration reactor. The ultrasonic transducers are located in this reactor. By means of an agitator the sludge suspension is treated by continuously being passed through the ultrasonic oscillators. Depending on the residence time of the sludge in the reactor, the flow rate, the rotation speed of the agitator and the energy input of the integrated ultrasonic elements, the disintegration rate can be selected. The treated sludge is subsequently transferred to the digestion process.

According to VTA, the Reverse Flow Disintegration Unit® gives the following benefits:

- reduction of organic substances in the digested sludge up to 25%

- increase of manure gas quantity up to 30% - increase of electricity production up to 30%

- reduction of total sludge production and therefore of the disposal costs up to 20%

- increase of dry matter in the discharge of the drain system up to 15%

- decrease of the employed auxiliary products (polymers) for the ludge dewatering up to 30%

- a more stable digestion is accomplished, with reduction of the digestion time and foam-control in the digestion tank caused by filamentous bacteria

Figure 2.13: The VTA Reverse Flow Disintegration Unit®

- sustainable process-improvements in the biological sewage plant operation by control of filamentous bacteria

VTA installed the Reverse Flow Disintegration Unit® in many wastewater plants throughout central Europe, mainly in Austria and Germany.

2.3 Enzymic hydrolysis

Enzymic hydrolysis is a special treatment which may actually be considered as a secondary effect of a sludge thickening process known as inverted phase fermentation, that is briefely explained below.

2.3.1 Inverted phase fermentation

The inverted phase fermentation (IPF) is a sludge thickening process developed by United Utilities Group and the School of Applied Science of Cranfield University. The aim is to provide an alternative to gravity belt thickeners, whose usage has disadvantages such as high energetic and manteinance costs, necessity of polymers to flocculate the sludge, rising the expenses and modifying the sludge rheology, making it very difficult to pump and mix.

The main idea of IPF is to exploit the carbon dioxide produced in the early stage of a digestion process to float the dry solids, thus producing a phase separation: a floating cake-layer with a notably high solid content and an underneath liquor phase (fig. 2.14).
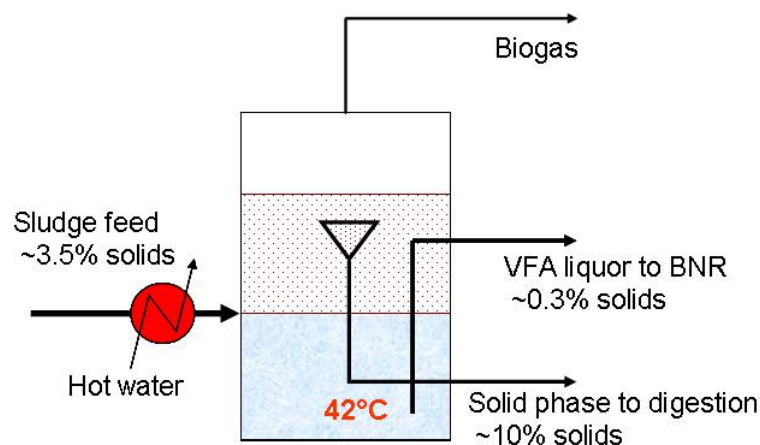
Figure 2.14: Inverted phase fermentation scheme [Le et al., 2009]

There are many variables that influence the IPF process, the two most important being the fermentation time and temperature

[Le et al, 2009] analyzed the effect of fermentation time on the IPF yield. Results are reported in table 2.6. Results clearly show that there is a significant increase in soluble COD. The fermentation reduced the ratio of soluble COD/VFA from 2,08 to 1,23 after two days, suggesting that most of the soluble COD was converted to VFA. Moreover, authors report that, even if the phase separation was essentially complete after

Table 2.6: Variations of IPF performance with different retention time [Le et al., 2009]

Retention time (hours)	Liquid phase volume (mL)	pH	VFA (mg/L)	COD (mg/L)	DS of liquid phase (w/w)	DS of solid phase (w/w)
0	0	5,45	1020	2122	(2,3%)	(2,3%)
12	350	5,41	1860	2456	0,37%	5,1%
18	500	5,38	2176	2621	0,28%	6,0%
24	550	5,28	2228	2692	0,24%	6,4%
42	600	5,27	2360	2862	0,20%	6,8%
48	600	5,28	2323	2873	0,20%	6,9%

24 hours with a 2,78-fold increase in the sludge solid concentration, increasing the fermentation time up to 48 hours was beneficial in reducing the suspended solids level in the liquor.

Regarding the effect of fermentation temperature, seven sludge samples were fermented at different temperatures (from 20 to 50°C) for 24 hours, in order to investigate the phase separation performance. This latter can be described with a concentration factor, calculated as ratio between solid phase and initial sludge concentration. Results are reported in table 2.7 and show that temperatures in the range 30-45°C produce a good phase separation.

Table 2.7: Variation of phase separation performance with temperature [Le et al., 2009]

Temperature	Liquid phase volume (mL)	DS of liquid phase (w/w)	DS of solid phase (w/w)	concentration factor
20°C	250	0,8%	3,6%	1,04
25°C	350	0,5%	4,4%	1,08
30°C	450	0,4%	5,7%	2,03
35°C	550	0,3%	6,9%	2,08
40°C	600	0,2%	7,6%	3,00
45°C	600	0,2%	7,8%	3,01
50°C	600	0,3%	7,5%	3,00

[Le et al., 2009] carried out other experiments in order to compare the performance of sludge samples coming from different wastewater plants, with different initial dry solid concentrations (table 2.8). The separation factor (ratio solid/liquid phase DS concentration) was about 32 in all cases where the initial sludge was over 3% in DS. Better separation (43-44) was observed at lower initial DS concentrations. Other tests confirmed that sludges with lower initial solid concentration separate more rapidly and with a better separation factor than sludges with higher solid concentrations.

Table 2.8: Phase separation with different sludge sources [Le et al., 2009]

Sludge source	DS of initial sludge (w/w)	DS of liquid phase (w/w)	DS of solid phase (w/w)	separation factor
St. Helens	2,40%	0,20%	8,60%	43
St. Helens	2,90%	0,20%	8,90%	44
Weaverham	3,30%	0,30%	9,10%	30
Ellesmere P.	3,80%	0,30%	9,80%	32
Blackburn	4,30%	0,30%	10,10%	33
Ellesmere P.	4,90%	0,30%	10,40%	34

IPF treatment proved to be effective also on E. coli reduction. Results (fig. 2.15) show that after a 48 hours treatment at 42°C, over 99,9% of E. coli was destructed and that most of this reduction takes place during the second half of the period. This is a very interesting result of the IPF process, as this E. coli reduction capability would obviate the need for pathogen attenuation in secondary digesters, thereby eliminating the biggest source of green house gas emissions from the sludge digestion process.

Finally, a viscosity comparison was carried out on two sludges with similar initial solid concentration
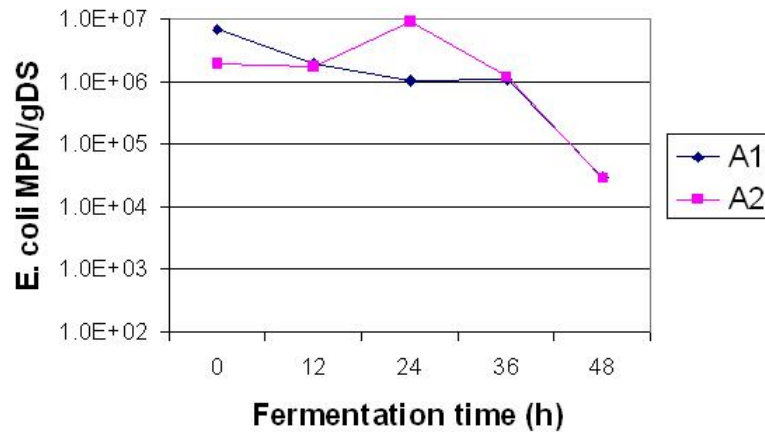
Figure 2.15: E. coli reduction during IPF treatment of two samples [Le et al., 2009]

(around 7,5), one resulting from the IPF process and the other treated with a gravity belt thickener (GBT). It was shown that IPF sludge viscosity was at least 50% lower than the GBT sludge, because poly-electrolites were not used to flocculate the sludge, and therefore ther is no ionic interaction between the sludge particles and the polymers. This is an important result, as a fluider sludge cuts energy requirements for pumping and mixing, not considering the costs for poly-electrolites supplies.

A lower viscosity should allow existing digestion assets to be intensified operating with sludge feeds in excess of 8-9% DS without the need of other pretreatments. It would allow digesters to operate with organic loading rates up to 4,9 $kg_{VS}/m^3/d$.

[Le et al., 2009] conclude that IPF appears to give the highest yield in phase separation at low initial DS concentration (2-5%), leading to a solid phase with a 8-11% DS concentration and a liquid phase with approx. 0,3% DS concentration (fig. 2.16). The phase separation start within two hours of the start of fermentation, and significantly slows down after 18 hours. Therefore in most cases, IPF optimum treatment is probably of about 24 hours at a temperature of 30°C or higher.

In case IPF is considered also to remove E. coli, higher temperatures and a longer fermentation time is needed because E. coli reduction starts around the 18th fermentation hour.
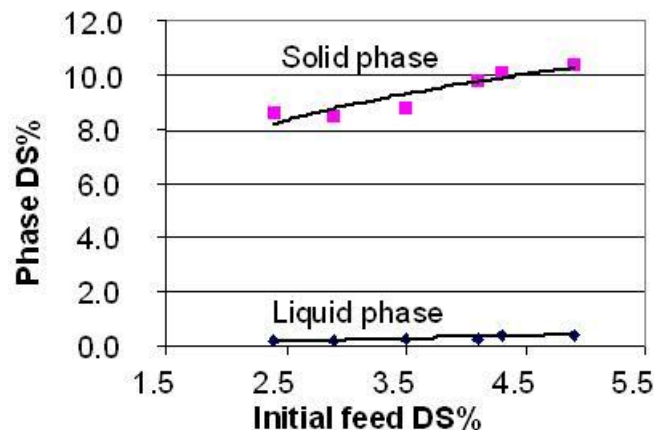
Figure 2.16: Phase separation performance relative to initial sludge concentration [Le et al., 2009]

2.3.2 Enzymic hydrolysis

Enzymic hydrolysis (EH) is a proprietary process based on patented technologies by United Utilities PLC.. The process main idea is to harness the actions of several types of bacteria to accelerate the mineralization process of complex organic matter. The bacteria work by producing and releasing the necessary enzymes, in particular those involved in the hydrolytic reactions.
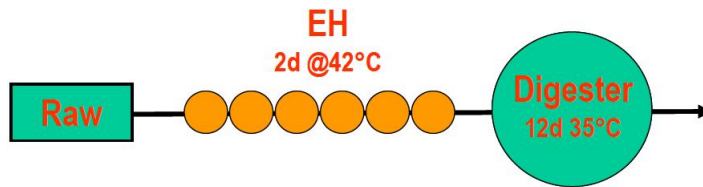
Figure 2.17: The sludge digestion with Enzymic Hydrolysis pre-treatment [United Utilities PLC]

The enzymic hydrolysis takes place during the fermentation step of the IPF, and it is said to have the highest yield with a treatment duration of two days at 42˚C. Fermentation is implemented in a sequence of 6 batch reactors to ensure a plug flow profile that allows a higher pathogen reduction performance. The enhanced enzymic hydrolysis process (EEH) provides higher pathogen reduction (up to 6-Log E. coli reduction) and allows some of the enzymes to continue working for maximum VS destruction by running the last three tanks at 55˚C.

The EH is considered an interesting option for sludge pretreatment as it is a process relatively simple to install and to manage. Moreover it does not need any specific high-tech device to be carried out and its operative costs are limited to the heating.

2.3.3 Macclesfield EH demonstration plant

United Utilities first full scale plant provided with the EH treatment was built in Macclesfield (UK), represented in fig. 2.18. It has a treatment capacity of 207 m^3/day with feed dry solids of up to 8% giving a total treatment capacity of 16,5 t/d. It is provided with six reactors of 80 m^3. In a first run trial in 2005, form 80 m^3 of a sludge with a 3,57% DS concentration, the formation of a 6 t solid cake was achieved, with a 27% DS concentration.
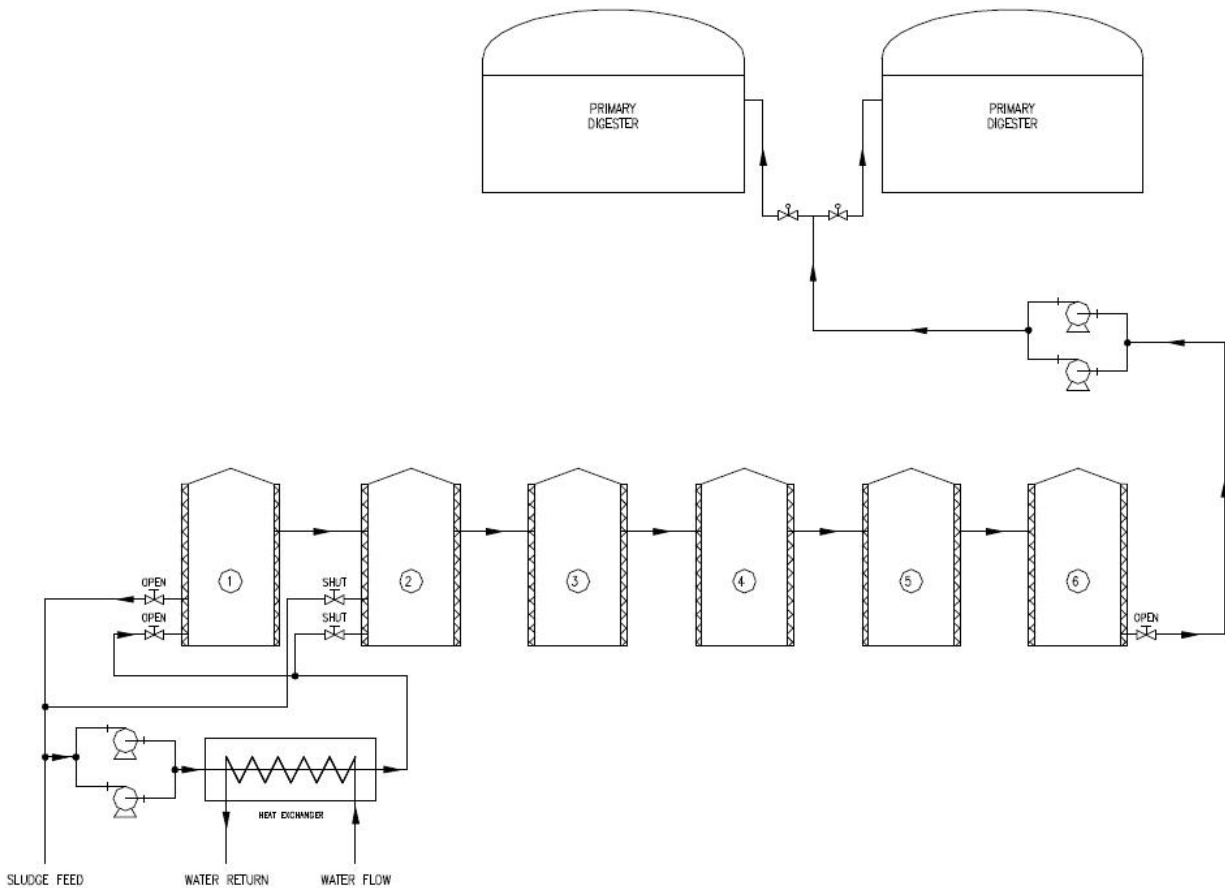
Figure 2.18: Schematic of Macclesfield EH Plant

At least two other plants equipped with EH or EEH system are now under construction by United Utilities, all of them in the UK (Blackburn and Bromborough).

Figure 2.19: Fermented cake (left) and liquor (right) proceding from Macclesfield EH Plant

Chapter 3

Materials and methods

The research was carried out at the Laboratory of Environmental Engineering of the University of Oviedo in the period March-July 2011 with the supervision of Prof. Elena Marañón and the help of the laboratory staff.

Specifically, the research was aimed to compare the action of different pretreatments (sonication, enzymic hydrolysis and a combined treatment of both) on two different types of wastewater sludges, to study the effects in terms of enhancement of methane production.

The laboratory work was divided into two rounds, corresponding to the two sludges that were analyzed:

1. March - May 2011: mix of primary and secondary wastewater sludges undergone to a thickening process through filter press with the addition of ferric chloride

2. May - July 2011: mix of primary and secondary wastewater sludges without the thickening process

3.1   Pretreatment descripton

Sonication   This treatment aims to provide a specific amount of energy (15000 kJ/kg of total solids) in the form of ultrasound waves. This was done the first round with a Bandelin Sonoplus HD 3200, at a fixed power of 150 W and the second round with a Hielscher UP400S that operated at a variable power range, starting at around 259 W and decreasing up to around 124 W at the end of the sonication process (fig. 3.1).

Enzymic hydrolysis   The enzyic hydrolysis of the sludge was achieved by leaving it for 48 hours at 42°C in special sealed bottles whose top is linked with a rubber tube to a beaker filled with water. During the heating, the sludge expands through the tube and goes up to the water removing all the air, thus assuring perfect anaerobic conditions. The result of this process, in addition to the energy subministration that is the aim of the pretreatment, is the separation of the liquid and the solid phases of the sludge, as solids will form a floating cake in the upper part of the bottle, so that it is necessary to homogenize the phases before using it in the BMP tests.

Figure 3.1: (left) Bandelin Sonoplus HD 3200 and (right) Hielscher UP400S sonication equipments

Figure 3.2: Enzymic hydrolysis in progress

3.2 Biochemical methane potential (BMP) tests

In both rounds, the anaerobic digestion was carried out under mesophilic conditions (37°C). The following mixtures were digested, all in duplicate:

1. Blank: digestion of manure only, substituting the sludge with an equivalent amount of water. This was done to measure the volume of methane produced by the inoculum, to be subtracted to the other BMP experiments.

2. Untreated: co-digestion of sludge and manure, to which no pretreatment was applied. This was done to be able to compare the effective enhancement of methane production of the pretreatment processes.

3. Ultrasounds: co-digestion of sonicated sludge and manure. The sonication treatment was performed by appling an energy of 15000 kJ per kg of total solids.

4. Enzymic hydrolysis: co-digestion of sludge and manure, in which the sludge was submitted to a process of enzymic hydrolysis.

5. Combined: co-digestion of sludge and manure, resulting as combination of the the two previous cases. In the first turn of the experiments, the first treatment was the sonication and the second the enzymic hydrolysis; in the second turn it was the other way round.

Both the first and the second experiments were carried out according to the following experimental procedure:

1. Characterize the sludge and the manure (pH, alkalinity, total and soluble COD, total and volatile solids, ammonia nitrogen concentration, total nitrogen, total phosphorus)

2. Determine the volume of sludge, manure and (if needed) distilled water to fulfill the next three conditions:

   - total volume of 4,5 L, as 1,75 L are necessary for each bottle, and 1 more L to characterize the mixture
   - total solid concentration around 5-6%
   - ratio of volatile solids sludge/manure $\leq$ 0,5

3. Prepare the mixtures

4. Characterize the mixtures (same parameters as in #1.)

5. Fix alkalinity to 9,1 g_{CaCO_3}/L by adding the correct amount of a solution of $NaHCO_3$ 1M

6. Addition of trace metals: 10 mL/$L_{mixture}$ of a solution of 0,1 g_{Co} and 0,101 g_{Ni} in 250 mL; 2 mL/$L_{mixture}$ of a solution of 0,22 g_{Se}, 0,18 g_W and 0,025 g_{Mo} in 250 mL

7. Seal the bottles with silicon and place them in the oven at 37°C. The top of the bottle is linked with a rubber tube to a plastic Tedlar bag where biogas will accumulate

8. Bottles remain in the oven for approximately 30-40 days, while the anaerobic digestion takes place (fig. 3.3). Meanwhile:

   (a) Measure of the volume of produced biogas every 1-2 days

   (b) Manual shake of the bottles every 1-2 days

   (c) Analyze the biogas composition every 7 days

9. Characterize the digested mixtures.

Figure 3.3: BMP tests in progress

3.3 Analytical methods

Standard methods and instruments were applied to measure the following parameters. .

- pH, measured with a CRISON 507

- Total Chemical Oxygen Demand: measured according to method 5220 D (closed reflux, colorimetric method) of the [APHA 1998], using a Perkin Elmer Lambda 35 Visible-UV system and a Hach DR/2010 Spectrophotometer.

- Soluble Chemical Oxygen Demand: same procedure as total COD, with previous centrifugation at 4350 rpm for 40 minutes and vacuum filtration with 0,45 μm filters. In the second round of the experiments (chapter 4.2), this method was slightly changed, as centrifugation was set to 3500 rpm for 15 minutes and 1,2 μm pore filters were used. This does not affect the results of the analysis, as the interest is focused on the variation of COD and not on the single value.

