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ADVANCED TREATMENTS OF ORGANIC SOLID WASTE FOR ANAEROBIC DIGESTION

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ABSTRACT

In the last years the increasing concern toward environmental protection and source exploitation has guided the legislative development of measures aiming to the sustainable management of municipal solid waste, which implies increasing material recovery and diminishing landfill disposal.

Biodegradable waste represents the prevailing fraction of municipal solid waste: in Europe, it can reach up to 40% of the total amount of waste produced. Therefore, its proper handling is fundamental.

In this context, anaerobic digestion has become one of the major developments in waste treatment facilities.

The interest in this technology is mainly due to the production of methane, which can be used as a renewable energy source in front of aerobic stabilization that requires energy consumption. Anaerobic digestion followed by an aerobic phase represents a recent treatment option, which allows not only biogas but also compost production.

Although anaerobic digestion is a well-established technology, extensive research proved that the methane yield of solid organic material is significantly affected by both the mass transfer in each biological step and substrate availability. It is recognized that the rate limiting step is the hydrolysis of the complex organic matter into soluble compounds.

Therefore, a proper treatment of the organic material before the digestion stage can promote substrate solubilisation and increase the amount of matter that is readily available to anaerobic microbial species.

To this end, several technologies can be applied, including ozonation and sonolysis, which have been widely investigated as sludge pretreatment for anaerobic digestion.

The use of ozone, which is well known as a strong oxidant, besides noticeable reduction of sludge production, was proved to modify sludge properties and characteristics and increase biogas production from pretreated substrates.

Sonolysis relies on the use of ultrasound to provide substrate disintegration by acoustic cavitational phenomena, which involves the formation and expansion of micro-bubbles and their consequent

collapse. As a result, both chemical and physical reactions can occur resulting in solubilisation and digestibility enhancement.

The analysis of scientific literature highlighted that both ozone and sonolysis applications represent innovative pretreatment options for solid organic substrates to be treated by means of anaerobic digestion.

Furthermore, research development has recently pointed out that the enhancement of methane production after ultrasonic treatment is highly variable according to the different parameters affecting the combined US/anaerobic process.

Therefore, the research activity discussed in this work aimed to:

- the comparative evaluation of ozonation and sonolysis as pretreatment of the organic fraction of municipal solid waste (OFMSW) for anaerobic digestion;
- the assessment of the technical and economic feasibility of the optimal OFMSW pretreatment option.

To this end, experimental activity was structured in two main steps:

- the first one was focused on the assessment of both ozonation and sonolysis effectiveness in promoting solubilisation and anaerobic biodegradability of OFMSW;
- the second part, performed on the basis of the results of the previous phase, was focused on sonolysis effects. In particular, experimental activity was addressed towards the definition of main parameters, in order to assess the technical and economic feasibility of the full scale US pretreatment.

Moreover the relation between sonication pretreatment effects and organic matter composition was assessed.

The first part of the research, focused on the comparative assessment of ozonation and sonolysis, was performed at the Sanitary Environmental Engineering division (SEED) of Salerno University.

For both ozonation and sonolysis, different operating conditions were studied in order to highlight the relation between process extent and the induced solubilisation, which was estimated through soluble COD. The reduction in volatile solid content, which indicates the occurrence of mineralization phenomena, was also monitored for the investigated operating conditions.

Results show that both ozonation and sonolysis are effective in increasing solubilisation of the organic fraction of municipal solid waste. However, higher ozone doses resulted in the formation of by-products which are less biodegradable than the untreated substrate.