- Ammonium nitrogen: in the first round, this parameter was measured by titration with 0,1 N sulfuric acid after distillation using a FOSS Tecator Kjeltec 2200 Auto Distillation System. In the second round, due to breakdown of the distillator, the ammonium nitrogen concentration was measured with a selective electrode Orion Mod 720 Aplus.

- Alkalinity: measured by titration with 0,1 N sulfuric acid as variation of pH of the supernatant of a sample previously centrifuged at 4350 rpm for 15 minutes, brought to boiling for 3 minutes, cooled to room temperature and then titrated with 0,1 N sodium dioxide (Degrémont method No. 805).

- Total and volatile solids. The sample of about 10 g is first dried at 105°C for 24 hours to drive off water, cooled and weighed to get the total solids value. It is then dried at 550°C to drive off volatile solids and again cooled and weighed. The difference between final weight and total solids gives the volatile solids value. [EPA Method 1684]

- Total Nitrogen and Phosphorus content: determined by ion chromatography (861 Advanced Compact IC 2.8610.0010) after transformation into, respectively, nitrates and phosphates by digestion under pressure in a microwave equipment (Milestone Ethos 1 Advanced Microwave Digestion Labstation) with H_2O_2 and HCOOH. Due to breakdown of the microwave in the month of June, measures of these parameters are incomplete.
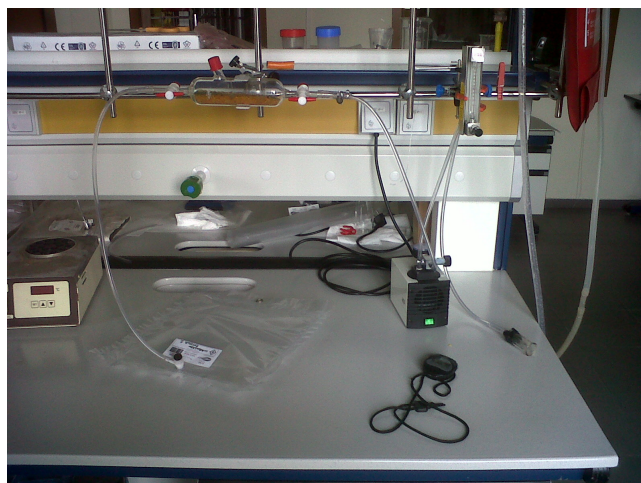
Figure 3.4: Equipment to measure the volume of biogas contained into the Tedlar bag

- The volume of the biogas produced was measured after the time necessary to vacuum the Tedlar bag in which it cumulated, using a pump equipped with a previously calibrated ball flow meter (fig. 3.4).

- The biogas composition (O_2,N_2,CO_2,CH_4) was measured on samples of 1 mL by gas chromatography (Agilent Technologies mod. 7890) using a TCD detector and a Poropack N packed column plus a molecular sieve, employing the following temperature ramp: starting temperature 35°C (1,5 min) increasing up to 55°C at a rate of 1,5°C per minute.

Chapter 4

Results

4.1   1st round: Frieres sludge

4.1.1   Sludge characterization

- Date of collection: April 15th, 2011

- Origin: Frieres wastewater plant (Asturias, Spain)

- Process: primary and secondary sludges mixed with ferric chloride to form flakes, with subfollowing thickening through press filters

- total COD = 262000 mg/L

- soluble COD = 43900 mg/L

- $[N\text{-}NH_4^+]$ = 3740 mg/L

- Total solids = 230,98 g/L

- Volatile solids = 152,66 g/L

- Total N = 2,01 g/kg

- Total P = 1,25 g/kg

4.1.2   Manure characterization

- Date of collection: February 7th, 2011

- Origin: Ganadería "Casa Viña", Albandi-Carreño (Asturias, Spain)

- Process: crushed cattle manure

- total COD = 65800 mg/L

- soluble COD = 27300 mg/L

- $[N\text{-}NH_4^+]$ = 1180 mg/L

- Total solids = 79,72 g/L

- Volatile solids = 40,61g/L

- pH = 7,52

- Alkalinity = 8,81 g_{CaCO_3}/L

- Total N = 2,01 g/L

- Total P = 1,25 g/L

4.1.3 Bottle composition

The best bottle composition was estimated to be the one reported in table 4.1, so to have the conditions mentioned in chapter 3.2, i.e.:

- total volume of 1,75 L per bottle

- total solid concentration of 60,05 g/L (equivalent to 6 %)

- ratio of volatile solids sludge/manure of 0,44

Table 4.1: Bottle composition

	[L]	[g_{sample}]	%
manure	0,985	1009	56,3%
sludge	0,115	126	6,6%
water	0,65	650	37,1%
total	1,75	1792	100,0%

In the "blank" bottles, sludge was replaced by an equivalent amount of distilled water.

4.1.4 Mixtures characterization

In table 4.2 the initial characterization (day zero) is reported.

Table 4.2: Initial characterization of the five mixtures - 1st round

		<1> blank	<2> untreated	<3> sonicated	<4> enzym.	<5> combined
pH	-	8,5	8,3	8,1	7,6	7,7
Total solids	g/L	44,87	60,05	60,05	60,05	60,05
Volatile solids	g/L	22,86	32,89	32,89	32,89	32,89
Alkalinity	g_{CaCO_3}/L	7,3	7,1	7,8	8,5	8,9
total COD	mg/L	21350	17200	17200	17200	17200
soluble COD	mg/L	8380	10200	11800	16900	16800
[N-NH$_4^+$]	mg/L	1288	1523	1411	2218	1983

In table 4.3 the characterization of the mixtures after an incubation period of 24 hours at 37°C in forced anaerobic conditions is reported. Note that this characterization was done using samples specifically prepared, as bottles had already been sealed.

Table 4.3: Mixture characterization at 24 h and variation from the initial values

		<1> blank	<2> untreated	<3> sonicated	<4> enzym.[1]	<5> combined
soluble COD	mg/L	8780	11700	13700	16400	15400
variation[2]	%	+5	+14	+15	0	-23
variation[3]	%	+12	+30	+19	-4	-8
[N-NH$_4^+$]	mg/L	1422	1809	1809	1904	2122
variation	%	+10	+19	+28	-14	+7

(1) it is possible that this sample was not in proper anaerobic conditions

(2) referred to values measured with Hach Spectrophotometer in glass tubes

(3) referred to values measured with Perkins Spectrophotometer in quartz cells

4.1.5 Biogas production

Biogas composition [%] is reported in the appendix (table A.7).

Graphics (fig. 4.1 - 4.5) show the net cumulated production of biogas and methane calculated as the difference between the methane production of each bottle and the one of the blanks. As productions of the pair of bottles of the same mixture are very similar, graphics represent the average of the two.
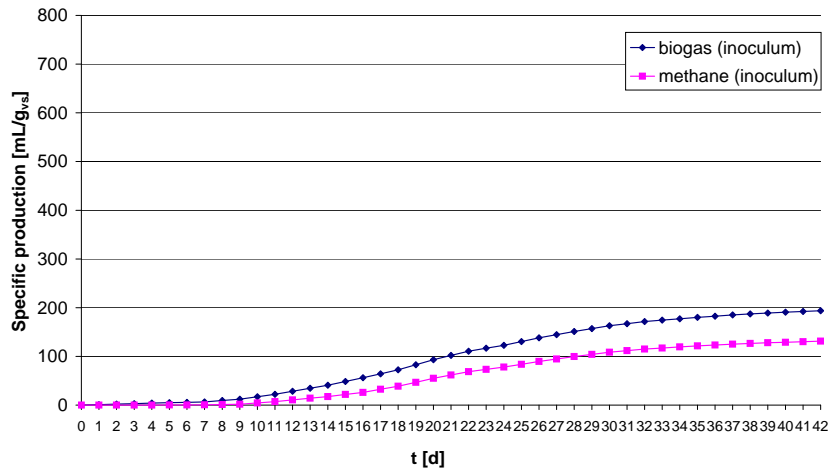
Figure 4.1: Cumulated biogas production - blank bottles <1>

Figure 4.2: Cumulated biogas production - untreated bottles <2>

Figure 4.3: Cumulated biogas production - sonicated bottles <3>

Figure 4.4: Cumulated biogas production - enzymic hydrolysis bottles <4>

Figure 4.5: Cumulated biogas production - combined treatment bottles <5>

4.1.6 Final characterization

For each mixture the average value of the two bottles, measured the final (42nd) day (tab. 4.4), is given.

Table 4.4: Final characterization. Variations are referred to the initial characterization

		<1> blank	<2> untreated	<3> sonicated	<4> enzym.	<5> combined
pH	-	8,2	8,2	8,2	8,2	8,2
variation	%	*-4*	*-1*	*+2*	*+8*	*+7*
Total solids	g/L	23,63	59,74	66,08	49,05	52,23
variation	%	*-47*	*-1*	*+10*	*-18*	*-13*
Volatile solids	g/L	10,12	20,48	18,85	18,62	18,77
variation	%	*-56*	*-38*	*-43*	*-43*	*-43*
Alkalinity	g_{CaCO_3}/L	8,8	10,1	10,4	9,8	9,5
variation	%	*+21*	*+41*	*+34*	*+16*	*+7*
total COD	mg/L	15300	23900	22700	25200	25800
variation	%	*-28*	*+38*	*+32*	*+46*	*+50*
soluble COD	mg/L	3950	2400	4760	5190	4920
variation	%	*-53*	*-76*	*-60*	*-69*	*-72*
[N-NH$_4^+$]	mg/L	1460	1880	2080	2130	2040
variation	%	*+14*	*+23*	*+47*	*-4*	*-3*

4.2 2nd round: Baiña sludge

4.2.1 Sludge characterization

- Date of collection: May 29th, 2011

- Origin: Baiña wastewater plant (Asturias, Spain)

- Process: mix of primary and secondary sludges

- total COD = 44700 mg/L

- soluble COD = 5281 mg/L

- [N-NH$_4^+$] = 287 mg/L

- Total solids = 39,76 g/L

- Volatile solids = 27,43 g/L

- pH = 6,1

- Alkalinity = 1,85 g_{CaCO_3}/L

4.2.2 Manure characterization

- Date of collection: April 7th, 2011

- Origin: Ganadería "Casa Viña", Albandi-Carreño (Asturias, Spain)

- Process: crushed cattle manure

- total COD = 38000 mg/L

- soluble COD = 22783 mg/L

- [N-NH$_4^+$] = 1571 mg/L

- Total solids = 59,64 g/L

- Volatile solids = 31,63 g/L

- pH = 8,3

- Alkalinity = 12,27 g_{CaCO_3}/L

- Total N = 2,14 g/L

- Total P = 1,65 g/L

4.2.3   Bottle composition

The best bottle composition was estimated to be the one reported in table 4.5, so to have the conditions mentioned in chapter 3.2, i.e.:

- total volume of 1,75 L per bottle

- total solid concentration of 52,26 g/L (equivalent to 5,23 %). Due to poor solid concentration in both sludge and manure, it was impossible to reach a total solid concentration of 6%. For this same reason, no water was used in these experiments.

- ratio of volatile solids sludge/manure of 0,5

Table 4.5: Bottle composition

	[L]	[g_{sample}]	%
manure	1,1	1120	62,86%
sludge	0,65	660	37,14%
total	1,75	1781	100%

In the "blank" bottles, sludge was replaced by an equivalent amount of distilled water.

4.2.4   Mixtures characterization

In table 4.6 the initial characterization (day zero) is reported.

Table 4.6: Initial characterization of the five mixtures - 2nd round

		<6> blank	<7> untreated	<8> sonicated	<9> enzym.	<10> combined
pH	-	8,2	8,6	8,3	8,0	8,4
Total solids	g/L	37,49	52,26	52,26	52,26	52,26
Volatile solids	g/L	19,88	30,07	30,07	30,07	30,07
Alkalinity	g_{CaCO_3}/L	7,8	8,6	8,2	9,0	9,1
total COD	mg/L	23200	41000	41500	42300	44700
soluble COD	mg/L	14800	16400	21000	20400	22200
[N-NH$_4^+$]	mg/L	1540	1790	1810	1800	1760

In table 4.7 the characterization of the mixtures after an incubation period of 24 hours at 37°C in forced anaerobic conditions is reported. Note that this characterization was done using specifically prepared samples, as bottles had already been sealed.

Table 4.7: Mixture characterization at 24 h. Variations are referred to the initial characterization

		<1> blank	<2> untreated	<3> sonicated	<4> enzym.	<5> combined
soluble COD	mg/L	14700	18600	19200	17000	17100
variation[4]	%	*+7*	*+19*	*-5*	*-18*	*-24*
variation[5]	%	*+10*	*+17*	*-5*	*-17*	*-17*
[N-NH$_4^+$]	mg/L	1510	1770	1690	1750	1940
variation	%	*-2*	*-1*	*-8*	*-3*	*+9*

(4) referred to values measured with Hach Spectrophotometer in glass tubes

(5) referred to values measured with Perkins Spectrophotometer in quartz cells

4.2.5 Biogas production

Biogas composition [%] is reported in the appendix (table A.14).

Graphics (fig. 4.7 - 4.11) show the net cumulated production of biogas and methane caomputed as difference between the methane production of each bottle and the one of the blanks. Again, as productions of the pair of bottles of the same mixture are very similar, graphics represent the average of the two, with the exception of the first bottle of mixture <10> that which test cannot be considered valid because biogas production resulted to be very irregular, as can be seen in fig. 4.6. The reason of this is not known, it may be due to a particular condition of the sludge or the manure contained in the bottle, or to imperfect anaerobic conditions.
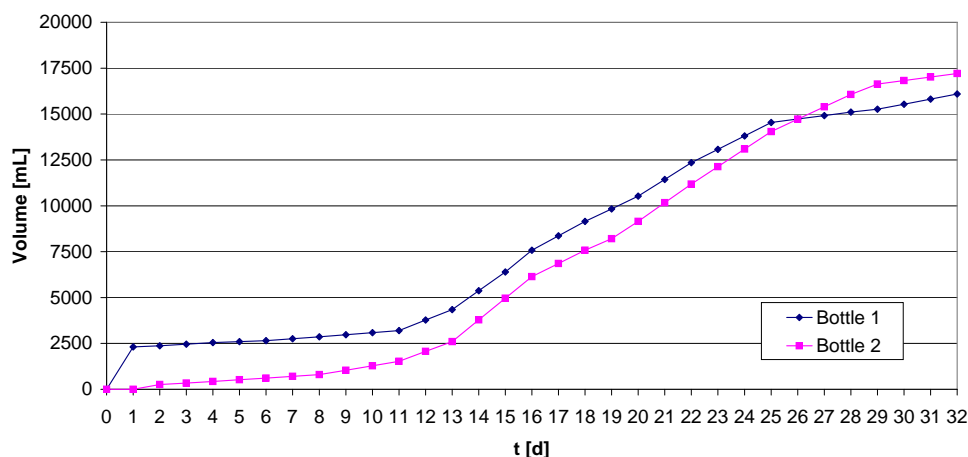
Figure 4.6: Mixture <10>, biogas production of bottles 1 and 2

4.2.6 Final characterization

For each mixture the average value of the two bottles, measured the final (32nd) day, is given: refer to table 4.8.

Table 4.8: 2nd round final characterization. Variations are referred to the initial characterization

|		<6> blank	<7> untreated	<8> sonicated	<9> enzym.	<10> combined
pH	-	8,3	8,3	8,1	8,2	8,2
variation	%	*+2*	*-3*	*-2*	*+1*	*-2*
Total solids	g/L	27,70	42,93	40,07	36,85	34,37
variation	%	*-26*	*-18*	*+23*	*-29*	*-34*
Volatile solids	g/L	11,19	20,05	19,01	17,62	17,31
variation	%	*-40*	*-33*	*-37*	*-41*	*-42*
Alkalinity	g_{CaCO_3}/L	8,9	10,1	10,3	10,2	10,4
variation	%	*+14*	*+17*	*+25*	*+13*	*+13*
total COD	mg/L	37500	55100	57000	47400	53400
variation	%	*+61*	*+35*	*+39*	*+16*	*+30*
soluble COD	mg/L	7820	9270	8560	11100	11700
variation	%	*-47*	*-43*	*-59*	*-45*	*-47*
[N-NH$_4^+$]	mg/L	1500	2000	2020	1980	2140
variation	%	*-3*	*+12*	*+11*	*+10*	*+22*
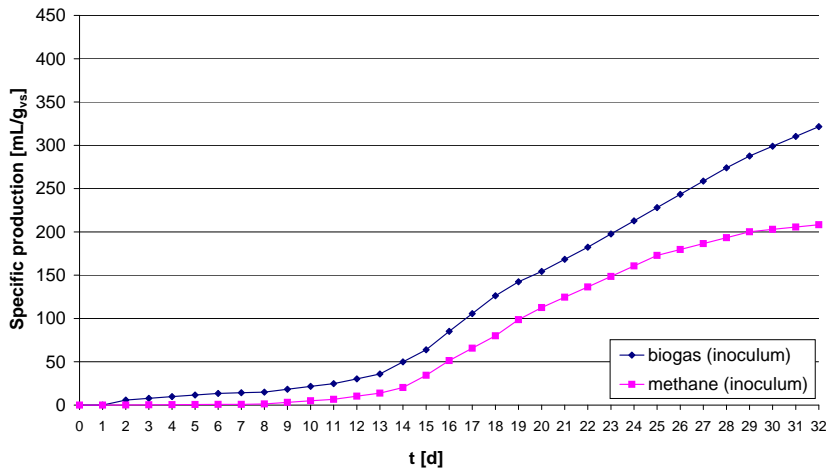
Figure 4.7: Cumulated biogas production - blank bottles <6>
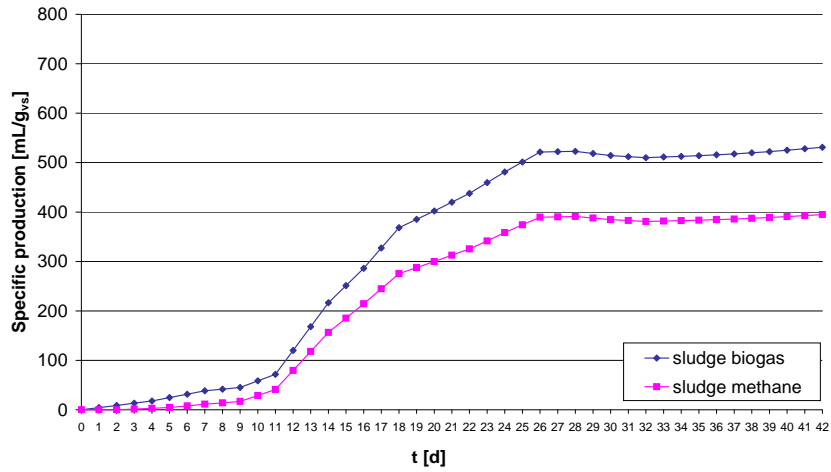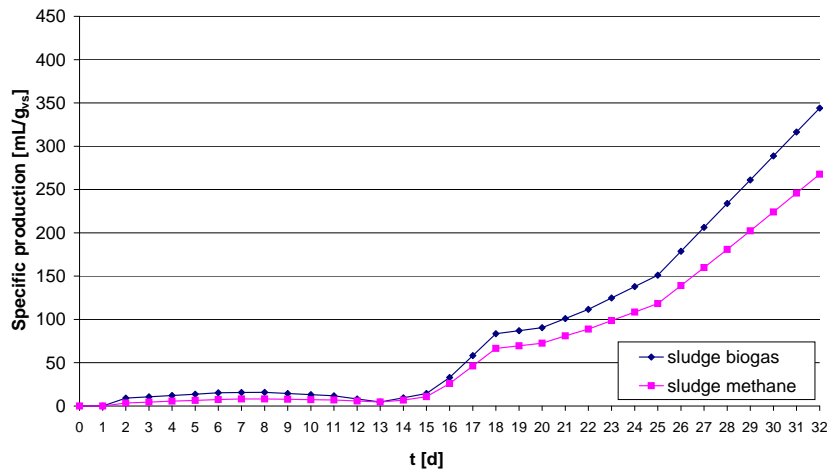

Figure 4.8: Cumulated biogas production - untreated bottles <7>
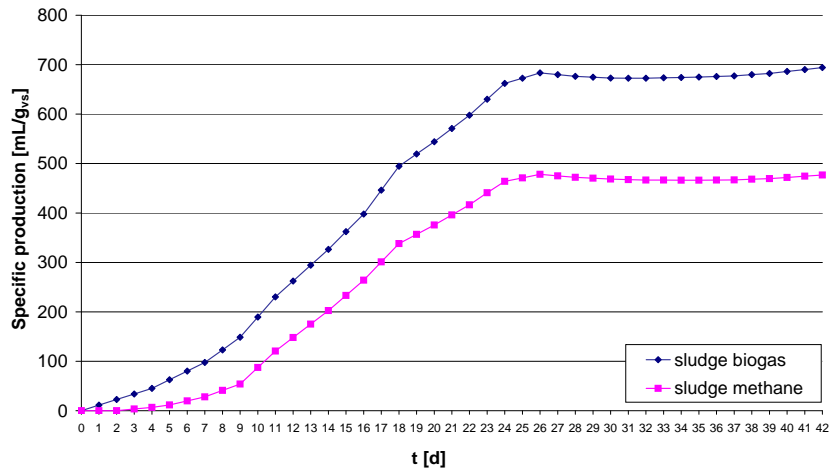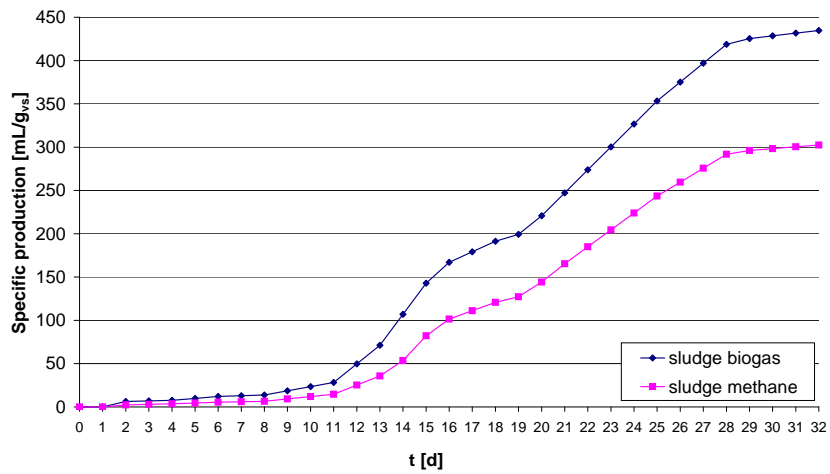

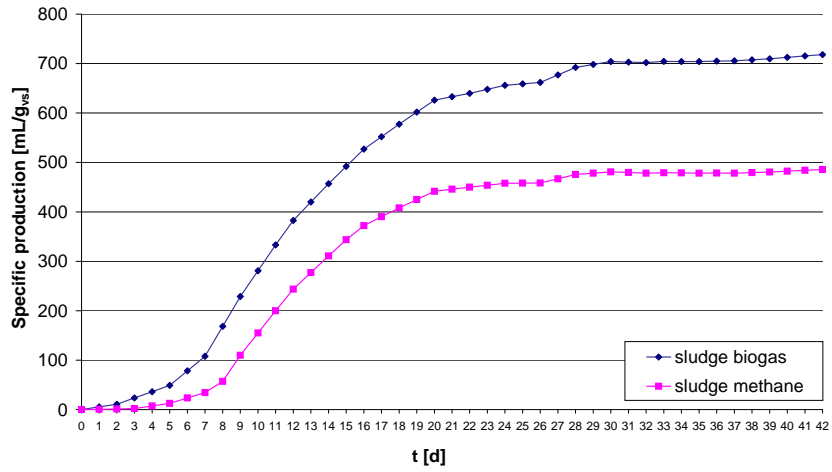Figure 4.9: Cumulated biogas production - sonicated bottles <8>

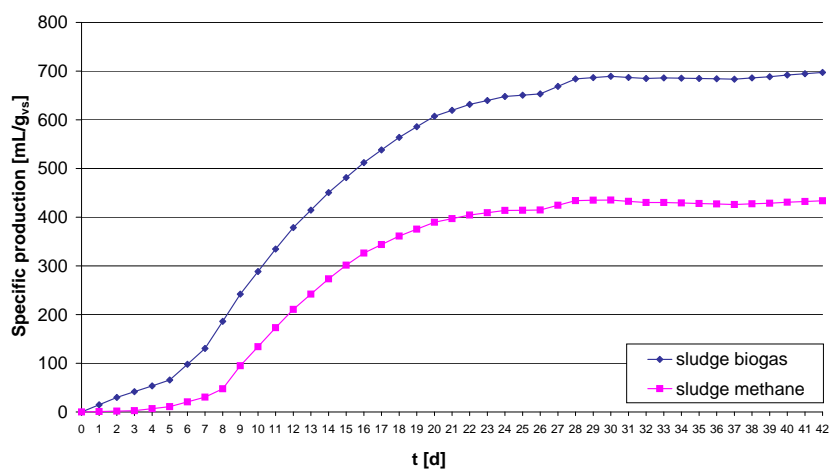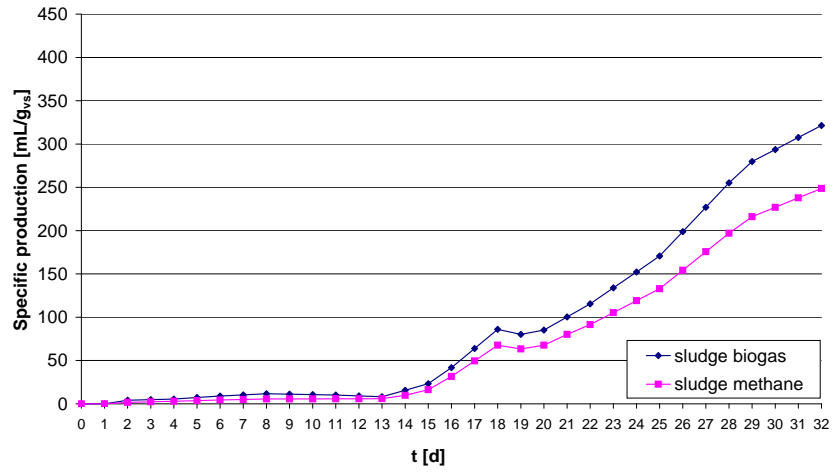Figure 4.10: Cumulated biogas production - enzymic hydrolysis bottles <9>
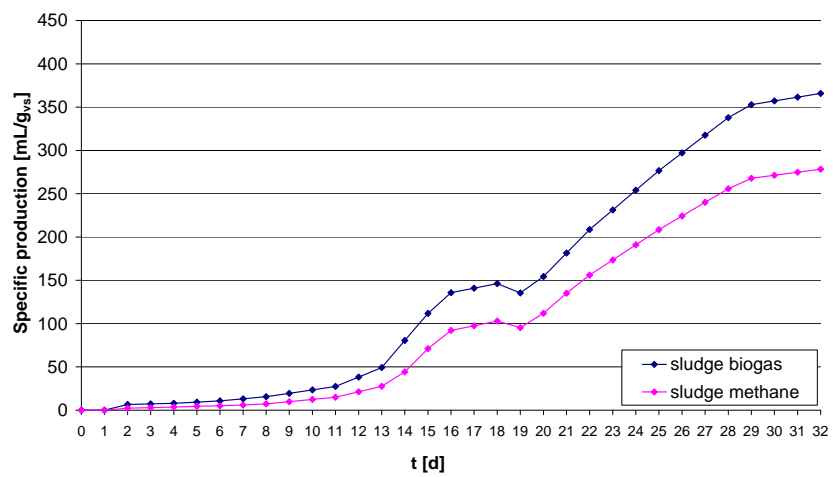
Figure 4.11: Cumulated biogas production - combined treatment <10>, bottle #2

Table 4.9: Comparison of the pretreatments effect

		total COD		soluble COD		[N-NH$_4^+$]		pH		Alkalinity	
		mg/L	Δ	mg/L	Δ	mg/L	Δ	value	Δ	g$_{CaCO_3}$/L	Δ
<2>	untreated	-	-	-	-	1523	-	8,3	-	7,1	-
<3>	sonicated	-	-	-	-	1411	-7,4%	8,1	-3%	7,8	8,6%
<4>	enzym.	-	-	-	-	2218	45,6%	7,6	-8%	8,5	18,4%
<5>	combined	-	-	-	-	1982	30,1%	7,7	-8%	8,9	24,3%
<7>	untreated	41000	-	16400	-	1786	-	8,6	-	8,6	-
<8>	sonicated	41500	1,3%	21000	28%	1814	1,6%	8,3	-4%	8,2	-5,4%
<9>	enzym.	42300	3,3%	20400	24%	1807	1,2%	8,1	-6%	9,0	4,0%
<10>	combined	44700	9,1%	22200	35%	1757	-1,6%	8,4	-2%	9,1	5,8%

Columns Δ report the effect of the treatment (comparison of mixtures <3>, <4>, <5> with <2>, and <8>, <9>, <10> with <7>).

4.3   Effect of pretreatments

It is possible to analyze the effect of pretreating the sludge by comparing values of COD, nitrogen-ammonia, pH and alkalinity measured before and after the treatment (tab. 4.9). Concerning COD, the comparison is more accurate if only the 2nd round values are considered, as COD measures for 1st round appear to be not reliable.

- Total COD should theoretically remain constant as pretreatment does not change the COD concentration. However, total COD measures show a slight increase, and this is consistent as pretreatment partially disrupted the solid matter, allowing a more reliable measure of this parameter.

- Soluble COD increases considerably (roughly by 30%), showing that the treatment achieved the desired effect: to release substrate into water by hydrolyzing the organic matter.

- Ammonium nitrogen concentration is affected by a remarkable increment in the 1st round (except for sonication), while almost does not change in the 2nd round. This parameter represents the effect of the pretreatment in terms of hydrolyzed proteins, and therefore it should increase, greatly or slightly according to the protein concentration of the sludge. Sludge used in the 2sst round had an ammonium nitrogen concentration much higher than the one used in the 3nd round, so that results are consistent.

- Alkalinity increases considerably in all cases except for the sonicated sludge.

4.4   Result analysis

Due to the high amount of data collected and to the impossibility of a simple/direct comparison between the two rounds (due to different initial VS concentration and different running time), a specific analysis of the experimental data is necessary to better understand the accuracy of the data itself and the actual methane potential.

4.4.1   Total COD mass balance

The chemical oxygen demand represents the amount of oxygen necessary to oxidize the organic and inorganic compounds in water. It is usually expressed as mg of oxygen per L of mixture. Total COD concentration value should be measured at the beginning and at the end of the digestion, considering residual COD of the mixture plus the fraction of COD that was transfered to the gas phase and therefore is evaluated by the CH$_4$ production.

The COD balance is therefore assessed by the following equation

$$CODt_f = CODt_i + COD_{CH_4} = CODt_i + V_{CH_4} \cdot f_c \qquad (4.1)$$

in which $CODt_f$ and $CODt_i$ represent the mass of COD at the final and initial stage of the digestion; COD_{CH_4} is the COD evaluated by the volume of CH$_4$ (V_{CH_4}) produced through a theoretical conversion factor ($f_c = 2,86\,g_{COD}/nL_{CH_4}$).

In detail, COD_{CH_4} may be calculated directly from the definition of COD, knowing that two moles of oxygen are necessary to oxidize a mole of methane:

$$CH_4 + 2\,O_2 \longrightarrow CO_2 + 2\,H_2O$$
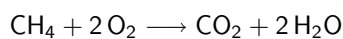
which means that, by stoichiometry, 1 mole of methane, is equivalent to 2 moles of oxygen or 2 moles of COD, which have a mass of $2\,mol \cdot 32\frac{g}{mol_{O_2}} = 64\,g_{COD}$. But 1 mole, at STP of 273 K and 1 atm, has a volume of 22,414 nL, so it is possible to state the following expression

$$1\,mol_{CH_4} = 64\,g_{COD} = 22,414\,nL_{CH_4}$$

and therefore to calculate the conversion factor as

$$f_C = \frac{64\,g_{COD}}{22,414\,nL_{CH_4}} = 2,86\,\frac{g_{COD}}{nL_{CH_4}}.$$

Results (tab. 4.10) are very poor, particularly the ones coming from the first round (mixtures <1> to <5>). Variations are calculated as comparison between the initial and the total-final COD values.

Table 4.10: total COD mass balance

		<1> blank	<2> untreated	<3> sonicated	<4> enzym.	<5> combined
$CODt_i$	g	37,36	30,18	30,18	30,18	30,18
$CODt_f$	g	26,82	41,75	39,74	44,06	45,19
$CODt_{CH_4}$	g	13,97	32,41	36,23	36,65	34,23
$total CODt_f$	g	40,77	74,16	75,97	80,71	79,42
$variation$	%	+9%	+146%	+152%	+167%	+163%

		<6> blank	<7> untreated	<8> sonicated	<9> enzym.	<10.2> combined
$CODt_i$	g	40,66	71,70	72,66	74,06	78,20
$CODt_f$	g	65,61	96,49	99,79	83,03	93,96
$CODt_{CH_4}$	g	19,27	31,97	33,62	31,07	28,58
$total CODt_f$	g	84,88	128,46	133,41	114,10	122,09
$variation$	%	+109%	+79%	+84%	+54%	+56%

It is very probable the presence of a mistake in the COD measures of the mixtures <2> to <5>. This also may be attributed to the measurement method used. In fact, the procedure employed to measure this data is usually applied to evaluate COD of waters, and therefore it is not the best one for the solid matter of the sludges.

A check of this assertion can be made by considering a theoretical COD value obtained by the COD of the manure and the sludge considered separately, taking into account the volumes of manure and sludge used to prepare the mixture. By knowing that

- for the first round

    - $CODt_{sludge} = 262,32\,g/L$
    - $CODt_{manure} = 65,76\,g/L$
    - Bottle composition: $0,115\,L_{sludge} + 0,985\,L_{manure} + 0,65\,L_{water} = 1,75\,L_{mixture}$

- for the second round

    - $CODt_{sludge} = 65,76\,g/L$
    - $CODt_{manure} = 43,22\,g/L$
    - Bottle composition: $0,65\,L_{sludge} + 1,1\,L_{manure} = 1,75\,L_{mixture}$

and remembering that, for blank mixtures, the sludge volume is replaced by an equivalent amount of water (for which COD = 0), it is possible to assess a theoretical COD measure ($th - CODt$, tab. 4.11):

$$th - CODt = CODt_{sludge} \cdot \frac{V_{sludge}}{V_{tot}} + CODt_{manure} \cdot \frac{V_{manure}}{V_{tot}}$$

This may be considered a theoretical proof of the inaccuracy of the initial COD measures, particularly for mixtures <2> to <5>, which values were highly underestimated.

It is now possible to recalculate the total COD mass balance considering the $th - CODt_i$ as initial total COD values (tab. 4.12). Results are better, but there is still a variation of about -15 to +70%, indicating uncertainty of the measures. It is important to note that COD measures referred to pretreated mixtures are

Table 4.11: Theoretical COD assessed by the proportion of manure and sludge and variation from the measured value

		<1> blank	<2> untreated	<3> sonicated	<4> enzym.	<5> combined
$CODt_i$	g/L	21,35	17,25	17,25	17,25	17,25
$th-CODt_i$	g/L	37,01	54,25	54,25	54,25	54,25
$variation$	%	+73%	+215%	+215%	+215%	+215%

		<6> blank	<7> untreated	<8> sonicated	<9> enzym.	<10> combined
$CODt_i$	g/L	23,24	40,97	41,52	42,32	44,69
$th-CODt_i$	g/L	27,16	44,96	44,96	44,96	44,96
$variation$	%	+17%	+10%	+8%	+6%	+1%

Table 4.12: total COD mass balance considering $th-CODt_i$ and variation from the final total COD value

		<1> blank	<2> untreated	<3> sonicated	<4> enzym.	<5> combined
$th-CODt_i$	g	64,77	94,94	94,94	94,94	94,94
$CODt_f$	g	26,82	41,75	39,74	44,06	45,19
$CODt_{CH_4}$	g	13,97	32,41	36,23	36,65	34,23
$totalCODt_f$	g	40,79	74,16	75,97	80,71	79,42
$variation$	%	-37%	-22%	-20%	-15%	-16%

		<6> blank	<7> untreated	<8> sonicated	<9> enzym.	<10> combined
$th-CODt_i$	g	47,54	78,67	78,67	78,67	78,67
$CODt_f$	g	65,61	96,49	99,79	83,03	93,96
$CODt_{CH_4}$	g	19,27	31,97	33,62	31,07	32,47
$totalCODt_f$	g	84,88	128,46	133,41	114,10	126,43
$variation$	%	+79%	+63%	+70%	+45%	+61%

better estimated, and this appears to be correct, as pretreatment carries out a first cell disruption, allowing a better measure of the COD of the particulate matter. Moreover, it is important to consider that manure is a poorly degradable substance for which COD measures cannot be considered very reliable. Therefore, it is not strange that blank measures have the highest initial-final variation, as blank mixtures are composed by the only manure.

4.4.2 Degradability

Many indicators can be considered when evaluating the yield of the anaerobic digestion process. In particular, it is usually evaluated considering the sludge COD consumption, the sludge volatile solid consumption and the volume of produced methane.

Degradability is calculated as the ratio between the COD_{CH_4} (same as eq. 4.1 but only referred to the sludge) and the initial sludge COD:

$$D(\%) = \frac{COD_{sludge,CH_4}\ [g]}{COD_{sludge,i}\ [g]} \cdot 100$$

Another form to consider the degradability is the ratio between the variation and the initial value of the sludge volatile solids:

$$D_{VS}(\%) = \frac{\Delta VS_{sludge}\ [g/L]}{VS_{sludge,i}\ [g/L]} \cdot 100$$

Another indicator, and probably the most common one, is the so-called Biochemical Methane Potential, calculated as ratio between the volume of methane produced by the sludge and the initial mass of the sludge volatile solids:

$$BMP = \frac{CH_{4,sludge}\ [nL]}{COD_{sludge,i}\ [g]}$$

The BMP has a theoretical maximum value of 0,35, that corresponds to the maximum stoichiometric volume (L) of methane achievable by a gram of COD.

Table 4.13: Degradability

		<2> untreated	<3> sonicated	<4> enzym.	<5> combined
COD_{sludge,CH_4}	g	18,44	22,26	22,68	20,26
$COD_{sludge,i}$	g	30,17	30,17	30,17	30,17
D	%	61%	74%	75%	67%
$VS_{sludge,f}$	g/L	1,42	1,30	1,29	1,30
$VS_{sludge,i}$	g/L	10,03	10,03	10,03	10,03
D_{VS}	%	85,9%	87,0%	87,2%	87,1%
$CH_{4sludge}$	nL	6,46	7,80	7,94	7,10
$COD_{sludge,i}$	g	30,17	30,17	30,17	30,17
BMP	L/g	0,21	0,26	0,26	0,24

		<7> untreated	<8> sonicated	<9> enzym.	<10> combined
COD_{sludge,CH_4}	g	12,70	14,35	11,80	13,20
$COD_{sludge,i}$	g	31,14	31,14	31,14	31,14
D	%	41%	46%	38%	42%
$VS_{sludge,f}$	g/L	5,14	4,87	4,52	4,50
$VS_{sludge,i}$	g/L	10,19	10,19	10,19	10,19
D_{VS}	%	50%	52%	56%	56%
$CH_{4sludge}$	nL	4,45	5,03	4,13	4,62
$COD_{sludge,i}$	g	31,14	31,14	31,14	31,14
BMP	L/g	0,14	0,16	0,13	0,15

Table 4.14: Degradability parameters variation comparison treated vs. untreated mixtures

	<3> sonicated vs. <2>	<4> enzym. vs. <2>	<5> combined vs. <2>	<8> sonicated vs. <7>	<9> enzym. vs. <7>	<10> combined vs. <7>
ΔD	20,7%	23,0%	9,9%	13,0%	-7,1%	3,9%
ΔD_{VS}	1,3%	1,5%	1,4%	5,3%	12,3%	12,6%
ΔBMP	20,7%	23,0%	9,9%	13,0%	-7,1%	3,9%

Results indicate that digestion of 1st round mixtures was more complete, this being explained by the higher tests time (42 days) compared to the 2nd round (32 days), so that in the 2nd round digestions were still ongoing.

Both rounds results show that pretreatments increased the digestion yield (tab. 4.14). Concerning 1st round, sonication and enzymic hydrolysis appear to give very similar results, as D and BMP increased by 21-23% and D_{VS} by 1,3-1,5%. With the combined pretreatment, D_{VS} had the same increment, but D and BMP variation was lower. Concerning the 2nd round, results are more scattered and therefore it is more difficult to come to a conclusion. Anyway, pretreatments still appear to increase the degradability, with the exception of D and BMP results for the enzymic hydrolysis. In particular, enzymic hydrolysis and the combined pretreatment give better enhancement in terms of D_{VS}.

4.4.3 COD/VS

The ratio between COD and volatile solids provides an important information about the organic composition of the sludge, the inoculum and the mixture.

According to [Angelidaki, 2004], average values of the COD/VS ratio can be referred to standard values representing the main component of the substance. For instance, carbohydrates have a COD/VS ratio around 1,19, proteins 1,42, lipids 2,90, ethanol 2,09, acetic acid 1,07 and propionic acid 1,51.