This aspect represents a limitation to the scale up of ozonation as pretreatment of OFMSW for anaerobic digestion, as the effectiveness of the process requires strict operating conditions in terms of ozone doses.
Moreover, this drawback was not reported to affect other chemical pretreatments, which can also be handled more easily.
Conversely, the ultrasound-induced disintegration determines an increase of the soluble compounds, which results in improved biodegradability of sonicated samples and, consequently, in higher biogas volumes from the anaerobic digestion of pretreated substrates.
The results of the comparative evaluation of ozonation and sonolysis addressed the research towards the in-depth analysis of sonolysis mechanisms on organic solid matter destined to anaerobic digestion.
In this second phase, the experimental activity was performed at the Technical University of Hamburg-Harburg (TUHH - Germany), in cooperation with Ultrawaves GmbH (Hamburg, Germany), acknowledged since 1995 for its valuable work on the development of new procedures for treating water, wastewater and sludge by means of ultrasound. This work led to the design of one of the most highly developed ultrasonic reactors, which is internationally commercialized. The cooperation allowed the acquisition of an over ten years experience accrued by prof. Uwe Neis and dr Klaus Nickel in the field of sonolysis as well as the use of the basic device of their ultrasonic reactor for the experimental activity.
The effects of the application of different operating conditions were assessed through the quantitative and qualitative characterization of the soluble fraction of sonicated samples as well as by means of anaerobic biodegradability tests.
Experimental results proved that, although the effects of sonolysis on organic substrates depend on the composition of the substrate itself, ultrasound is a valid technical option in anaerobic digestion facilities for OFMSW treatment, as it allowed relevant solubilisation enhancement as well as the improvement of methane production from pretreated substrates. Moreover, its proper application can imply several operating advantages and proved to be economically feasible.
The results of this research are of relevant interest, as they highlight advantages and limits of these innovative pretreatment technologies as well as the high operative potential of sonolysis as pretreatment of solid waste for anaerobic digestion.

SOMMARIO

Negli ultimi decenni, il crescente interesse verso le problematiche di protezione ambientale e utilizzo delle risorse naturali ha indirizzato l'azione del legislatore verso l'emanazione di provvedimenti finalizzati a garantire una gestione sostenibile dei rifiuti solidi urbani.
Tale strategia comporta l'implementazione di politiche volte alla massimizzazione del recupero di materia e alla riduzione del ricorso allo smaltimento in discarica, soprattutto per le matrici che comportano i maggiori impatti ambientali, come la frazione organica dei rifiuti solidi urbani.
L'individuazione di corrette modalità di gestione di tale matrice è tanto più necessaria se si considera che la frazione organica rappresenta l'aliquota predominante del rifiuto prodotto.
In questo contesto la digestione anaerobica, processo di degradazione biologica della sostanza organica in condizioni anaerobiche, si è affermata come una valida opzione di trattamento della frazione organica dei rifiuti solidi urbani (FORSU).
L'interesse verso tale tecnologia è legato, principalmente, alla possibilità di produrre metano impiegabile come fonte di energia rinnovabile. L'attuale tendenza, in particolare, prevede l'integrazione del processo anaerobico con una successiva fase di stabilizzazione aerobica del digestato: in tal modo, qualora il substrato destinato a digestione sia costituito da una matrice selezionata alla fonte, è possibile anche la produzione di compost.
Sebbene la digestione anaerobica si configuri come una tecnologia consolidata per il trattamento della FORSU, è stato dimostrato che i rendimenti del processo, in termini di produzione di metano, sono estremamente influenzati dal trasferimento di massa nell'ambito delle singoli fasi del processo di degradazione del substrato. È noto, in particolare, che lo step limitante l'intero processo è l'idrolisi delle molecole organiche complesse in monomeri solubili.
Tale limite può essere superato mediante l'applicazione di opportuni pretrattamenti che, agendo sul substrato alimentato ai digestori, ne favoriscano la solubilizzazione, incrementando, così, la quantità di