Results are reported in table 4.15.

Results show that main component of both of the mixtures are proteins, as it is common for wastewater sludges. It is important as well to point out that the second round final value indicates that the main component of the mixture at that stage were the lipids, and this might be read as a confirm that the second round was terminated when the digestion process was still at an initial-intermediate state, as lipids are the molecules with the lowest degradation speed [IWA, 2002].

On the other hand, the final value of 1st round is lower than the initial one. This can be explained by considering that 1st round mixtures had time for a complete digestion, so that all the compounds contained

Table 4.15: COD/VS ratio

	1st round	2nd round
COD/SV manure	1,62	1,37
COD/SV sludge	1,72	1,75
COD/SV mixture	(0,52)	1,41
th-COD/SV mixture	1,65	1,49
COD/SV mixture, final	1,27	2,88

in the mixture at the initial stage were degraded to simpler molecules, hence a lower COD/VS value.

Moreover, 1st round initial value is totally out of range, confirming the probable incorrectness of COD measures of the mixtures. Theoretical COD value appears, once again, more reliable.

4.4.4 Digestion kinetics

Methane production of particulate and slowly hydrolysable oraganic matter can usually be represented with an exponential model such as:

$$CH_4(t) = CH_{4\infty} \cdot (1 - exp^{-k_h \cdot t}) \tag{4.2}$$

where $CH_4(t)$ is the methane production at time t, $CH_{4\infty}$ is the asymptote to which methane production tends at the end of the digestion process, k_h is the hydrolysis constant.

In some cases, a 0-order kinetics fits data better. When this is the case, the model assumes the simple equation of a line and the parameters to be assessed are the slope a, representing the hydrolysis average speed, and the intercept b, that has no physical meaning:

$$CH_4(t) = a \cdot t + b \tag{4.3}$$

In the case of this research, experimental data from the 1st round are fairly well represented by the exponential model (fig. 4.12), while 2nd round values fit better a zero-order model (fig. 4.13), as the digestion process was interrupted so that there are no values to represent the asymptotic part of the model.

In both cases, data collected during the initial lag phase were discarded. As a consequence, only the following data are considered:

- mixture <2>, untreated: from day 11 to 42, for a total of 32 days

- mixture <3>, sonicated: from day 11 to 42, for a total of 32 days

- mixture <4>, enzymic hydrolysis: from day 8 to 42, for a total of 35 days

- mixture <5>, combined: from day 8 to 42, for a total of 35 days

- mixture <7>, untreated: from day 16 to 32, for a total of 17 days

- mixture <8>, sonicated: from day 11 to 32, for a total of 22 days

- mixture <9>, enzymic hydrolysis: from day 14 to 32, for a total of 19 days

- mixture <10>, combined: from day 11 to 32, for a total of 22 days

Lag times are generally remarkable, corresponding up to 47% of the whole BMP test time, and can be attributed to the oldness of the manure (62 days in the 1st round, 55 in the 2nd), so that it provided an inoculum with a low initial bacterial activity.

The values, deprived of the initial lag, were then used to assess the parameters of the model with the least square errors technique. Results are reported in tables 4.16 and 4.17 and in fig. 4.14, each one with the corresponding 95% confidence interval.
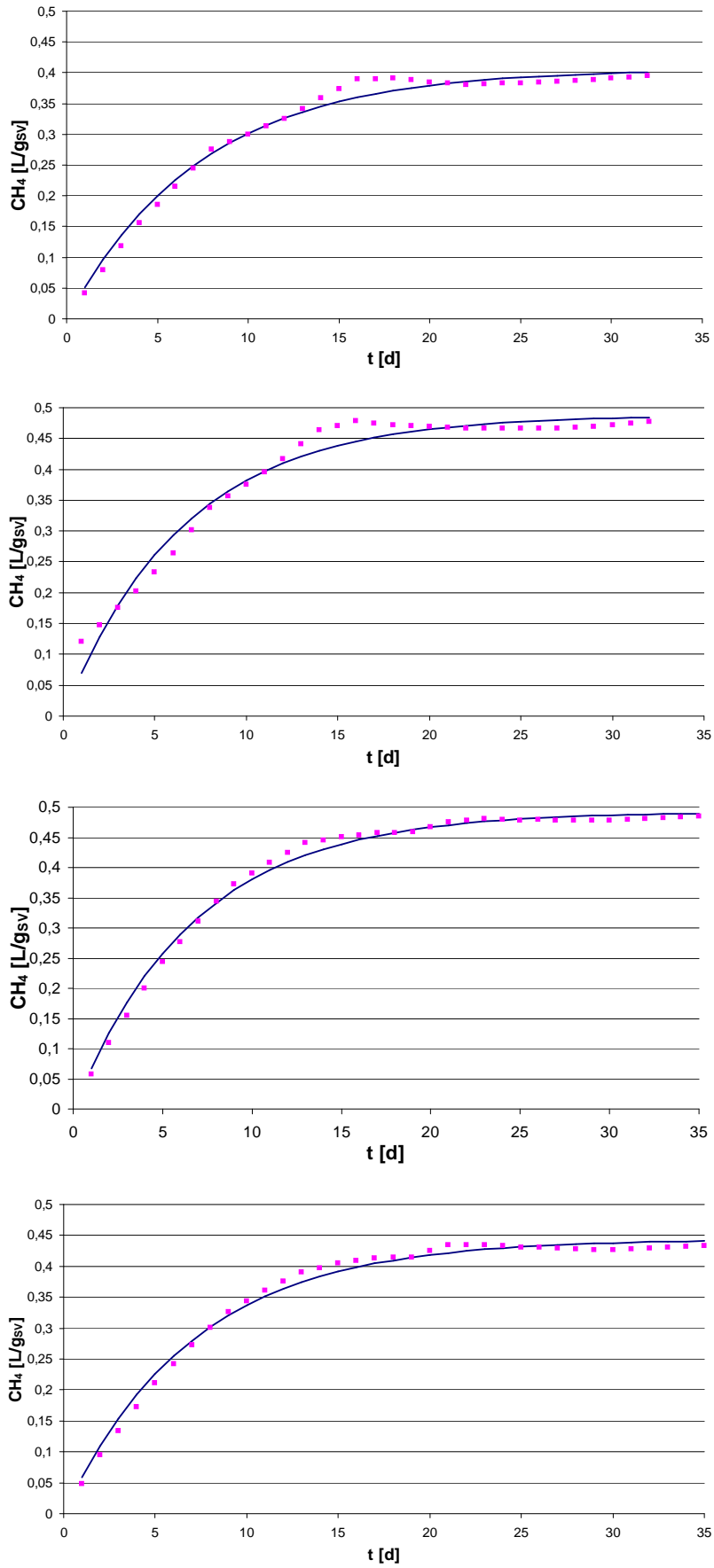
Figure 4.12: mixtures <2>, <3>, <4> and <5> digestion kinetics data (dots) and model (line)
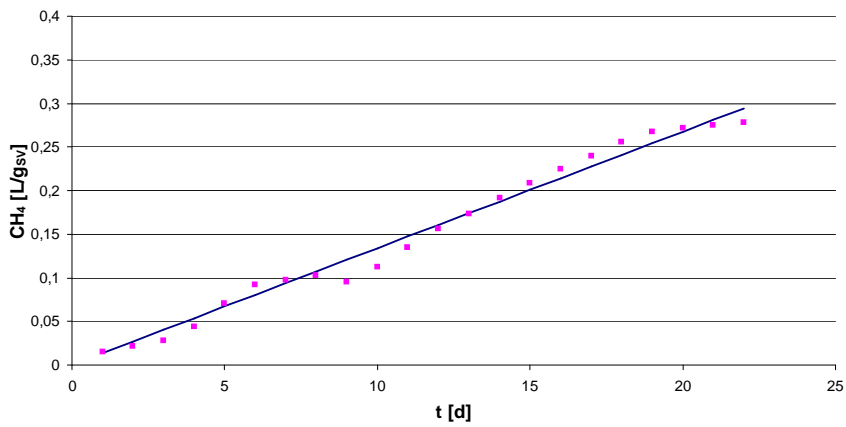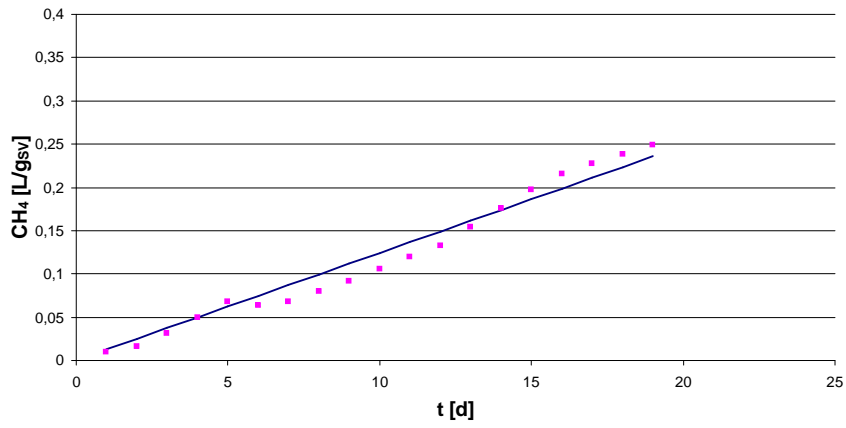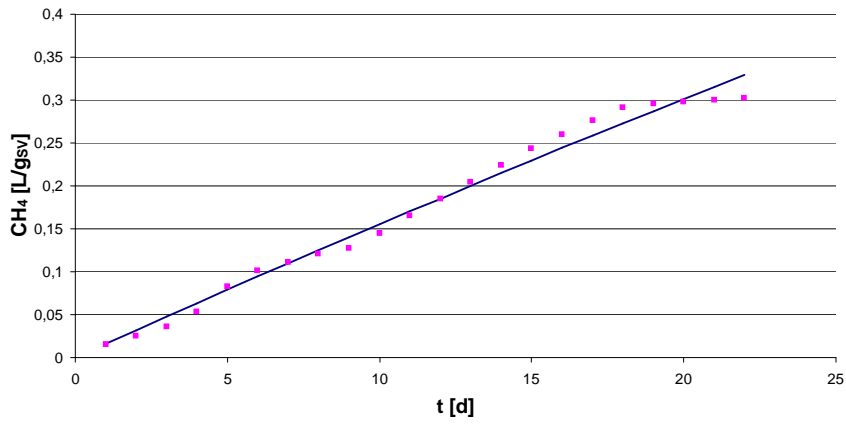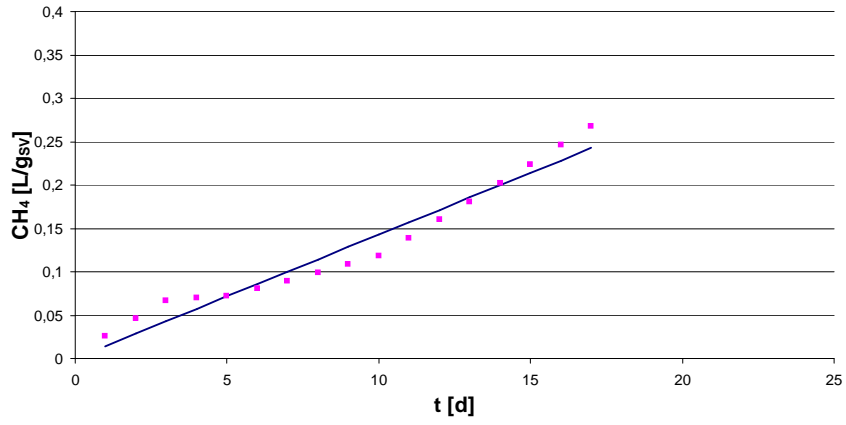
Figure 4.13: mixtures <7>, <8>, <9> and <10> digestion kinetics data (dots) and model (line)

Table 4.16: BMP model parameters - 1st round

mixture	$CH_{4\infty}$ [L/g$_{vs}$]	95% confidence interval	k_h [1/d]	95% confidence interval
<2> untreated	0,406	$0,397 - 0,416$	0,136	$0,125 - 0,146$
<3> sonicated	0,487	$0,474 - 0,500$	0,154	$0,139 - 0,169$
<4> enzym.	0,493	$0,486 - 0,499$	0,148	$0,141 - 0,155$
<5> combined	0,444	$0,437 - 0,451$	0,142	$0,134 - 0,150$

Table 4.17: BMP model parameter - 2nd round

mixture	a [1/d]	95% confidence interval
<7> untreated	0,014	$0,012 - 0,016$
<8> sonicated	0,015	$0,014 - 0,016$
<9> enzym.	0,014	$0,013 - 0,015$
<10> combined	0,014	$0,013 - 0,015$

Regarding 1st round results, it is possible to notice that $CH_{4\infty}$ increases comparing untreated to treated mixtures, and so does its confidence interval, providing statistical significance to this assumption. This can lead to the conclusion that energy supplied with pretreatments entailed a partial hydrolysis of the substrate, providing then a more easily digestible mixture from which a higher biogas volume will be produced. The maximum increase is the +21,2% due to the enzymic hydrolysis, followed by the +19,9% of the sonication and the +9,3% of the combined pretreatment.

Also k_h values appear to increase with the pretreatment (+13,3% with sonication, +9,1% with enzymic hydrolysis and +5,0% with combined pretreatment), but confidence intervals partially overlap, so that there is no statistical evidence regarding the influence of pretreatments in the biogas yield production and no firm conclusion can be given.

2nd round results do not give any relevant information, as values obtained for the hydrolysis average rate are scattered (+8,1% for sonication, -2,8% for enzymic hydrolysis and -1,8% for the combined pretreatment) and confidence intervals totally overlap, and therefore these values cannot lead to any firm conclusion.

4.4.5 Methane production

The volume of produced methane was estimated from methane percentages of biogas. It is although important to consider that, during the digestion process, part of the carbon dioxide naturally solubilized in water to bicarbonate, following the reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \tag{4.4}$$
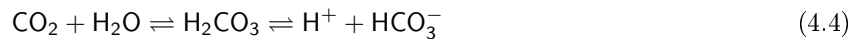
thus providing an underestimation of the percentage of carbon dioxide produced and so an overestimation of the methane. It is possible to estimate the rate of solubilized carbon dioxide by comparing initial and final alkalinity measures that provide the concentration of bicarbonate in the mixture (in g_{CaCO_3}/L).

Real methane production can then be calculated with the following equation

$$CH_4(\%) = \frac{CH_{4\,measured}}{CH_{4\,measured} + CO_{2\,measured} + CO_{2\,dissolved}}$$
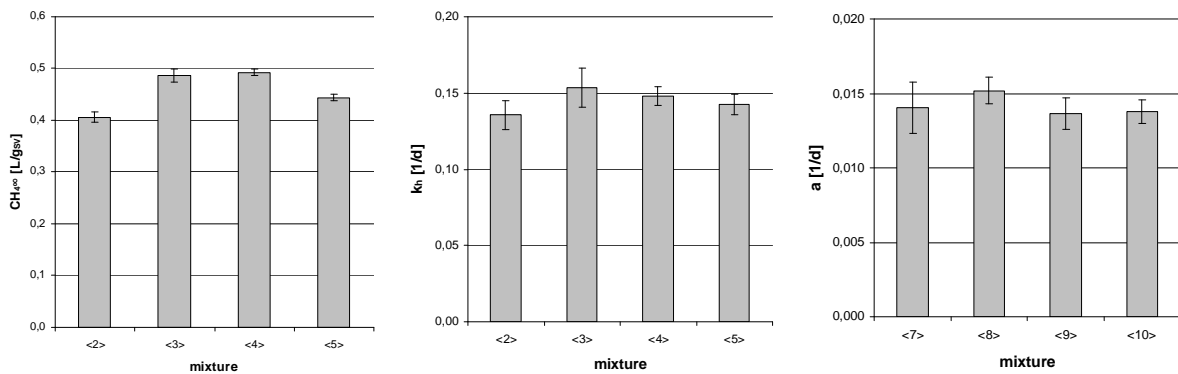
Figure 4.14: $CH_{4\infty}$, k_h and a parameter comparison

Data and results are reported in table 4.18.

Table 4.18: Real methane production

		$<2>$ untreated	$<3>$ sonicated	$<4>$ enzym.	$<5>$ combined
net ΔAlkalinity[6]	g_{CaCO_3}/L	1,44	1,14	-0,16	-0,86
	g_{CO_2}/L	0,63	0,50	-0,07	-0,38
dissolved CO_2	$L_{CO_2}/L_{mixture}$	0,32	0,26	-0,04	-0,19
	L_{CO_2}/g_{vs}[7]	0,032	0,025	-0,004	-0,019
measured CH_4	nL_{CH_4}/g_{vs}	0,368	0,444	0,452	0,404
measured CO_2	nL_{CO_2}/g_{vs}	0,120	0,170	0,178	0,166
$CH_{4\ meas}+CO_{2\ meas}+CO_{2\ diss}$	nL/g_{vs}	0,520	0,640	0,627	0,551
CH_4 production	%	71%	69%	72%	73%

		$<7>$ untreated	$<8>$ sonicated	$<9>$ enzym.	$<10>$ combined
net ΔAlkalinity	g_{CaCO_3}/L	0,34	0,97	0,08	-0,20
	g_{CO_2}/L	0,15	0,43	0,03	-0,09
dissolved CO_2	$L_{CO_2}/L_{mixture}$	0,08	0,22	0,02	-0,05
	L_{CO_2}/g_{vs}	0,008	0,021	0,002	-0,004
measured CH_4	nL_{CH_4}/g_{vs}	0,249	0,282	0,232	0,259
measured CO_2	nL_{CO_2}/g_{vs}	0,071	0,112	0,082	0,099
$CH_{4\ meas}+CO_{2\ meas}+CO_{2\ diss}$	nL/g_{vs}	0,328	0,416	0,316	0,354
CH_4 production	%	76%	68%	73%	73%

(6) Alkalinity variation for the only sludge, result of subtracting the blank alkalinity variation to the other mixtures (blank 1 for mixtures 2-5, blank 6 for mixtures 7-10)

(7) Sludge volatile solids in 1,75 $L_{mixture}$: 10,03 $g_{vs}/L_{mixture}$ for mixtures 2-5 and 10,19 $g_{vs}/L_{mixture}$ for mixtures 7-10, so respectively 17,56 g_{vs} and 17,83 g_{vs}

Results show that variation of methane production is very little, as the portion of CO_2 that dissolved in water was roughly the 5-10% of total CO_2 production. Moreover, percentage of methane in biogas is similar for all the (sonication always being the lowest), indicating that pretreatments might enhance the overall amount of methane produced, but not at the expense of the CO_2.

In three cases ($<4>$, $<5>$ and $<10>$) dissolved CO_2 is negative, meaning that the variation of alkalinity in the mixture of manure and sludge is inferior to the one measured for the manure-only blank. It appears that the CO_2 produced by the sludge was directly released as gas, with no solubilization into water. As a consequence, the negative variation indicates that part of the CO_2 measured as gas has to be assigned to the CO_2 that was dissolved.

4.4.6 Hydrolyzed proteins

Initial and final measures of ammonia concentration can be used to evaluate the amount of hydrolyzed proteins. This is possible because proteins are the only compound containing nitrogen atoms of all compounds forming the mixture that participated to the digestion. Proteins are usually considered to have a constant proportion of nitrogen, empirically assessed to 16% [Metcalfe & Eddy, 2003], so that one gram of proteins corresponds to $1/0,16 = 6,25$ grams of nitrogen.

Due to the breaking of the microwave, total nitrogen measures are available only for initial characterizations, and therefore in this paragraph only ammonia nitrogen measures can be considered.

Results are reported in tab. 4.19.

As variation is very small compared to instrument sensitivity, reported values refer to the whole mixture, that is blank value was not subtracted to mixture values. The interest of this thesis is focused on comparing untreated an treated mixtures, which is still possible by comparing results in this way, avoiding the propagation of the possible measurement error of the blank samples.

Table 4.20 shows that proteins are approx. in the range of 10-20%, so that the majority of the hydrolyzed matter is composed by different substances.

Regarding the hydrolyzed protein values, there is no possibility to conclude whether pretreatments enhanced protein hydrolysis or not, as results are very scattered and it is not possible to spot any kind of trend.

Table 4.19: Hydrolyzed proteins

		\<2\> untreated	\<3\> sonicated	\<4\> enzym.	\<5\> combined
initial [N-NH_4^+]	mg_N/L	1523	1411	2218	1982
final [N-NH_4^+]	mg_N/L	1881	2081	2134	2043
Δ[N-NH_4^+]	mg_N/L	357	670	-84	61
hydrolyzed proteines	$mg_{proteins}/L$	2234	4188	-525	379

		\<7\> untreated	\<8\> sonicated	\<9\> enzym.	\<10\> combined
initial [N-NH_4^+]	mg_N/L	1786	1814	1807	1757
final [N-NH_4^+]	mg_N/L	2002	2021	1980	2080
Δ[N-NH_4^+]	mg_N/L	216	207	173	323
hydrolyzed proteins	$mg_{proteins}/L$	1352	1292	1080	2018

Table 4.20: Protein content in the hydrolized volatile solids

important to notice

		\<2\> untreated	\<3\> sonicated	\<4\> enzym.	\<5\> combined
hydrolyzed proteins	$mg_{proteins}/L$	2234	4188	-525	379
hydrolyzed VS	g_{SV}/L	12,41	14,04	14,27	14,12
hydrolyzed proteins/VS	$g_{proteins}/g_{SV}$	18%	30%	-4%	3%

		\<7\> untreated	\<8\> sonicated	\<9\> enzym.	\<10\> combined
hydrolyzed proteins	$mg_{proteins}/L$	1352	1292	1080	2018
hydrolyzed VS	g_{SV}/L	10,02	11,06	12,45	12,51
hydrolyzed proteins/VS	$g_{proteins}/g_{SV}$	13%	12%	9%	16%

This may be caused by an error propagation, as the amount of hydrolyzed proteins are computed from the difference between two measures of ammonia concentration, an it is possible that this difference is too close to the instrument sensitivity.

4.4.7 Biogas production comparison

Table 4.21 reports a comparison between the two sludges total biogas productions at the 32nd day, that is the final day for the 2nd round.

According to this data it is possible to analyze the sludge degradability in terms of total biogas and methane production.

- 1st round thickened sludge specific production was considerably higher (up to +48% with enzymic hydrolysis), but it is important to consider that 2nd round mixtures had longer inital lag times, and a

Table 4.21: Sludge degradability - 1st and 2nd round comparison [mL/g_{SV}]

		1st round		2nd round	comparison at day 32
		day 32	day 42	day 32	1st $\rightarrow$ 2nd round
\<1\> and \<6\>, blank	biogas	172	194	307	79%
(manure only)	methane	115	131	208	81%
	total biogas	275	297	320	16%
\<2\> and \<7\>, untreated	sludge biogas	510	531	344	-33%
	sludge methane	381	395	268	-30%
	total biogas	324	346	350	8%
\<3\> and \<8\>, sonicated	sludge biogas	673	694	435	-35%
	sludge methane	467	477	303	-35%
	total biogas	333	354	312	-6%
\<4\> and \<9\>, enzym.	sludge biogas	702	718	321	-54%
	sludge methane	478	486	249	-48%
	total biogas	328	347	327	0%
\<5\> and \<10\>, combined	sludge biogas	685	697	366	-47%
	sludge methane	430	434	278	-35%

lower solid concentration (approx. 5,2%, while 1st round sludge was 6%)

- by contrast, 2nd sludge biogas had a higher methane concentration, except for the sonicated mixture, which production was the same as the 1st round one

- considering blank mixtures, it is possible to notice that the second manure produced much more biogas (and methane) than the first one, while considering the mixtures, total biogas production does not change much. It is therefore possible to conclude that the biogas produced by the manure balanced the lower production given from the sludge

- comparing pretreated mixtures between 1st and 2nd round it is possible to notice that methane production lowered by 30-35% (with the only exception of the enzymic hydrolysis) and this is valid for the untreated mixtures too, meaning that pretreatment effect is roughly the same on the two sludges

- this data confirm that pretreatments enhance methane production, with no particular difference between the pretreatments. It is also possible to conclude that combining sonication and enzymic hydrolysis does not improve biogas production more than sonication or enzymic hydrolysis alone: the cost and effort of a double treatment is not balanced by an enhancement in methane production.

This information cannot for sure be considered as conclusions, as they are based only on two rounds of BMP tests. However, this may be a useful starting point for future tests.

4.5 Discussion

In the previous sections a large amount of information was presented, that is useful to analyze jointly. In fact, results show that pretreatments enhanced the hydrolyzation by disrupting the solid matrix and releasing organic matter into water. As a consequence, soluble COD and ammonia concentration increased (refer to chapter 4.3 for the specific values).

The anaerobic digestion carried out an effective degradation of the sludges, reducing soluble COD (approx. -70% in 1st round and -50% in the 2nd) and volatile solids (approx. -40% in both rounds) and increasing the ammonia concentration by approx. 10-20%[8] (and therefore the amount of hydrolyzed proteins).

Concerning biogas, and in particular methane production, it was computed that 1st round sludge had a higher degradability, as it produced roughly 30% more methane than the 2nd. It is also possible to state that pretreatments enhanced methane production, as it was always higher in the case of pretreated sludge, with the only exception of the enzymic hydrolysis of the 2nd round (table 4.22). Moreover, in chapter 4.4.5 was computed that the composition of the biogas is not affected by the treatments, and it settled around 70% in both rounds.

Finally, the kinetics analysis of 1st round showed that pretreatments increased the volume of methane that is possible to produce by approx. 20%, and this is the most important result, as it is an actual demonstration of the usefulness of pretreating the sludge. The methane production rate also appears to increase (approx. by 10%), but confidence intervals partially overlap, so that this aspect needs to be analyzed further with other researches.

Table 4.22: Sludge methane production - enhacement effect of pretreatments

	1st round		2nd round	
	methane production [mL/g_{SV}]	variation after pretreatment	methane production [mL/g_{SV}]	variation after pretreatment
<2> and <7>, untreated	395	-	268	-
<3> and <8>, sonicated	477	21%	303	13%
<4> and <9>, enzym.	486	23%	249	-7%
<5> and <10>, combined	434	10%	278	4%

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[8]values for 2nd round. For the 1st round, measures are quite scattered, approx. ranging from 0 up to 47%.

Chapter 5

Energetic analysis

A plant scale energetic analysis was carried out to compute the impact that a pretreatment has over an hypothetical wastewater treatment plant in order to assess the economical feasibility.

The analysis considers the kinetic data computed for 1st round sludge (table 4.16) and is expressed per unit of incoming COD, i.e. the COD of the sewage water measured at the plant entrance. In fact, it is possible to consider how the incoming COD is divided into the products of the wastewater treatment (fig. 5.1): in particular, 25% of the incoming COD is assigned to primary sludge and 30% to secondary sludge. Considering a mixed sludge composed of equal parts of primary and secondary sludge, the percentage of COD transferred from sewage water to sludge will be 55%.

A list of all the assumptions made is given in table 5.1.

As sludge with higher solid concentration gives a higher yield in terms of biogas production, a thickener is necessary previous to the pretreatment equipment. It was supposed an initial solid concentration of 3,5%, growing up to 5% after the thickening. Because of this higher concentration, and under the assumption of using the same anerobic digestor in case of applying a pretreatment, the hydraulic residence time will rise of a factor $5/3,5$, and therefore the heating necessary will decrease of the inverse $(3,5/5)$. A schematic of the anareobic digestion with or without pretreatment is reported in figure 5.2.
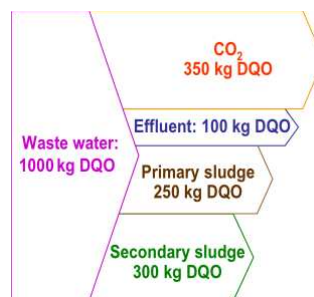
Figure 5.1: Organic matter flow for a conventional activated sludge process [Fdez-Polanco et al, 2009]
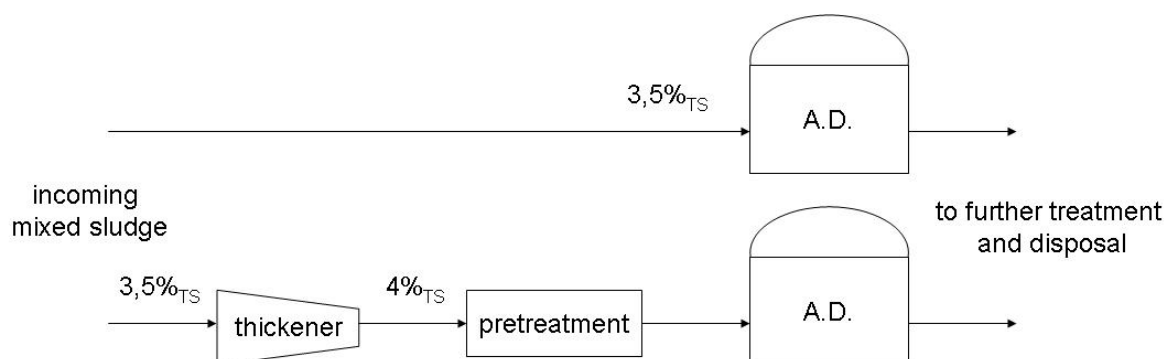
Figure 5.2: Anaerobic digestion of untreated sludge (first line) and with previous treatment (second line)

Table 5.1: Assumptions

value	units	description	reference
55%	$\frac{gCOD_{sludge}}{gCOD_{sewage}}$	part of the incoming sewage COD transferred to the mixed sludge (25% to primary sludge and 30% to the secondary)	[Fdez-Polanco et al, 2009]
$\theta_H = 20$	d	Hydraulic residence time for the untreated sludge	-
$\theta_H = 28,6$	d	Hydraulic residence time for the treated sludge (higher than the case of untreated sludge because of the thickening process applied to bring solid content from 3,5 to 5%)	-
0,4	$\frac{kWh_{el}}{kWh_{th}}$	conversion efficiency from thermal to electric energy	-
0,4	$\frac{kWh_{th}}{kWh_{el}}$	conversion efficiency from electric to thermal energy (typical values are between 33% and 48%)	-
0,35	$\frac{kWh_{el}}{kgCOD_{sewage}}$	energy cost for aeration	[Bonomo, 2007]
50	$\frac{kWh_{el}}{p.e.\cdot year}$	specific aerobic cost for wastewater treatment plants in Milano	[Bonomo, 2007]
43,8	$\frac{kgCOD_{sewage}}{p.e.\cdot year}$	specific COD discharges	[Bonomo, 2007]
90	$\frac{€}{t_{sludge}}$	averrage cost for sludge disposal	[Bonomo, 2007]
0,9	$\frac{€}{kgCOD_{sewage}}$	average treatment cost	[Bonomo, 2007]
24%	$\frac{kg_{TS}}{kg_{sludge}}$	output thickening concentration	-
1,26	$\frac{kWh}{m^3}$	typical cost of a thickening process	-
100%	-	heat loss of the reactors (overall need for heating will be twice the theoretical one)	-

5.1 Electric energy production

The electric energy production (per unit of incoming sewage COD) was computed in few steps.

First of all, it is necessary to calculate the volume of methane produced at the residence time θ_H of 20 or 28,6 days, taking into account that:

- as hydrolysis is the limiting stage, a 1st-order kinetics was considered,

- the methane production achievable by a complete degradation and the kinetic constant were assessed by the results of the BMP tests ($CH_{4\infty}$ and k_θ in table 4.16),

- methane production process is complementary to the COD removal.

COD removal follows the equation:

$$\frac{dX}{dt} = \frac{X_{in} - X}{\theta_H} - k_h \cdot X \qquad (5.1)$$

where X is the COD concentration, X_{in} is the input COD concentration, θ_H is the hydraulic retention time and k_h is the kinetics constant.

At stationary conditions, $\frac{dX}{dt} = 0$ and therefore:

$$X = \frac{X_{in}}{1 + k_h \cdot \theta_H} \qquad (5.2)$$

From equation 5.2 it is possible to state a conversion yield of the process, that will take different values if a pretreatment was applied:

$$\eta_{AD} = \left(1 - \frac{X}{X_{in}}\right)$$

As said, the methane production process is complementary to the COD removal, therefore:

$$CH_{4,\theta} = CH_{4\infty} \cdot \eta_{AD}$$

Values of $CH_{4,\theta}$ considering a $\theta_H = 20$ days are reported in table 5.2.

From these values it is also possible to compute the amount of COD that was transfered to methane, referred to the COD concentration of the incoming sewage:

$$Y\left[\frac{kg_{COD\to CH_4}}{kg_{COD_{sludge}}}\right] = \frac{CH_{4,\theta=20\,d}\left[\frac{L_{CH_4}}{g_{VS}}\right]}{0,35\left[\frac{L_{CH_4}}{g_{COD\to CH_4}}\right] \cdot 1,72\left[\frac{g_{COD_{sludge}}}{g_{VS}}\right]}$$

Table 5.2: Actual methane production over 20 days

	$CH_{4\infty}$ $[L_{CH_4}/g_{VS}]$	k_h [1/d]	θ_H [d]	η_{AD}	$CH_{4,\theta=20\,d}$ $[L_{CH_4}/g_{VS}]$	Y $[kg_{COD\to CH_4}/kg_{COD_{sludge}}]$
<2> untreated	0,406	0,136	20	0,73	0,30	0,49
<3> sonicated	0,487	0,154	28,6	0,81	0,40	0,66
<4> enzym.	0,493	0,148	28,6	0,81	0,40	0,66
<5> combined	0,444	0,142	28,6	0,80	0,36	0,59

Table 5.3: Electric energy production

	E_{th} $[kWh_{th}/kg_{COD_{sewage}}]$	E_{el} $[kWh_{el}/kg_{COD_{sewage}}]$	variation after pretreatment
<2> untreated	0,95	0,38	-
<3> sonicated	1,26	0,51	34%
<4> enzym.	1,27	0,51	34%
<5> combined	1,13	0,45	20%

where, in particular, $0,35 \frac{m^3_{CH_4}}{kg_{COD}}$ is the stoichiometric conversion factor from COD to methane, i.e. the inverse of the f_c of equation 4.1. Results are reported in table 5.2.

Moreover, considering that the inferior calorific power of methane is $PCI_{CH_4} = 9,96\,kWh_{th}/m^3_{CH_4}$, that the efficiency of thermal to electric energy conversion is $0,4\,kWh_{el}/kWh_{th}$, that the COD/SV ratio for the mixed sludge used is 1,72 and that only 55% of the sewage COD is transferred to the sludge, it is possible to compute the electric energy E_{el} that may be produced:

$$E_{th}\left[\frac{kWh_{th}}{g_{COD_{sewage}}}\right] = \frac{CH_{4,\theta=20\,d}\left[\frac{L_{CH_4}}{g_{VS}}\right] \cdot 0,00996\left[\frac{kWh_{th}}{L_{CH_4}}\right]}{1,72\left[\frac{g_{COD_{sludge}}}{g_{VS_{sludge}}}\right]} \cdot 0,55\left[\frac{g_{COD_{sludge}}}{g_{COD_{sewage}}}\right]$$

$$E_{el}\left[\frac{kWh_{el}}{g_{COD_{sewage}}}\right] = E_{th}\left[\frac{kWh_{th}}{g_{COD_{sewage}}}\right] \cdot 0,4\left[\frac{kWh_{el}}{kWh_{th}}\right]$$

Results are reported in table 5.3. Pretreatments appear to enhance energy production by 34% for sonication, 34% for enzymic hydrolysis and only 20% for the combined.

5.2 Impact on the plant management costs

The electric energy production values may be used to assess the impact on the plant management costs.

For example, energy production appear to balance correctly the cost for the aeration, which is generally the highest in the plant budget (tab. 5.4).

In table 5.5 are reported the results of the economic analysis. Values computed are the following:

- energy production increment with pretreatment, as difference between the energy produced by the treated samples and the energy produced by the untreated ones:

$$\Delta E_{el}\left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right] = E_{el}\left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]_{treated} - E_{el}\left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]_{untr.}$$

- impact of the energy increase on the plant overall energy needs, as ratio between ΔE_{el} and the energy needs, calculated as ratio between the energy requirement per population equivalent per year and the

Table 5.4: energy production vs. energy cost for aeretion$[kWh_{el}/kg_{COD_{sewage}}]$

	E_{el}	aeration	net energy production
<2> untreated	0,38	0,35	0,03
<3> sonicated	0,51	0,35	0,16
<4> enzym.	0,51	0,35	0,16
<5> combined	0,45	0,35	0,10

Table 5.5: Economic analysis

	ΔE_{el} $\left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]$	$i_{\Delta E}$ [%]	$\Delta Sludge$ $\left[\frac{kg_{COD_{sludge}}}{kg_{COD_{sewage}}}\right]$	$Saving$ $\left[\frac{\text{€}}{kg_{COD_{sewage}}}\right]$	i_{Saving} [%]
<3> sonicated	0,127	11,2%	0,091	0,030	3,4%
<4> enzym.	0,130	11,4%	0,093	0,031	3,4%
<5> combined	0,076	6,6%	0,054	0,018	2,0%

COD production per population equivalent per year [values by Bonomo, 2007]:

$$i_{\Delta E} \ [\%] = \frac{\Delta E_{el} \left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]}{\frac{50 \left[\frac{kWh_{el}}{p.e.\cdot year}\right]}{43,8 \left[\frac{kg_{COD_{sewage}}}{p.e.\cdot year}\right]}} \cdot 100 = \frac{\Delta E_{el} \left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]}{1,14 \left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]} \cdot 100$$

- reduction in sludge production per unit of sewage COD, computed as difference between the COD not transfered to methane of the untreated sludge and the one undergone ultrasounds, enzymic hydrolysis or combined pretreatment:

$$\Delta Sludge \left[\frac{kg_{COD_{sludge}}}{kg_{COD_{sewage}}}\right] =$$

$$= \left[\left(1 - Y \left[\frac{kg_{COD \to CH_4}}{kg_{COD_{sludge}}}\right]\right)_{untr.} - \left(1 - Y \left[\frac{kg_{COD \to CH_4}}{kg_{COD_{sludge}}}\right]\right)_{treated}\right] \cdot 0,55 \left[\frac{g_{COD_{sludge}}}{g_{COD_{sewage}}}\right]$$

- economic saving corresponding to the sludge reduction, computed as ratio between the sludge reduction and the COD content of the sludge after thickening[1], multiplied by an average cost for sludge disposal of 90 €/ton [Bonomo, 2007]:

$$Saving \left[\frac{\text{€}}{kg_{COD_{sewage}}}\right] = \frac{\Delta Sludge \left[\frac{kg_{COD_{sludge}}}{kg_{COD_{sewage}}}\right]}{0,27 \left[\frac{kg_{COD}}{kg_{sludge}}\right]} \cdot \frac{90 \left[\frac{\text{€}}{t}\right]}{1000 \left[\frac{kg}{t}\right]}$$

- saving on the overall wastewater treatment plant management, computed as the economic saving per kg of incoming COD divided by an average treatment cost of 0,9 €/$kg_{COD_{sewage}}$ [Bonomo, 2007]:

$$i_{Saving} \ [\%] = \frac{Saving \left[\frac{\text{€}}{kg_{COD_{sewage}}}\right]}{0,9 \left[\frac{\text{€}}{kg_{COD_{sewage}}}\right]} \cdot 100$$

5.3 Heat balance

It is important to assess if the thermal energy produced balances the heating requirements for the anaerobic digestion.

Heating requirement per kg of sludge is computed as:

$$Q \left[\frac{kJ}{kg_{sludge}}\right] = c \cdot \Delta T \tag{5.3}$$

where $c \left[\frac{kJ}{kg\cdot K}\right] = 4,186$ is the heat capacity (assumed equal to that of water) and $\Delta T = T_f - T_i$ is the temperature increment necessary. In mesophilic anaerobic digestion $T_f = 37°C$, while the initial sludge temperature T_i was supposed to be 20°C. Under these conditions,

$$Q_{untreated} = 71,162 \left[\frac{kJ}{kg_{sludge}}\right] = 0,0198 \left[\frac{kWh}{kg_{sludge}}\right].$$

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[1] under the assertion of a thickening process giving an output sludge with a 24% solid content:

$$1,72 \frac{kg_{COD}}{kg_{VS}} \cdot 0,66 \frac{kg_{VS}}{kg_{TS}} \cdot 0,24 \frac{kg_{TS}}{kg_{sludge}} = 0,27 \frac{kg_{COD}}{kg_{sludge}}$$

Table 5.6: Heat balance $[kWh_{th}/kg_{COD_{sewage}}]$

	produced	necessary	balance (surplus)
<2> untreated	0,945	0,547	0,398
<3> sonicated	1,263	0,268	0,995
<4> enzym.	1,269	0	1,269
<5> combined	1,134	0	1,134

When computing the heat necessary for the anaerobic digestion of pretreated sludge, it is necessary to consider tha higher HRT, therefore:

$$Q_{pretreated} = \frac{3,5}{5} \cdot 71,162 \left[\frac{kJ}{kg_{sludge}} \right] = 0,0138 \left[\frac{kWh}{kg_{sludge}} \right] .$$

By knowing that the COD/SV ratio for the mixed sludge used is 1,72 and that only 55% of the sewage COD is transferred to the sludge, it is possible to compute the heating requirement per unit of sewage COD (it is multiplied by 2 to take into account the heat losses):

$$Q \left[\frac{kWh}{kg_{COD_{sewage}}} \right] = 2 \cdot \frac{Q_{untreated} \, or \, Q_{pretreated} \left[\frac{kWh}{kg_{sludge}} \right]}{COD \left[\frac{g_{COD_{sludge}}}{kg_{sludge}} \right]} \cdot 0,55 \left[\frac{g_{COD_{sludge}}}{g_{COD_{sewage}}} \right] \cdot 1000 \frac{g}{kg} \tag{5.4}$$

in which the COD concentration value come after the solid concentration of the sludge, and therfore will be different in the case of treated or untreated sludge:

$$COD_{untreated} = 35 \left[\frac{g_{TS}}{kg_{sludge}} \right] \cdot 0,66 \left[\frac{g_{VS}}{g_{TS}} \right] \cdot 1,72 \left[\frac{g_{COD_{sludge}}}{g_{VS}} \right] = 39,73 \left[\frac{g_{COD_{sludge}}}{kg_{sludge}} \right]$$

$$COD_{pretreated} = 50 \left[\frac{g_{TS}}{kg_{sludge}} \right] \cdot 0,66 \left[\frac{g_{VS}}{g_{TS}} \right] \cdot 1,72 \left[\frac{g_{COD_{sludge}}}{g_{VS}} \right] = 56,76 \left[\frac{g_{COD_{sludge}}}{kg_{sludge}} \right]$$

Results are reported in table 5.6. Heat balance is fully positive for all the four cases. Note that there is no need for heating sludge pretreated with enzymic hydrolysis, as the enzymic hydrolysis process temperature is higher than the one needed for the anaerobic digestion.