materiale prontamente disponibile nell'ambito dei processi di degradazione biologica anaerobica.
L'attività di ricerca oggetto del presente lavoro riguarda l'applicazione di processi di ossidazione avanzata come pretrattamento di rifiuti organici destinati a digestione anaerobica e, in particolare, di ozonizzazione e sonolisi.
La letteratura scientifica evidenzia che l'impiego dell'ozono, noto per il suo elevato potere ossidante, è in grado di indurre modifiche della struttura di substrati organici, utili a favorire incrementi della produzione di biogas dal successivo trattamento anaerobico di tali substrati.
La sonolisi, invece, sfrutta l'impiego degli ultrasuoni per promuovere la disintegrazione della matrice organica sottoposta a trattamento mediante il fenomeno della cavitazione acustica, che consiste nella formazione, espansione e successivo collasso di micro-bolle, con conseguente sviluppo di effetti di natura fisica e chimica. Tali fenomeni determinano all'interno della matrice organica oggetto di trattamento un incremento della solubilizzazione e, di conseguenza, un miglioramento della degradabilità anaerobica della matrice stessa.
Dal momento che l'esperienza maturata nel campo dell'applicazione di ozonizzazione e sonolisi è relativa a substrati liquidi, quali fanghi di depurazione e reflui civili ed industriali di varia natura, l'applicazione di tali processi si configura come un'opzione innovativa per il trattamento di substrati organici solidi.
La ricerca, dunque, ha inteso perseguire i seguenti obiettivi:

- la valutazione comparativa di ozonizzazione e sonolisi come pretrattamento della FORSU destinata a digestione anaerobica;
- l'analisi della fattibilità tecnico-economica del processo rivelatosi il migliore tra i due come pretrattamento della FORSU.

A tal fine, l'attività sperimentale è stata articolata in due fasi principali:

- il primo step è stato incentrato sulla stima dell'efficacia di ozonizzazione e sonolisi nell'incrementare la solubilizzazione e la degradabilità biologica, in condizioni anaerobiche, della FORSU;
- la seconda parte, sviluppata a partire dai risultati della prima è stata, quindi, focalizzata sugli effetti della sonolisi e, in particolare, sulla stima dei principali parametri utili alla valutazione della fattibilità tecnico-economica del processo combinato sonolisi/digestione anaerobica per il trattamento della FORSU.

Inoltre, al fine di approfondire i meccanismi di azione degli ultrasuoni, è stata condotta una campagna sperimentale finalizzata a identificare le relazioni tra gli effetti della sonolisi e la composizione del substrato organico sottoposto a trattamento.
La prima parte dell'attività di ricerca, dedicata all'analisi comparativa di ozonizzazione e sonolisi, è stata condotta presso il Laboratorio di Ingegneria Sanitaria Ambientale (SEED - Sanitary Environmental Engineering Division) dell'Università degli Studi di Salerno.
Per entrambi i trattamenti sono state considerate diverse condizioni operative, al fine di evidenziare la relazione esistente tra l'entità del processo considerato e la solubilizzazione indotta, stimata attraverso il COD solubile. È stata, inoltre, monitorata la riduzione del contenuto di solidi volatili dei campioni sottoposti ai trattamenti di ozonizzazione e sonolisi, indicativa del verificarsi di fenomeni di mineralizzazione.
I risultati ottenuti evidenziano che entrambi i processi sono efficaci nell'incrementare la solubilizzazione della frazione organica dei rifiuti solidi urbani. Tuttavia, nel caso dell'ozonizzazione, è stata rilevata l'assenza di una relazione diretta tra l'incremento di solubilizzazione e quello di biodegradabilità anaerobica dei campioni sottoposti al trattamento. L'impiego di ozono, infatti, può determinare, secondo le specifiche condizioni di trattamento applicate, sottoprodotti meno biodegradabili della matrice non trattata. Tale aspetto rende il processo di ozonizzazione meno competitivo della sonolisi.
La disintegrazione indotta mediante gli ultrasuoni, invece, determina un incremento della quantità di materiale organico presente in forma solubile, che si traduce in un aumento della degradabilità biologica di tale materiale, con corrispondente aumento della produzione di biogas derivante dalla digestione anaerobica di matrici pretrattate.
I risultati dell'analisi comparativa hanno, dunque, indirizzato la ricerca verso l'approfondimento dei meccanismi di azione degli ultrasuoni su matrici organiche destinate a digestione anaerobica.
In questa seconda fase, l'attività sperimentale è stata condotta presso l'Università Tecnica di Amburgo (Germania), in collaborazione con l'azienda Ultrawaves GmbH (Amburgo, Germania), attiva fin dal 1995 nello sviluppo di nuove procedure per l'impiego degli ultrasuoni in combinazione con processi biologici, sia di natura aerobica sia anaerobica. Tale collaborazione ha consentito l'acquisizione dell'esperienza maturata, dal prof. Uwe Neis e dal dr Klaus Nickel, nel campo della sonolisi dei fanghi di depurazione e concretizzatasi nella

realizzazione di uno dei più efficaci reattori ultrasonici presenti sul mercato internazionale.
In particolare, l'attività sperimentale discussa nel presente lavoro è stata realizzata mediante l'unità base del sistema commercializzato a scala reale.
Gli effetti dell'applicazione di differenti condizioni operative sono stati valutati in riferimento alla caratterizzazione quali-quantitaviva della frazione solubile dei campioni sottoposti a sonolisi, nonché mediante la valutazione della biodegradabilità anaerobica di tali campioni.
I risultati della sperimentazione evidenziano che, sebbene comporti effetti fortemente dipendenti dalla composizione del substrato, la sonolisi rappresenta una valida opzione tecnica per il trattamento della FORSU da avviare a digestione anaerobica.
La corretta applicazione del trattamento, infatti, comporta numerosi vantaggi operativi e risulta economicamente sostenibile.
Le conclusioni della presente attività di ricerca sono, dunque, di rilevante interesse scientifico ed evidenziano l'elevato potenziale applicativo dell'innovativo processo studiato come pretrattamento di substrati organici destinati a digestione anaerobica.

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ABOUT THE AUTHOR

Alessandra Cesaro got her Master Degree in Environmental Engineering, with honours, in 2009, discussing a thesis entitled "Anaerobic do-digestion processes of the organic fraction of municipal solid waste". In the same year, she performed a post-graduating activity at Stuttgart University (Germany) and was admitted to the PhD course in Civil and Environmental Engineering at Salerno University.
Her research interest has been focused on the study of advanced treatment of organic solid waste for anaerobic digestion. In 2012, she spent a semester as visiting researcher at the Technical University of Hamburg-Harburg (Germany), working in cooperation with Ultrawaves GmbH (Hamburg, Germany).
She is co-author of several papers dealing with solid waste management, published in international journals and congress proceedings. Currently, she is involved in initiatives promoted by SEED (Sanitary Environmental Engineering Division), at Salerno University.

Alessandra Cesaro ha conseguito, con lode, la laurea specialistica in Ingegneria per l'Ambiente e il Territorio nel 2009, discutendo una tesi dal titolo "Processi di co-digestione anaerobica della frazione organica dei rifiuti solidi urbani". Nello stesso anno ha svolto attività di formazione post-laurea presso l'Università di Stoccarda (Germania) ed è stata ammessa al Corso di Dottorato di Ricerca in Ingegneria Civile per l'Ambiente e il Territorio presso l'Università degli Studi di Salerno.
La sua attività di ricerca è stata incentrata sullo studio di processi innovativi per il trattamento di matrici organiche solide destinate a digestione anaerobica. Nel 2012, ha trascorso un semestre presso l'Università Tecnica di Amburgo (Germania), lavorando come ricercatore ospite in collaborazione con Ultrawaves GmbH (Amburgo, Germania).
È coautrice di diversi articoli scientifici relativi alla gestione dei rifiuti solidi urbani, pubblicati in atti di convegni e riviste scientifiche internazionali. Attualmente collabora alle iniziative promosse dal SEED (Sanitary Environmental Engineering Division), presso l'Università di Salerno.