5.4 Pretreatment energy balance

Another other energy balance that is useful to compute is to assess wheather the energy used to pretreat the sludge may be provided by the surplus methane the pretreatment contributed to produce.

Before all the pretreatments the sludge undergoes a thickening process. Under the assumption that the thickener energy consumption is $1,26 \, kWh/m^3_{sludge}$, by knowing that the initial sludge concentration is $35 \, g_{TS}/kg_{sludge}$, that the COD/SV ratio for the sludge is 1,72, that the ratio VS/TS is 0,66 and that 55% of the sewage COD is transferred to the sludge, the thickening process may be assessed to have the following energy requirement:

$$E_{thick} = \frac{1,26 \left[\frac{kWh_{el}}{t_{sludge}} \right] \cdot 10^{-3} \frac{t}{kg}}{0,35 \left[\frac{kg_{TS}}{kg_{sludge}} \right] \cdot 0,66 \left[\frac{kg_{SV}}{kg_{ST}} \right] \cdot 1,72 \left[\frac{kg_{COD_{sludge}}}{kg_{SV}} \right]} \cdot 0,55 \left[\frac{kg_{COD_{sludge}}}{kg_{COD_{sewage}}} \right] = 0,0017 \left[\frac{kWh_{el}}{kg_{COD_{sewage}}} \right]$$

The energy requirement for the sonication treatment may be computed considering that $15000 \, kJ/kg_{TS} = 4,167 \, kWh/kg_{ST}$ were supplied as ultrasounds. Moreover, the sludge ratio between volatile and total solid is 0,66, the COD/SV ratio is 1,72 and 55% of the sewage COD is transferred to the sludge, therefore:

$$E_{US} = E_{thick} + \frac{4,167 \left[\frac{kWh_{el}}{kg_{TS}} \right]}{0,66 \left[\frac{kg_{SV}}{kg_{ST}} \right] \cdot 1,72 \left[\frac{kg_{COD_{sludge}}}{kg_{SV}} \right]} \cdot 0,55 \left[\frac{kg_{COD_{sewage}}}{kg_{COD_{sludge}}} \right] = 2,02 \left[\frac{kWh_{el}}{kg_{COD_{sewage}}} \right]$$

For the enzymic hydrolisis, the energy requirement is that of the heating (42°C for 24 h), computed with the same equation 5.3 used above, considering a temperature increase of $\Delta T = 42 - 20 = 22$°C:

$$Q = 92,09 \left[\frac{kJ}{kg_{sludge}} \right] = 0,0256 \left[\frac{kWh_{th}}{kg_{sludge}} \right]$$

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Table 5.7: Pretreatments energy balance

	surplus after AD heating		necessary for treatments	balance
	$E_{th}\,[kWh_{th}/kg_{COD_{sewage}}]$	$E_{el}\,[kWh_{el}/kg_{COD_{sewage}}]$	$E_{el}\,[kWh_{el}/kg_{COD_{sewage}}]$	$E_{el}\,[kWh_{el}/kg_{COD_{sewage}}]$
<2> untreated	0,398	0,159	0	0,159
<3> sonicated	0,995	0,398	2,020	-1,622
<4> enzym.	1,269	0,508	1,241	-0,733
<5> combined	1,134	0,454	3,262	-2,808

so that (multiplied by two to consider the heat losses)

$$Q_{EH} = 2 \cdot \frac{0,0256\left[\frac{kWh_{th}}{kg_{sludge}}\right]}{56,76\left[\frac{g_{COD_{sludge}}}{kg_{sludge}}\right]} \cdot 0,55\left[\frac{g_{COD_{sewage}}}{g_{COD_{sludge}}}\right] \cdot 1000\,\frac{g}{kg} = 0,496\left[\frac{kWh_{th}}{kg_{COD_{sewage}}}\right]$$

converted to electric energy and adding the energy for thickening

$$E_{EH} = E_{thick} + 0,496\left[\frac{kWh_{th}}{kg_{COD_{sewage}}}\right]/0,4\left[\frac{kWh_{th}}{kWh_{el}}\right] = 1,24\left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]$$

For the combined treatment, the energy requirement will be the sum of the two:

$$E_{UE} = E_{US} + E_{EH} = 2,02 + 1,24 = 3,26\left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]$$

The energy balance may now be computed. The thermal energy surplus after heating for the anaerobic digestion is converted to electric energy, to which the pretreatment energy requirement is subtracted (tab. 5.7).

The overall balance is negative for all the pretreated sludges.

5.5 Discussion

It is important to point out that the following assumptions were made while computing the energetic balance:

- heat lost during enzymic hydrolysis or anaerobic digestion is 100%, meaning that twice the theoretical heating (eq. 5.3) is needed. This assumption is quite pessimistic, mostly regarding the enzymic hydrolysis that as it has a duration of only 48. By contrast, an initial sludge temperature of 20°C is high, and can be true only for warm weather periods.

- regarding to the enzymic hydrolysis, it was considered to send to anaerobic digestion all the sludge treated, i.e. both the fermented cake and the liquor phase, and not only the fermented (solid) one. This corresponds to what was done in the experimental work, but, at plant scale, the core idea of the complete process of inverted phase fermentation + enzymic hydrolysis is to digest only the fermented phase (see chapter 2.3), because actually the phase separation is the major goal of the pretreatment, and the possibility to concentrate virtually all the solid content into a smaller volume increases notably the digestible matter-concentration, and therefore the biogas production, while the solid free liquor phase is recirculated at the head of the plant.
  When this is the case, the overall energetic balance would be much higher, as a greater volume of is expected to be produced. In fact, considering that the solid concentration in the fermented phase is approx. 10% (i.e. almost three times the untreated sludge solid content), and that the fermented phase is approx. 40% of the initial total sludge volume, the same anaerobic reactor will contain more than twice the initial solid content.

- regarding again to the enzymic hydrolysis, it was supposed to use electric energy to heat up the enzymic hydrolysis digester, but if it was possible to use directly the thermal energy produced by the anaerobic digester, there would be no need for this conversion, which implies an energy loss because of the efficiency of $0,4\,kWh_{el}/kWh_{th}$:

$$Q - Q_{EH} = 1,269 - 0,496 = 0,774\,\frac{kWh_{th}}{kg_{COD_{sewage}}}$$

Converting this surplus of thermal energy to electric energy and taking into account of the energy needed for thickening, we have:

$$\Delta E_{EH} = 0,774 \left[\frac{kWh_{th}}{kg_{COD_{sewage}}}\right] \cdot 0,4 \left[\frac{kWh_{el}}{kWh_{th}}\right] - E_{thick} = 0,308 \left[\frac{kWh_{th}}{kg_{COD_{sewage}}}\right]$$

meaning a positive energy balance even higher than the case of the untreated sludge.

One of the major outcomes of the energetic analysis is that energy balance for sonication is very negative. It is to be said that the sonication energy provided of $15000\,kJ/kg_{TS}$ is very high, as optimal energy is usually not higher than $7500\,kJ/kg_{TS}$ (see chapter 2.2.2; [Bougrier et al., 2005]). However, to achieve a positive energetic balance in the case of this work, the ultrasound energy suppy should be lowered to approx. $2950\,kJ/kg_{TS} = 0,818\,kWh_{el}/kg_{ST}$, which is probably a too low energy dose to achieve an actual enhancement in biogas production. In fact,

$$E'_{US} = E_{thick} + \frac{0,818 \left[\frac{kWh_{el}}{kg_{ST}}\right]}{0,66 \left[\frac{kg_{SV}}{kg_{ST}}\right] \cdot 1,72 \left[\frac{kg_{COD_{sludge}}}{kg_{SV}}\right]} \cdot 0,55 \left[\frac{kg_{COD_{sewage}}}{kg_{COD_{sludge}}}\right] = 0,398 \left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]$$

$$E^*_{el} - E'_{US} = 0,398 - 0,398 = 0 \left[\frac{kWh_{el}}{kg_{COD_{sewage}}}\right]$$

where E^*_{el} is the residual energy available after the heating requirement for the anaerobic digestion.

It appears therefore important to understand better the actual energetical feasibility of sonication by further studies about the optimal dose of ultrasound to enhance the biogas production without resulting in a negative energetic balance. Another option would be a part-stream approach, and therefore would be important to study the optimal ratio of sonicated/untreated sludge to have a positive energetic balance.

The same consideration, for sure, are valid for the combined pretreatment, in which the greatest part of the energy requirement is due to sonication. Moreover, energy production of the sludge treated with combined pretreatment is not higher than that of the others, suggesting that the energetic balance of the combined pretreatment is unlikely to ever become positive.

On the other hand, the energetic analysis pointed out some very useful information indicating the feasibility of the pretreatments at plant scale. The two most important are:

- the heat balance is fully positive in all the four cases, with a higher surplus energy in the case of pretreated sludge.

- the economic analysis shows that the impact of the energy increase over the plant energy needs $(i_{\Delta E})$ might be high enough to make the investment profitable, particularly in the case of enzymic hydrolysis. An economical feasibility analysis would be necessary to assess if this energy production increase balances the investment and management costs.

Chapter 6

Conclusions

The aim of this thesis was to verify the effectiveness of the pretreatments to better understand if they represent an actual option to enhance the sludge degradability and so the biogas production.

There are several treatment alternatives, the majority of which are described in chapter 2, some of them are at state of the art and implemented in many wastewater treatment plants around the world, while others are still at research stage, and at present they are implemented only at laboratory scale. Greater focus was given to ultrasound treatment and enzymic hydrolysis, as they were lated implemented in BMP test (chapter 3).

As described in paragraph 4.3, pretreatments achieved an initial hydrolysis of the sludge, i.e. to destroy the cell membrane of the organic matter releasing intracellular matter to the aqueous phase, making it available for subsequent degradation to CH_4 and CO_2 in anaerobic digestion. The effectiveness was confirmed by the increase in soluble COD (approx. +30%) and in ammonium nitrogen concentration (in this case data is more scattered, but an increasing trend is evident).

BMP tests were performed in two different rounds, corresponding to the two different sludges that were used (1st round: thickened mixed sludge, 2nd round: mixed sludge). Cattle manure was used to start up the digestion. Volume proportion of sludge and inoculum was computed to fix the overall total solid content to 6% (5,25% in the 2nd round) and to have a food/inoculum ratio less or equal than 0,5 (0,44 in the 1st round, 0,5 in the 2nd).

The anaerobic digestion resulted in an effective degradation of the sludge. Volatile solid concentration lowered by approx. 40%, and in particular the sludge volatile solid content notably decreased by 87% (1st round, 42 days) and by 50-55% (2nd round, 32 days). Ammonia concentration also lowered by 10-20%, meaning that an effective proteins hydrolyzation was carried out.

Biogas production increased after pretreatments, as the volume of the biogas produced by treated sludge was higher than the volume obtained by untreated sludge. The best result was +23% of 1st round enzymic hydrolysis. Biogas production values of 1st round allowed to carry out a kinetics analysis (chapter 4.4.4), which demonstrated the enhancement effect of prestreatments, as the biochemical methane potential value $CH_{4\infty}$ increased by approx. 20%, meaning that pretreated sludge produces 20% more methane than the untreated one. The kinetics constant k_h also increased by approx. 5%, meaning that methane production is 5% faster when sludge is pretreated. However this last value needs to be confirmed by further studies because its 95%-confidence interval partially overlaps with the interval of the untreated sludge.

After that, an energetic/economic analysis was computed (chapter 5).

The heat balance showed that anaerobic digestion is fully self-heating, as heating necessary to bring sludge to 37°C is less than the thermal energy produced during the digestion (58% in the case of untreated sludge and 21% for the sonicated sludge).

The economical analysis showed that the energy increase impact over the plant needs ($i_{\Delta E}$, table 5.5) are around 11%, meaning that the energy production increment is still quite low, but might be enough to pay back the investment of a pretreatment installation, particularly in the case of enzymic hydrolysis, as the economical analysis does not consider that only the fermented-solid cake would be sent to the anaerobic digestion, allowing therefore a condiderable increment in biogas production. The reduction of sludge production is quite low, meaning that the cost for sludge disposal lowers by a probably negligible amount ($3\,cent\text{€}/kg_{incomingCOD}$). However, a deeper economical feasibility analysis is necessary to better assess these values.

Finally, the energetic analysis of the pretreatments show that sonication at $15000\,kJ/kg_{TS}$ is not energetically self-sufficient, whereas the enzymic hydrolysis appears to have a positive energetic balance if using the directly the thermal energy produced in the anaerobic digester. However, it must again be recalled that the anaerobic digestion carried out only on the fermented phase of the enzymic hydrolysis would increase

methane production considerably, meaning that a far more positive energetic balance is likely to be expected.

In this conclusive chapter is useful to give a final comparison between sonication and enzymic hydrolysis. Concerning biogas production, there are no substancial differences between the two: the biochemical methane potential value is very similar (around $0, 49\, L_{CH_4}/g_{VS}$) and the methane production content of biogas is in both cases around 70%. It is important however to remember that this is valid under the assertion of carring out the enzymic hydrolysis of both the solid and the liquid cake, and not of the solid one only.

When choosing between the two to implement a pretreatment at plant scale, the major difference stands in the fact that sonication is a well established technique, manifactured by various industries and implemented many wastewater treatment plants around the world. By contrast, enzymic hydrolysis is a proprietary method of United Utilities UK, it is still at development phase and only few demonstration plants have been implemented yet.

On the other hand, another important aspect is that sonication is not self-sufficient in terms of energetic balance, and this is why sonication devices are usually part-stream instruments, meaning that only a percentage of the incoming sludge is treated. This is for example the case presented by [Barber, 2005] (see chapter 2.2.3), which resulted in a positive energy balance.

Finally, combined ultrasound + enzymic hydrolysis pretreatment appears to be not profitable, as, from the data collected in this thesis, the energy gain is totally unbalanced by the energy that must be provided.

Future developements

Further studies need to be carried out in order to give more reliable conclusions, with particular focus on the most uncertain aspects highlighted in this thesis:

- first of all, to carry out at least another round of BMP tests on sludge treated with enzymic hydrolysis, and in particular using the only solid phase, in order to get reliable information about the effective methane potential. In this way, it would be possible to give better conclusion at plant-scale, by computing the energy balance again.

- a deeper study of the sonication energetic balance is due, to assess the optimal value for the sonication dose that does not cause an energy deficit. This might be done in alternative or combined with a study of a partial sonication, i.e. assessing the right percentage of the sludge volume to be treated.

- for sure, this was the first study of this kind and therefore there are no other values that are directly comparable. Other BMP tests should be carried out to confirm the results obtained here. One or more rounds of non-batch tests would be useful too.

Milan, November 2011

Appendix A

Laboratory results

A.1   1st round

Table A.1: 1st round - COD concentrations [mg/L] using a Perkin Elmer Lambda 35 Visible-UV system.

	t = 0		t = 24 h	t = 42 d			
mixture	total	soluble	soluble	bottle 1, total	bottle 1, soluble	bottle 2, total	bottle 2, soluble
<1> blank	24256	9188	10268	15081	2851	15408	4536
<2> untreated	*17247*	*10183*	13194	*26975*	*1998*	*20743*	*2798*
<3> sonicated	*17247*	*11817*	14053	*20296*	*3918*	*25122*	*5607*
<4> enzym.	*17247*	*16901*	16276	*25572*	*5761*	*24779*	*4619*
<5> combined	*17247*	16759	*15424*	25970	5054	25680	4988

Numbers in italics are values with lower standard deviation and so used in the results analysis.

Table A.2: 1st round - COD concentrations [mg/L] using a Hach DR/2010 Spectrophotometer.

	t = 0		t = 24 h	t = 42 d			
mixture	total	soluble	soluble	bottle 1, total	bottle 1, soluble	bottle 2, total	bottle 2, soluble
<1> blank	*21350*	*8375*	*8775*	*15100*	*2975*	*15550*	*4925*
<2> untreated	17700	10275	*11675*	28600	2725	21900	3550
<3> sonicated	17700	11900	*13650*	19950	3525	23700	5450
<4> enzym.	17700	16440	*16400*	24375	5763	23700	4913
<5> combined	17700	*17450*	13483	24000	*4750*	23725	*5088*

Numbers in italics are values with lower standard deviation and so used in the results analysis.

Table A.3: 1st round - Ammonium nitrogen concentrations [mg/L]

mixture	t = 0	t = 24 h	t = 42 d
<1> blank	1288	1422	1463
<2> untreated	1523	1809	1881
<3> sonicated	1411	1809	2081
<4> enzym.	2218	1904	2134
<5> combined	1982	2122	2043

Table A.4: 1st round - pH and alkalinity $[g_{CaCO_3}/L]$ concentrations

mixture	t = 0		t = 42 d	
	pH	alkalinity	pH	alkalinity
<1> blank	8,5	7,3	8,2	8,8
<2> untreated	8,3	7,1	8,2	10,1
<3> sonicated	8,1	7,8	8,2	10,4
<4> enzym.	7,6	8,5	8,2	9,8
<5> combined	7,7	8,9	8,2	9,5

Table A.5: 1st round - Total and volatile solids [g/L]

| mixture	t = 0						t = 42 d					
	TS			VS			TS			VS		
	sludge	manure	total	sludge	manure	total	sludge	manure	total	sludge	manure	total
<1> blank	0	44,87	44,87	0	22,86	22,86	0	23,63	23,63	0	10,12	10,12
<2> untreated	15,18	44,87	60,05	10,03	22,86	32,89	6,25	53,49	59,74	1,42	9,34	20,48
<3> sonicated	15,18	44,87	60,05	10,03	22,86	32,89	6,91	59,18	66,08	1,30	8,60	18,85
<4> enzym.	15,18	44,87	60,05	10,03	22,86	32,89	5,13	43,92	49,05	1,29	8,49	18,62
<5> combined	15,18	44,87	60,05	10,03	22,86	32,89	5,46	46,77	52,23	1,30	8,56	18,77
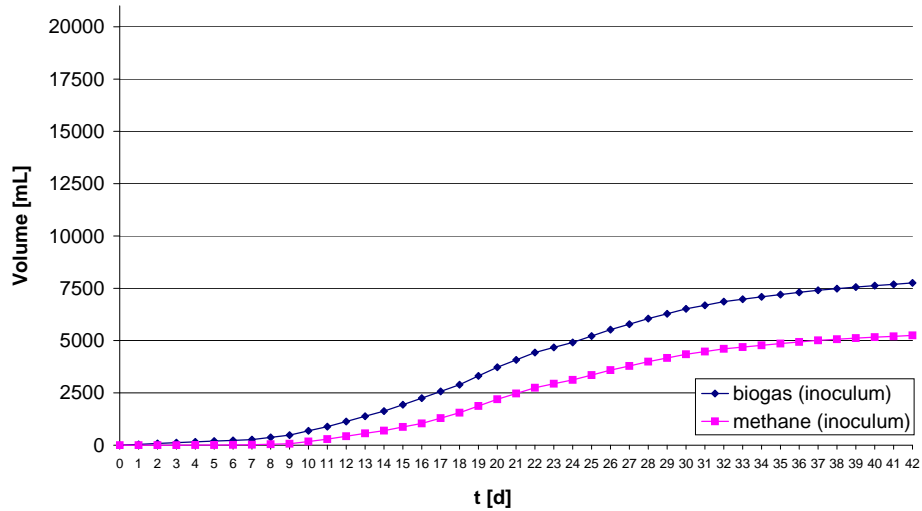
Figure A.1: Cumulated biogas production - blank bottles <1>
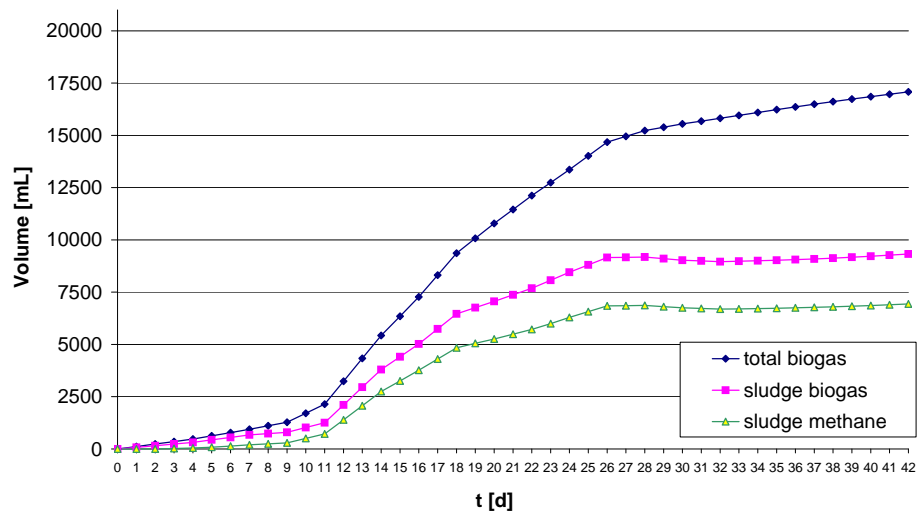

Figure A.2: Cumulated biogas production - untreated bottles <2>
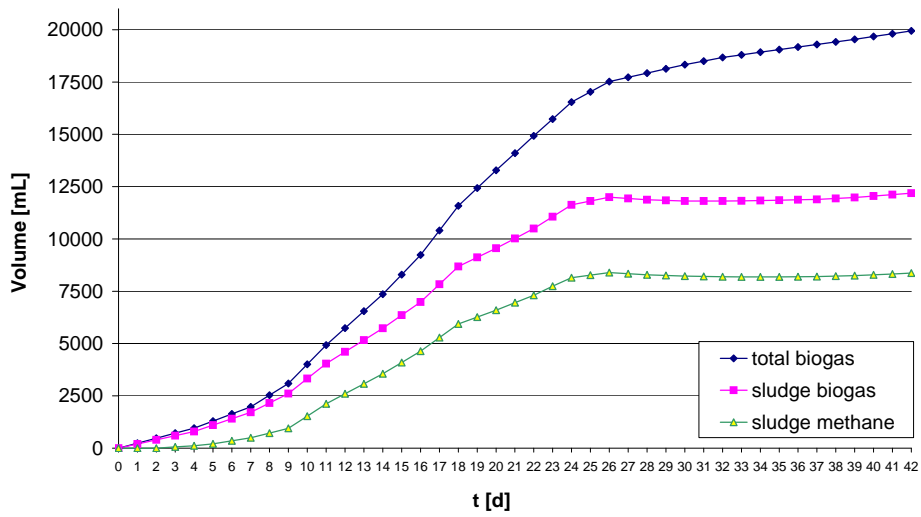
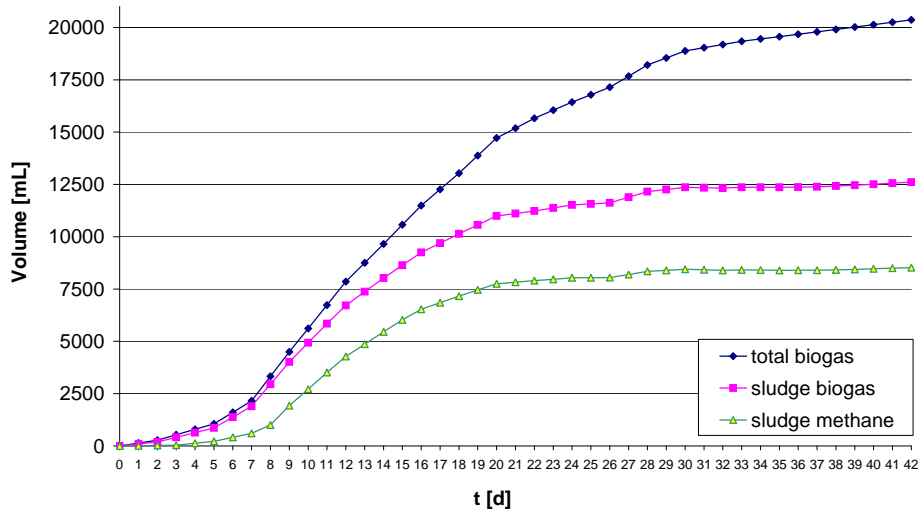Figure A.3: Cumulated biogas production - sonicated bottles <3>

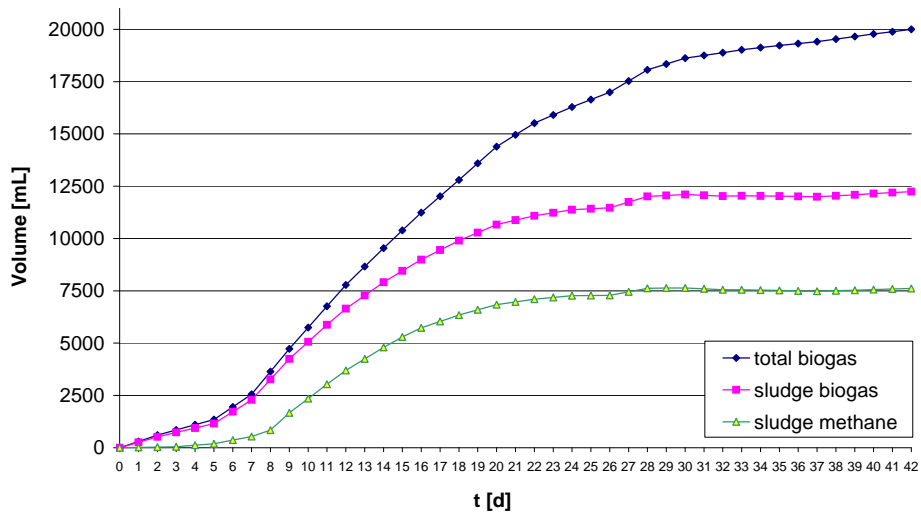Figure A.4: Cumulated biogas production - enzymic hydrolysis bottles <4>

Figure A.5: Cumulated biogas production - combined treatment bottles <5>

Table A.6: 1st round - Total cumulated biogas volumes [mL]

day	date	<1> blank		<2> untreated		<3> sonicated		date	<4> enzym.		<5> combined	
		bottle 1	bottle 2	bottle 1	bottle 2	bottle 1	bottle 2		bottle 1	bottle 2	bottle 1	bottle 2
0	11/04/11	0	0	0	0	0	0	13/04/11	0	0	0	0
1	12/04/11	34	47	109	128	221	257	14/04/11	126	146	170	434
2	13/04/11	67	95	217	257	442	513	15/04/11	253	292	339	868
3	14/04/11	101	142	326	385	663	770	16/04/11	513	555	610	1095
4	15/04/11	134	189	434	513	884	1026	17/04/11	774	818	881	1321
5	16/04/11	171	221	605	653	1208	1384	18/04/11	1034	1081	1152	1547
6	17/04/11	208	253	776	792	1531	1742	19/04/11	1575	1642	1666	2238
7	18/04/11	245	284	947	931	1855	2100	20/04/11	2115	2202	2179	2928
8	19/04/11	335	410	1109	1105	2408	2660	21/04/11	3280	3378	3260	4022
9	20/04/11	426	537	1271	1279	2960	3221	22/04/11	4444	4555	4341	5115
10	21/04/11	628	742	1666	1756	3888	4132	23/04/11	5549	5686	5404	6089
11	22/04/11	829	947	2060	2234	4815	5044	24/04/11	6654	6817	6467	7062
12	23/04/11	1118	1152	3110	3373	5652	5828	25/04/11	7759	7949	7530	8036
13	24/04/11	1408	1358	4160	4512	6488	6612	26/04/11	8651	8857	8422	8900
14	25/04/11	1697	1563	5210	5652	7325	7396	27/04/11	9543	9764	9314	9764
15	26/04/11	2080	1796	6137	6563	8280	8312	28/04/11	10459	10688	10207	10571
16	27/04/11	2463	2029	7065	7475	9235	9228	29/04/11	11375	11612	11100	11377
17	28/04/11	2721	2420	8079	8552	10398	10414	30/04/11	12170	12356	11937	12098
18	29/04/11	2980	2812	9093	9628	11561	11600	01/05/11	12965	13100	12775	12818
19	30/04/11	3308	3313	9833	10314	12407	12453	02/05/11	13861	13887	13558	13628
20	01/05/11	3637	3814	10573	10999	13252	13307	03/05/11	14758	14674	14341	14439
21	02/05/11	3923	4233	11250	11652	14035	14168	04/05/11	15275	15104	14940	14967
22	03/05/11	4209	4652	11927	12306	14818	15030	05/05/11	15792	15535	15539	15496
23	04/05/11	4440	4902	12503	12976	15617	15844	06/05/11	16183	15911	15919	15887
24	05/05/11	4671	5153	13078	13645	16416	16658	07/05/11	16575	16287	16299	16279
25	06/05/11	4980	5454	13775	14264	16921	17136	08/05/11	16892	16682	16620	16662
26	07/05/11	5289	5756	14472	14882	17426	17613	09/05/11	17209	17077	16941	17046
27	08/05/11	5571	6002	14777	15129	17629	17817	10/05/11	17663	17684	17508	17547
28	09/05/11	5853	6249	15083	15376	17833	18021	11/05/11	18117	18291	18076	18048
29	10/05/11	6088	6480	15247	15528	18025	18232	12/05/11	18489	18593	18393	18287
30	11/05/11	6323	6711	15411	15681	18216	18443	13/05/11	18861	18894	18710	18526
31	12/05/11	6562	6817	15541	15822	18385	18616	14/05/11	19004	19058	18856	18646
32	13/05/11	6801	6922	15670	15963	18553	18788	15/05/11	19148	19223	19003	18766
33	14/05/11	6926	7024	15811	16096	18676	18918	16/05/11	19292	19387	19149	18886
34	15/05/11	7051	7126	15952	16229	18798	19049	17/05/11	19389	19513	19258	18988
35	16/05/11	7176	7228	16093	16362	18921	19179	18/05/11	19487	19638	19368	19090
36	17/05/11	7255	7353	16253	16468	19039	19309	19/05/11	19593	19763	19450	19184
37	18/05/11	7333	7478	16414	16574	19156	19438	20/05/11	19699	19888	19532	19278
38	19/05/11	7392	7576	16543	16683	19277	19559	21/05/11	19803	20014	19663	19393
39	20/05/11	7450	7674	16672	16793	19399	19681	22/05/11	19908	20139	19793	19507
40	21/05/11	7505	7747	16795	16900	19532	19816	23/05/11	20012	20264	19924	19622
41	22/05/11	7560	7820	16917	17007	19665	19952	24/05/11	20114	20386	20030	19740
42	23/05/11	7615	7893	17040	17114	19798	20088	25/05/11	20216	20507	20135	19857

Table A.7: 1st round - Biogas composition [%]

t	<1> blank - bottle 1				<1> blank - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0%	0%	0%	0%	0%	0%	0%	0%
4	12,94%	77,40%	3,27%	6,38%	13,49%	77,71%	2,20%	6,61%
7	10,04%	63,17%	7,08%	19,71%	10,04%	62,65%	5,71%	21,58%
14	1,38%	6,42%	33,08%	59,11%	3,59%	11,76%	34,42%	50,22%
21	1,41%	1,09%	16,48%	81,01%	2,14%	6,51%	15,36%	75,98%
28	1,51%	4,50%	15,67%	78,32%	2,74%	7,94%	14,96%	74,36%
35	3,14%	9,06%	11,14%	76,66%	-	-	-	-
42	6,14%	19,26%	7,87%	66,73%	5,92%	16,51%	6,66%	70,90%

t	<2> untreated - bottle 1				<2> untreated - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0%	0%	0%	0%	0%	0%	0%	0%
4	5,50%	56,04%	14,51%	23,95%	7,07%	59,67%	11,27%	21,99%
7	3,09%	26,38%	30,70%	39,83%	3,86%	24,06%	27,75%	44,33%
14	0,42%	1,03%	26,26%	72,30%	0,61%	1,48%	21,98%	75,93%
21	0,53%	1,48%	22,69%	75,31%	0,81%	1,76%	21,89%	75,54%
28	1,01%	2,72%	19,24%	77,03%	1,79%	4,61%	16,39%	77,21%
35	2,43%	7,16%	18,76%	71,65%	3,29%	8,89%	15,20%	72,62%
42	2,73%	8,34%	21,01%	67,93%	3,91%	10,66%	15,67%	69,77%

t	<3> sonicated - bottle 1				<3> sonicated - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0%	0%	0%	0%	0%	0%	0%	0%
4	4,62%	40,75%	28,30%	26,33%	4,75%	40,03%	29,91%	25,32%
7	1,72%	7,73%	45,68%	44,87%	1,49%	6,13%	47,96%	44,43%
14	0,61%	1,48%	21,98%	75,93%	0,59%	1,56%	21,87%	75,98%
21	0,66%	1,63%	23,17%	74,54%	0,59%	1,83%	18,32%	79,26%
28	2,40%	6,18%	17,74%	73,68%	2,00%	5,91%	20,03%	72,07%
35	3,88%	10,00%	19,08%	67,03%	3,41%	9,87%	22,19%	64,53%
42	3,91%	10,49%	21,15%	64,45%	3,11%	9,81%	24,07%	63,01%

t	<4> enzym. - bottle 1				<4> enzym. - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0	0	0	0	0	0	0	0
2	9,75%	74,33%	9,50%	6,60%	7,20%	71,47%	13,07%	8,26%
5	2,60%	31,20%	30,63%	35,56%	1,79%	27,32%	34,28%	36,60%
12	0,44%	1,03%	17,75%	80,78%	0,25%	0,68%	18,26%	80,80%
20	0,64%	1,60%	25,05%	72,70%	0,41%	1,19%	23,81%	74,60%
26	1,61%	4,01%	27,81%	66,57%	0,86%	2,52%	30,16%	66,47%
33	3,19%	8,00%	20,22%	68,59%	1,81%	5,39%	25,76%	67,05%
40	4,83%	13,74%	17,68%	63,74%	2,29%	6,70%	25,27%	65,74%

t	<5> combined - bottle 1				<5> combined - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0	0	0	0	0	0	0	0
2	8,40%	69,29%	13,85%	8,45%	10,85%	74,50%	10,22%	4,43%
5	1,99%	27,01%	35,82%	35,18%	3,86%	46,32%	24,80%	25,02%
12	0,33%	1,08%	17,61%	80,99%	0,64%	6,96%	17,01%	75,38%
20	0,49%	1,69%	25,80%	72,02%	0,75%	1,94%	25,59%	71,71%
26	1,20%	4,24%	27,28%	67,27%	1,58%	4,11%	27,87%	66,44%
33	2,35%	8,13%	32,22%	66,30%	5,66%	16,22%	17,45%	60,67%
40	3,38%	10,13%	22,68%	63,81%	4,98%	13,22%	18,21%	63,59%

A.2 2nd round

Table A.8: 2nd round - COD concentrations [mg/L] using a Perkin Elmer Lambda 35 Visible-UV system.

mixture	t = 0 total	t = 0 soluble	t = 24 h soluble	t = 32 d bottle 1, total	bottle 1, soluble	bottle 2, total	bottle 2, soluble
<6> blank	23236	14819	16319	38095	8312	36880	7322
<7> untreated	40969	16390	19234	55261	9765	55012	8770
<8> sonicated	41521	20981	19991	56692	8549	57347	8579
<9> enzym.	42322	20403	17017	49099	11097	45790	11155
<10> combined	44685	22169	18458	53181	11597	53688	11870

Table A.9: 2nd round - COD concentrations [mg/L] using a Hach DR/2010 Spectrophotometer.

mixture	t = 0 total	t = 0 soluble	t = 24 h soluble	t = 32 d bottle 1, total	bottle 1, soluble	bottle 2, total	bottle 2, soluble
<6> blank	21250	13750	14675	35400	8320	33450	7280
<7> untreated	41550	15650	18575	50150	9390	49950	8510
<8> sonicated	42600	20200	19150	51400	8230	52000	8410
<9> enzym.	42650	20725	16950	44550	10060	41500	10120
<10> combined	44500	22400	17100	48200	10530	48750	10770

These values were not used in the result analysis. Please refer to COD concentrations of table A.8

Table A.10: 2nd round - Ammonium nitrogen concentrations [mg/L]

mixture	t = 0	t = 24 h	t = 32 d
<6> blank	1543	1513	1498
<7> untreated	1786	1771	2002
<8> sonicated	1814	1686	2021
<9> enzym.	1807	1750	1980
<10> combined	1757	1936	2140

Table A.11: 2nd round - pH and alkalinity $[g_{CaCO_3}/L]$ concentrations

mixture	t = 0 pH	t = 0 alkalinity	t = 32 d pH	t = 32 d alkalinity
<6> blank	8,2	7,8	8,3	8,9
<7> untreated	8,6	8,6	8,3	10,1
<8> sonicated	8,3	8,2	8,1	10,3
<9> enzym.	8,1	9,0	8,2	10,2
<10> combined	8,4	9,1	8,2	10,4

Table A.12: 2nd round - Total and volatile solids [g/L]

| mixture	t = 0 TS sludge	TS manure	TS total	t = 0 VS sludge	VS manure	VS total	t = 32 d TS sludge	TS manure	TS total	t = 32 d VS sludge	VS manure	VS total
<6> blank	0	37,49	37,49	0	19,88	19,88	0	27,70	27,70	0	11,91	11,91
<7> untreated	14,77	37,49	52,26	10,19	19,88	30,07	15,95	26,99	42,93	5,14	6,68	20,05
<8> sonicated	14,77	37,49	52,26	10,19	19,88	30,07	14,88	25,18	40,07	4,87	6,34	19,01
<9> enzym.	14,77	37,49	52,26	10,19	19,88	30,07	13,69	23,16	36,85	4,52	5,87	17,62
<10> combined	14,77	37,49	52,26	10,19	19,88	30,07	12,77	21,60	34,37	4,44	5,77	17,31
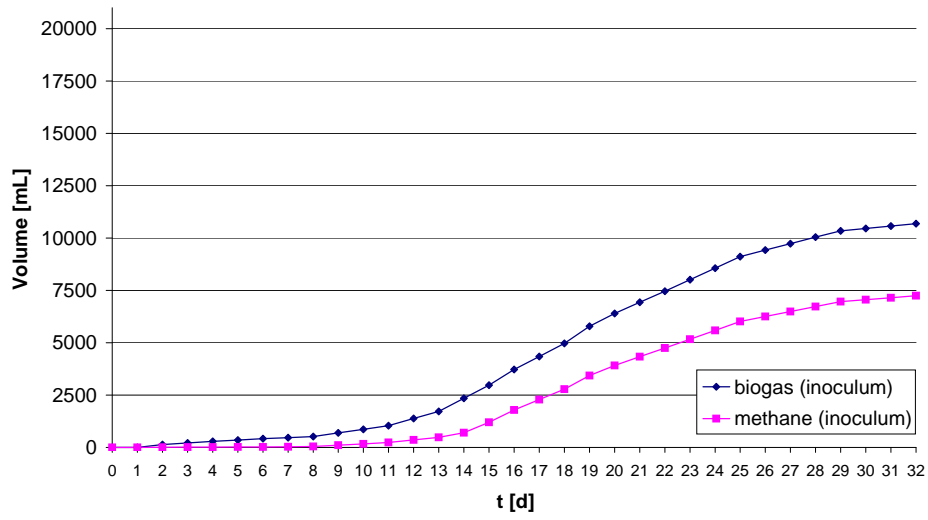
Figure A.6: Cumulated biogas production - blank bottles <6>
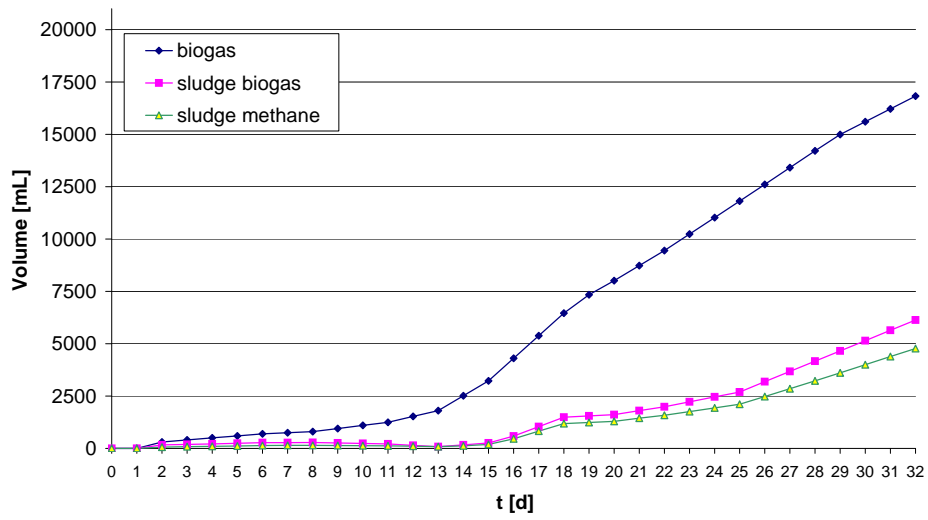