1 INTRODUCTION

Biodegradable waste represents the main fraction of municipal solid waste. In Europe, it varies between 24 and 37% of the total amount of waste produced (IPCC, 2006). In 2010, food waste generation in EU-Members States was estimated to be approximately 175 kg/capita/year, which corresponds to almost 84 million tons/year (JRC, 2011).
The need to handle great amount of organic solid waste and the requirement to avoid its direct landfilling, in accordance with the Council Directive 1999/31/EC, address towards the biological processing of this waste stream.
Biological treatments, which aim at the stabilization of organic waste, are considered the most effective management option for the organic fraction of municipal solid waste (OFMSW), especially when collected separately (Fricke et al., 2007).
In the last decades anaerobic digestion, which is the biological degradation process of organic substrates under anaerobic conditions, has become one of the major developments in the field of waste treatment facilities, as proved by the increase, both in number and size, of plants built throughout Europe (De Baere et al., 2008; Cesaro et al., 2010).
The interest in this technology is related to the possibility of producing methane gas, that can be used as a renewable energy source in order to obtain certified emission reduction (CER) credits by clean development mechanism (CDM) under the Kyoto Protocol (Tong et al., 2006). Therefore, the chance to attract foreign investments for renewable energy projects in developing countries is also provided (Poh et al., 2009). Moreover, the aerobic treatment of digestion effluent can produce a stabilized residue which can be employed as soil amendment or for environmental restorations (Castillo et al., 2006).
Since anaerobic digestion of organic solid waste represents a well-established technology, scientific research is mainly addressed to the in-depth analysis of the following aspects:

- methods to enhance biogas and/or hydrogen production from organic substrates processing under anaerobic conditions (Yadvika et al., 2004; Zheng et al., 2009; Sittijunda et al., 2010);
- anaerobic process modelling (Álvarez J.A. et al., 2010; Buendía et al., 2009; Habiba L., 2009);
- anaerobic co-digestion of different organic substrates (Ağdağ et al., 2005; Capela et al., 2008; Bouallagui et al., 2009; Martín-González et al., 2010);
- anaerobic treatment of specific organic waste (Hendriks et al., 2009; Valladão et al., 2007), often of industrial origin (Luste et al., 2009);
- possible use of the supernatant which is produced within anaerobic processes (Adani et al., 2010).

Among these topics, the enhancement of biogas through substrate pretreatment is of great interest, due to its relevant technical and economic implications.
To this end, several technologies can be applied, even in combination (Jian et al., 2008; Xu et al., 2010).
According to the basic mechanism of action, pretreatments can be distinguished in chemical, physical and biological processes.
Whatever the pretreatment is used, its objective is to obtain an extension or an acceleration of the anaerobic process, in order to achieve an increase in biogas production as well as in volatile solid reduction or the decreasing of digestion time.
Therefore, especially when considering solid substrates, such as the organic fraction of municipal solid waste, pretreatments mainly aim to promote the accessibility of hydrolytic microorganisms to the solid matter and hydrolysis of complex polymeric components (Delgenès et al., 2003).

1.1 OBJECTIVES

Most of the experience in the field of the treatment of organic matter before anaerobic digestion has been gained with reference to liquid substrates, such as sewage sludge and manure.
The overall aim of this research is the optimization of OFMSW anaerobic digestion by the application of advanced technologies, useful

to overcome obstacles related to hydrolysis of complex organic matter, thus achieving the increase of biogas production and the possibility of higher energy recovery as well.

Ozonation and sonolysis, which have been traditionally used as wastewater advanced oxidation processes (Sangave et al., 2007), have been widely investigated as pretreatment of liquid substrates addressed to anaerobic digestion.

The analysis of scientific literature points out that the application of both sonolysis and ozonation to complex solid organic substrates, such as the organic fraction of municipal solid waste, represents an innovative pretreatment option for anaerobic digestion.