Figure A.7: Cumulated biogas production - untreated bottles <7>
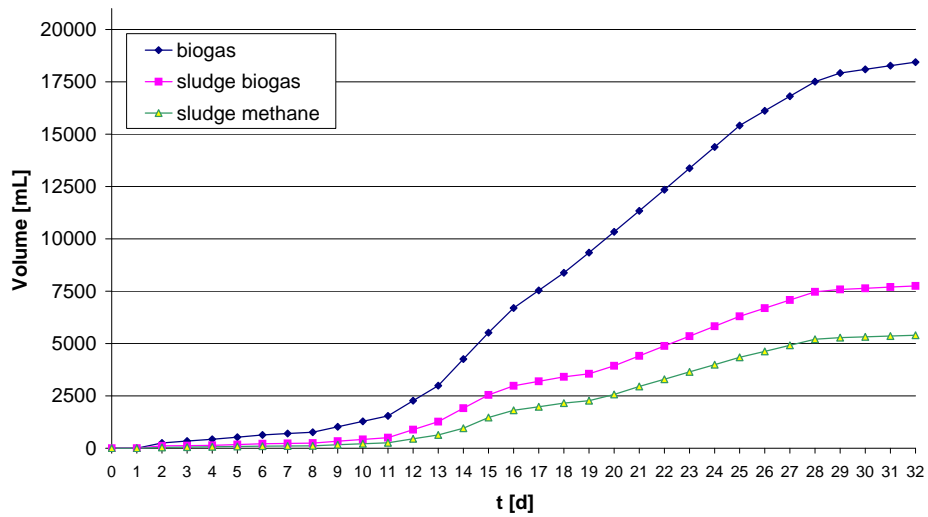

Figure A.8: Cumulated biogas production - sonicated bottles <8>
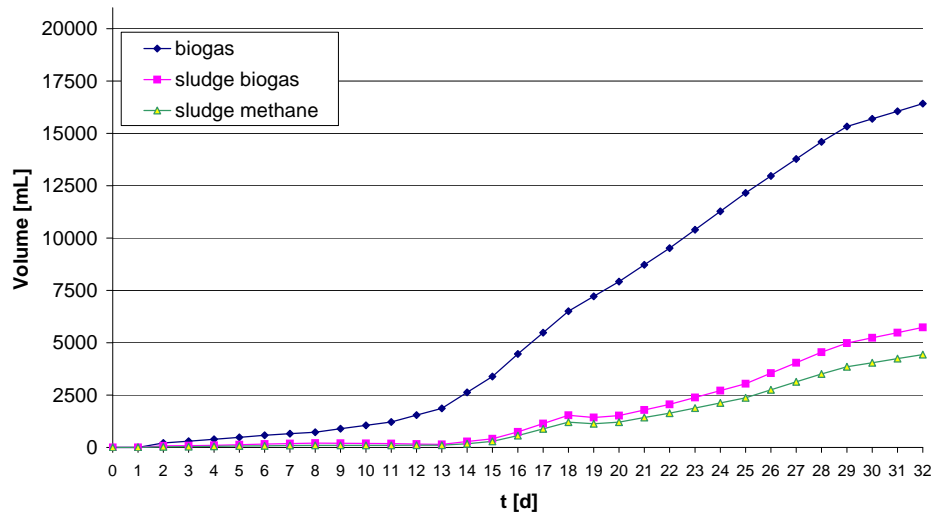
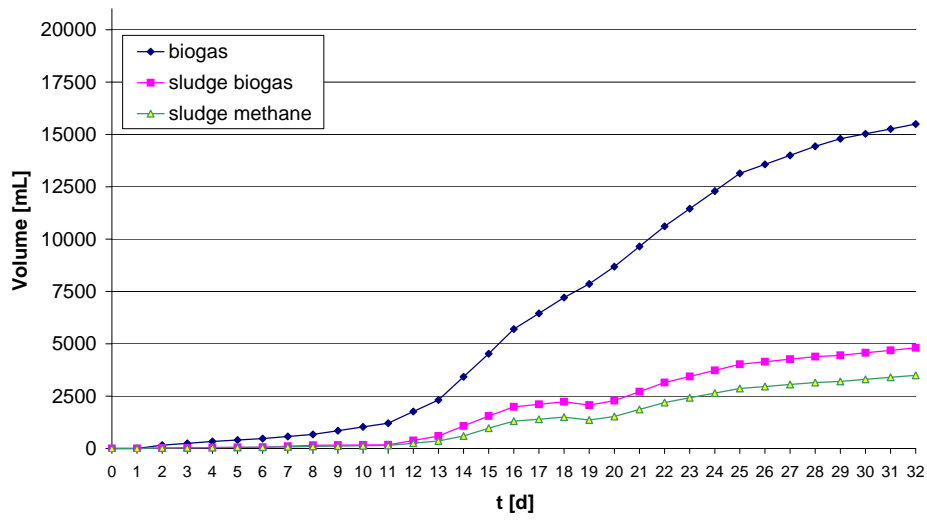Figure A.9: Cumulated biogas production - enzymic hydrolysis bottles <9>

Figure A.10: Cumulated biogas production - combined treatment second bottle <10>

Table A.13: 2nd round - Total cumulated biogas volumes [mL]

day	date	<6> blank		<7> untreated		<8> sonicated		<9> enzym.		<10> combined	
		bottle 1	bottle 2	bottle 1	bottle 2	bottle 1	bottle 2	bottle 1	bottle 2	bottle 1	bottle 2
0	02/06/11	0	0	0	0	0	0	0	0	0	0
1	03/06/11	0	0	0	0	0	0	0	0	2310	0
2	04/06/11	188	94	305	305	282	227	274	157	2372	258
3	05/06/11	254	176	411	399	356	325	364	239	2459	345
4	06/06/11	321	258	517	493	431	423	454	321	2545	431
5	07/06/11	384	325	615	583	529	532	556	411	2600	521
6	08/06/11	446	392	713	673	626	642	658	501	2654	611
7	09/06/11	497	442	752	744	697	705	740	568	2756	705
8	10/06/11	548	493	791	814	767	767	822	634	2858	799
9	11/06/11	736	650	963	940	1013	1039	979	804	2973	1041
10	12/06/11	924	806	1135	1065	1258	1310	1135	974	3088	1284
11	13/06/11	1112	963	1308	1190	1503	1582	1292	1143	3202	1527
12	14/06/11	1484	1276	1597	1456	2232	2302	1613	1472	3774	2063
13	15/06/11	1856	1589	1887	1723	2960	3022	1934	1801	4346	2600
14	16/06/11	2478	2216	2623	2412	4267	4244	2682	2572	5371	3782
15	17/06/11	3101	2842	3359	3101	5575	5465	3430	3343	6397	4964
16	18/06/11	3751	3688	4479	4134	6749	6648	4510	4416	7579	6139
17	19/06/11	4377	4314	5563	5203	7572	7509	5571	5399	8362	6859
18	20/06/11	5003	4941	6648	6272	8394	8370	6632	6381	9145	7579
19	21/06/11	5857	5724	7493	7188	9373	9318	7384	7055	9827	8206
20	22/06/11	6475	6327	8128	7900	10390	10281	8096	7744	10524	9153
21	23/06/11	7008	6855	8860	8605	11428	11252	8918	8523	11436	10167
22	24/06/11	7540	7384	9592	9310	12465	12223	9741	9302	12348	11181
23	25/06/11	8120	7906	10396	10077	13593	13141	10568	10231	13079	12137
24	26/06/11	8699	8428	11200	10845	14720	14060	11395	11160	13810	13092
25	27/06/11	9279	8950	12003	11612	15848	14979	12223	12090	14540	14047
26	28/06/11	9592	9255	12807	12411	16487	15736	13042	12893	14728	14720
27	29/06/11	9905	9560	13611	13209	17127	16493	13862	13697	14916	15394
28	30/06/11	10218	9866	14415	14008	17766	17249	14681	14501	15104	16067
29	01/07/11	10516	10163	15135	14854	18111	17735	15292	15362	15261	16631
30	02/07/11	10633	10278	15730	15477	18280	17910	15605	15777	15537	16824
31	03/07/11	10751	10393	16326	16101	18450	18085	15918	16192	15814	17017
32	04/07/11	10868	10508	16921	16725	18620	18260	16232	16607	16091	17210

Volumes referred to the first bottle of mixture <10> are not considered in the analysis

Table A.14: 2nd round - Biogas composition [%]

t	<6> blank - bottle 1				<6> blank - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0%	0%	0%	0%	0%	0%	0%	0%
4	11,24%	80,34%	2,96%	5,46%	6,77%	79,09%	6,96%	7,18%
11	5,40%	29,61%	30,49%	34,50%	3,68%	26,10%	32,59%	37,63%
18	0,53%	1,34%	17,27%	80,86%	1,06%	3,56%	17,77%	77,61%
25	0,48%	1,55%	21,04%	76,92%	0,52%	1,33%	21,29%	76,86%
32	1,18%	3,52%	15,05%	80,25%	0,99%	2,88%	16,56%	79,56%
t	<7> untreated - bottle 1				<7> untreated - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0%	0%	0%	0%	0%	0%	0%	0%
4	5,38%	55,23%	17,30%	22,08%	4,76%	55,52%	15,25%	24,47%
11	4,57%	25,43%	34,99%	35,01%	5,54%	21,80%	33,83%	38,83%
18	0,45%	1,30%	17,77%	80,48%	0,68%	2,02%	18,28%	79,02%
25	0,41%	1,01%	22,31%	76,26%	0,77%	2,15%	21,14%	75,94%
32	0,54%	1,40%	20,79%	77,28%	0,69%	1,87%	16,62%	80,82%
t	<8> sonicated - bottle 1				<8> sonicated - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0%	0%	0%	0%	0%	0%	0%	0%
4	5,93%	55,06%	16,07%	22,94%	10,47%	60,99%	12,60%	15,94%
11	2,26%	8,96%	42,69%	46,09%	4,63%	16,76%	38,94%	39,67%
18	0,35%	1,08%	17,88%	80,69%	1,09%	3,30%	17,51%	78,09%
25	0,28%	0,87%	22,44%	76,41%	1,04%	3,23%	21,62%	74,12%
32	1,10%	3,34%	17,73%	77,83%	1,93%	6,03%	17,70%	74,34%
t	<9> enzym. - bottle 1				<9> enzym. - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0	0	0	0	0	0	0	0
4	6,20%	57,65%	19,84%	16,31%	2,31%	53,57%	23,50%	20,62%
11	4,11%	18,30%	40,47%	37,13%	3,46%	14,36%	42,27%	39,91%
18	0,75%	2,60%	17,59%	79,06%	0,39%	1,08%	17,14%	81,40%
25	0,13%	0,31%	23,39%	76,17%	0,26%	0,80%	22,69%	76,25%
32	0,14%	0,29%	19,88%	79,69%	0,11%	0,32%	21,74%	77,83%
t	<10> combined - bottle 1				<10> combined - bottle 2			
[d]	O_2	N_2	CO_2	CH_4	O_2	N_2	CO_2	CH_4
0	0	0	0	0	0	0	0	0
4	2,97%	63,33%	16,75%	16,95%	4,77%	57,79%	17,93%	19,52%
11	3,02%	19,19%	38,28%	39,51%	1,79%	10,06%	44,06%	44,09%
18	0,84%	8,57%	15,54%	75,04%	0,36%	1,21%	16,18%	82,25%
25	0,11%	0,27%	23,11%	76,51%	0,08%	0,22%	22,74%	76,97%
32	0,64%	1,56%	15,73%	82,06%	0,20%	0,42%	19,06%	80,33%

Appendix B

Wastewater plants

The next few pages are a brief description of the two wastewater plants that provided the sludges used for the BMP tests: Frieres (1st round) and Baiña (2nd round). The two plants are very similar both in dimension and in technologies applied. Their description is based on official documentation of the Asturias' *Consorcio de Aguas* (Water Authority).

B.1   Frieres

Figure B.1: Schematic of the Frieres wastewater plant, bordered by the Nalón river: (1) raw water inlet and lift, (2) grinding, (3) grit and grease removal, (4) primary sedimentation, (5) biological treatment, (6) secondary sedimentation, (7) sludge treatment

Frieres wastewater plant is located in the municipality of Langreo, south-east of Oviedo, Asturias, Spain. It is a medium size plant, serving approx. 85000 p.e., corresponding to an average flow treatment of 1620 m^3/h (450 L/s). It is said to have a treatment efficiency of 95%.

Water treatment is divided in the following steps:

1. Sewage water enters the plant flowing through a stone trap where heavier materials settle to the bottom, and other floating and bulky materials are retained by a screen with bar spacing of 150 mm, placed upstream to an Archimedean screw pump that raises the water to the next treatment step.

2. Water flows through other two screens (bar spacing of 40 and 10 mm), to remove smaller solids that a conveyor belt system carries to a press to partially remove water, and then to a container where they are stored to be finally disposed.

3. In the next step, sand is removed from the bottom of a channel where it sediments naturally as water slowly flows. At the same time, fat and grease are removed by skimmers, as they float on the surface. Air diffusers at the base of the tank are used to speed up the flotation.

4. Sewage water reaches the primary sedimentation tanks, where most of the settleable solids and floating material that could not be removed in the earlier stages get eliminated. Settled sewage is collected into a channel and sent to biological treatment. Some sedimentator tanks are operated as storm tanks, where excess water due to heavy rainfalls accumulates to be treated in off-peak periods.

5. Once the majority of solids has been separed, the water flows through the biological reactors, where the organic matter degradation takes place under the effect of bacteria populations. The biological reactors are divided into two areas:

    (a) Aerobic zone, where carbonic organic matter is degraded by aerobic microorganisms. Oxygen is provided by blowers that release into the water small air bubbles

    (b) Anoxic zone (upstream of the aerobic one), where anoxic microorganisms convert nitrates to nitrogen. A set of stirrers prevent the sludge settling.

6. The last step in wastewater treatment is the secondary sedimentation, where treated water is separated from the biological sludge. Sludge settles at the bottom of the tank, from where is removed by a scraper and recirculated at the head of the anoxic zone. Clean water is discharged by a channel to the Nalón river. Disinfection is not provided.

7. Sludge treatment facilities differ according to the origin of sludge. Primary sludge is treated into thickeners, where part of the water is removed. Biological surplus sludge is thickened by flotation: sludge is pressurisedw with air and then released to achieve the separation between sludge and water. Later, primary and biological sludges are mixed together and with ferric chloride and calcium oxide and dewatered in filter presses. Thickened sludge can now be disposed to an appropriate landfill. All the water that was separated sludge during the sludge treatment is recirculated to the head of the plant.

B.2 Baiña

Baiña wastewater plant is located in the municipality of Mieres, Asturias, Spain. It is a medium size plant, serving approx. 85000 p.e., corresponding to an average flow treatment of 900 m^3/h (250 L/s). It is said to have a treatment efficiency of 97%.

Water treatment is divided in the following steps:

1. Sewage water enters the plant flowing through a stone trap where heavier materials settle to the bottom, and other floating and bulky materials are retained by a screen with bar spacing of 60 mm, placed upstream to an Archimedean screw pump that raises the water to the next treatment step.

2. Sewage flows through other two screens (bar spacing of 80 and 30 mm), to remove smaller solids that a conveyor belt system carries to a press to partially remove water, and then to a container where they are stored to be finally disposed.

3. In the next step, sand is removed from the bottom of a channel where it sediments naturally as water slowly flows. At the same time, fat and grease are removed by skimmers, as they float on the surface. Air diffusers at the base of the tank are used to speed up the flotation.

4. Sewage water reaches the primary sedimentation tanks, where most of the settleable solids and floating material that could not be removed in the earlier stages get eliminated. Settled sewage is collected into a channel and sent to biological treatment. Some of the sedimentators are operated as storm tanks, where excess water due to heavy rainfalls accumulates to be treated in off-peak periods, in order to keep organic load to the biological treatment as constant as possible.

5. Settled water flows through the biological reactors, where the organic matter degradation takes place under the effect of bacteria populations. The biological reactors are divided into two areas:
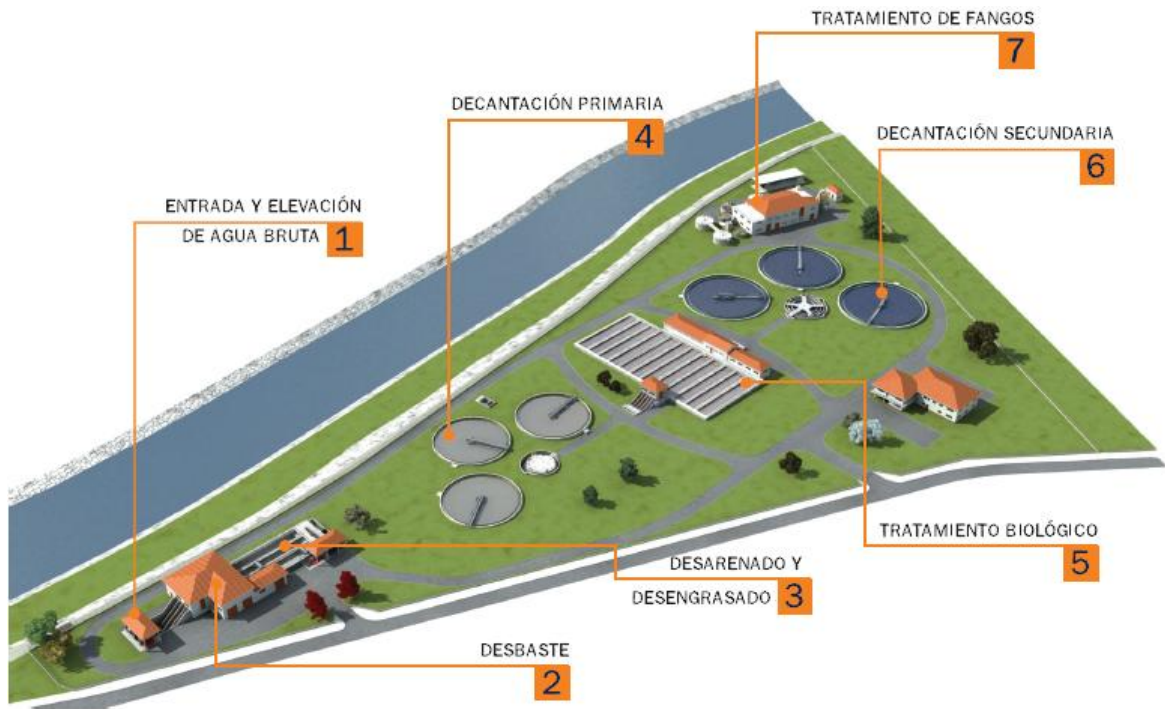
Figure B.2: Schematic of the Baiña wastewater plant, built next to the Caudal river: (1) raw water inlet and lift, (2) grinding, (3) grit and grease removal, (4) primary sedimentation, (5) biological treatment, (6) secondary sedimentation, (7) sludge treatment

    (a) Aerobic zone, where carbonic organic matter is degraded by aerobic microorganisms. Oxygen is provided by blowers that release into the water small air bubbles

    (b) Anoxic zone (upstream of the aerobic one), where anoxic microorganisms convert nitrates to nitrogen. A set of stirrers prevent the sludge settling.

6. The last step in wastewater treatment is the secondary sedimentation, where treated water is separated from the biological sludge. Sludge settles at the bottom of the tank, from where is removed by a scraper and recirculated at the head of the anoxic zone. Clean water is discharged by a channel to the Caudal river. Disinfection is not provided.

7. Sludge treatment facilities differ according to the origin of sludge. Primary sludge is treated into thickeners, where part of the water is removed. Biological surplus sludge is thickened by flotation: sludge is pressurised with air and then released to achieve the separation between sludge and water. Primary and biological sludges are mixed together and with ferric chloride and calcium oxide and then dewatered in filter presses. Subsequently, sludge is heat dried. Dried sludge can now be disposed to an appropriate landfill. All the water that is separated from sludge during the sludge treatment is recycled to the head of the plant.

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