Although action mechanisms are different, both ozone and ultrasound can promote disintegration and solubilisation of organic matter, thus improving its biological degradation and resulting in increased biogas production from the anaerobic digestion of pretreated substrates.

Therefore, both sonolysis and ozonation were chosen as OFMSW treatment options in order to compare their effectiveness on anaerobic digestion yields as well as to evaluate the technical and economic feasibility of the combined process providing the best performances.

Moreover, as research development has recently highlighted that the enhancement of methane production after ultrasonic treatment is highly variable according to the different parameters affecting the combined US/anaerobic process, the relation between sonication pretreatment effects and organic matter composition was assessed.

To this end, experimental activity was structured in two main steps:

- the first one was focused on the assessment of both ozonation and sonolysis effectiveness in promoting solubilisation and anaerobic biodegradability enhancement of OFMSW;
- the second part, performed on the basis of the results of the previous phase, was focused on sonolysis effects. In particular, experimental activity was addressed towards the definition of main parameters, in order to assess the technical and economic feasibility of the full scale US pretreatment.

The objectives of the first step, performed at the Sanitary Environmental Engineering division (SEED) of Salerno University, were:

- the definition of the optimal ozone doses, according to ozone demand determination as well as to the occurrence of mineralization phenomena, estimated through the variation of volatile solid content of pretreated samples;

- the definition of the optimal ultrasonic specific energy input, as the one providing the highest solubilisation as well as the lowest reduction in volatile solid content;
- the assessment of ozonation and sonolysis effectiveness, under the previously identified operating conditions, according to the quantitative and qualitative characterization of the soluble fraction of treated samples;
- the evaluation of both ozone and ultrasound application in improving anaerobic biodegradability of treated samples.

The results of the comparative evaluation of ozonation and sonolysis proved that the latter process was more competitive than the former one, thus directing towards the in-depth analysis of sonolysis mechanisms on organic solid matter destined to anaerobic digestion.

The second part of the research was, therefore, focused on sonolysis and it was performed at the Technical University of Hamburg-Harburg (TUHH - Germany), in cooperation with Ultrawaves GmbH (Hamburg, Germany).

Main aim of the second phase of the research was the assessment of the technical and economic feasibility of an ultrasonic installation as pretreatment step within OFMSW anaerobic digestion facilities.

To this end, the experimental activity pursued the following objectives:

- the evaluation of main chemical and physical effects promoted by the basic unit of a commercialized ultrasound device, operating under different conditions;
- the assessment of the anaerobic biodegradability enhancement of sonicated samples under the investigated operating conditions;
- the identification of the proper parameters to assess the economic feasibility of an ultrasonic installation within OFMSW anaerobic digestion facilities.

According to scientific literature development, the definition of the relation between sonolysis effects and substrate composition was stated as an additional objective, in order to better define ultrasound action mechanisms.

1.2 OUTLINES

The research discussed in this work follows an in-depth analysis of scientific literature.
Anaerobic digestion process, the kinetics of the different process stages as well as the main factors affecting its yields, with particular reference to substrate characteristics, were studied as reported in Chapter 2.
Main physical, chemical and biological pretreatments are overviewed in the third chapter, in order to define main advantages and drawbacks as well as to highlight the current status of their application on solid substrates, with particular reference to OFMSW. Details of some well-known commercially available pretreatment devices are also provided.
In Chapter 4, sonolysis and ozonation are analysed in details. For both processes, the basic action mechanisms are pointed out, with particular reference to the reactions with organic matter. Moreover, the factors influencing both ultrasound effects and the results of ozone application are discussed. In the field of sonolysis, particular attention was paid to prof. Neis studies, as the ultrasonic device commercialized by Ultrawaves GmbH and considered in the second part of this research was developed following those investigations.
The experimental activity is described in details in the last three chapters:

- Chapter 5 illustrates the investigation plan and the single stages in which it can be divided;
- equipments and analytical procedure are presented in Chapter 6. This section is divided in two parts, so that the first one is focused on the materials and methods used for the comparative assessment of ozonation and sonolysis as OFMSW pretreatment for anaerobic digestion; the second one deals with substrates, devices and modus operandi employed during the sonolysis focused studies.
- Chapter 7 discusses the results of the experimental activity.

The latter chapter is, in turn, divided in three section:

- the first one is focused on the results of the comparative evaluation of sonolysis and ozonation effectiveness as pretreatment of OFMSW to be anaerobically digested;
- the second section deals with the results of sonolysis focused studies;

- the third part discusses sonolysis scale up in order to highlight the technical and economic feasibility of the combined ultrasound/anaerobic digestion process.

Conclusive remarks are reported in Chapter 8.

2 ANAEROBIC DIGESTION OF ORGANIC SOLID WASTE

Anaerobic digestion is the biological degradation process of organic matter, in absence of oxygen. The main product is biogas, which is composed by methane in percentages ranging between 50 and 70% and carbon dioxide. Nitrogen, nitrogen oxides, hydrogen, ammonia, hydrogen sulphide and other volatile compounds are also biogas components: they usually constitute less than 1% of the total gas volume. Along with biogas generation, a digestate is also produced.
Traditionally, anaerobic digestion has been used to treat liquid wastes, such as sludge and manure.
The anaerobic processing of solid wastes was firstly investigated in 1970s, in the United States. In 1980s, studies on solid waste anaerobic digestion were undertaken in Europe and they were mainly devoted to the organic fraction of municipal solid waste (OFMSW), which is one of the most complex substrates to be degraded biologically.
This chapter is focused on the principles of the anaerobic digestion process, in order to highlight main biochemical reactions and to define the factors affecting the conversion of organic matter into biogas, with particular reference to the substrate characteristics.

2.1 KINETICS OF ANAEROBIC PROCESSES

Anaerobic digestion develops in four main steps, as plotted in Figure 2.1:

- hydrolysis, during which the insoluble, complex and polymeric organic matter is converted into soluble molecules and monomeric substances by hydrolytic bacteria;
- acidogenesis, which consists of the organic monomers fermentation, with production of volatile fatty acids, acetic acid, hydrogen and carbon dioxide. The prevailing pathway is the one leading to the formation of organic acids and alcools that are further converted in the subsequent stage;

- acetogenesis, during which the acid formers convert organic acids an alcools into acetate, carbon dioxide and hydrogen;
- methanogenesis, with the production of methane that can occur by means of cleavage of two acetic acid molecules to generate carbon dioxide and methane or by reduction of carbon dioxide with hydrogen.

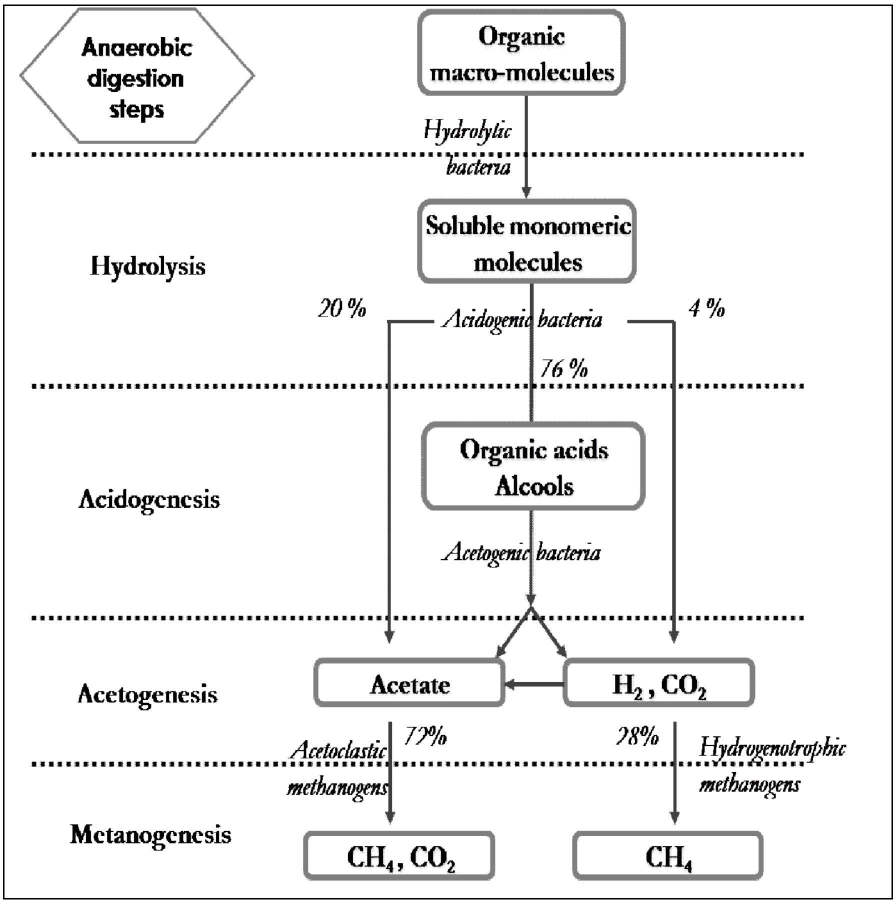


Figure 2.1 Anaerobic biodegradation scheme of complex organic matter

The overall conversion process of organic substrate to biogas is mediated by sequencing of biochemical reactions which do not necessarily share the same optimal environment and the methane yield of solid organic material anaerobic digestion is significantly affected by the mass transfer in each biological step as well as by substrate availability (Li and Noike, 1992). It is recognized that the rate limiting step is the hydrolysis of the complex organic matter to soluble compounds (Vandevivere et al., 2003; Appels et al., 2008), a process which is usually described by a first order kinetic, as the one proposed by Eastman and Ferguson (1981):

$$r = K \cdot X_S$$

where: r is the substrate hydrolysis rate, K is the maximum hydrolytic rate, X_S is the substrate concentration.
Hydrolyzing microorganisms can degrade particulate matter (Vavilin et al., 1996), or produce extracellular enzymes (Jain et al., 1992) that can be adsorbed on the complex molecule surface, thus breaking down complex substrates and making them available for transport within the cells of acidogenic microorganisms.
The latter operate the oxidation of simple organic substrates into pyruvate, which is then converted into volatile fatty acids, alcohols and ketones. These compounds represent the substrate for the subsequent phases.
Differently from the hydrolytic stage, which does not involve any microorganism increase, the net growth of acidogenic bacteria can be simplified through a Monod kinetic:

$$\left(\frac{dX}{dt}\right)_{growth} = \mu_{max} \frac{S}{K_S + S} \cdot I \cdot X - K_d \cdot X$$

and the substrate degradation kinetic can be expressed as:

$$r = K_{max} \frac{S}{K_S + S} \cdot I \cdot X$$

where: μ_{max} is the maximum specific growth rate of the biomass, S is the substrate concentration, X is the acidogenic biomass concentration, K_S is the semi-saturation constant, K_{max} is the maximum substrate conversion rate, K_d is the biomass removal rate, I is the inhibition factor.
Substrate characteristics play a fundamental role affecting the conversion rate of organic matter into organic acids, which are further degraded in the acetogenesis step. If the substrate is supposed to be composed only

by sugars and amino acids, typical numerical values of the model coefficients are in the ranges reported in Table 2.1.
However, if the composition of the substrate varies, experimental work is required to evaluate specific values, as hydrolysis products is highly dependent on the development of the hydrolysis itself (García-Hera, 2003).

Table 2.1 Acidogenic model coefficients (García-Hera, 2003)

Coefficient	Range
μ_{max} [d^{-1}]	3 - 9
K_{max} [g_{COD}/g_{COD} d]	24 - 120
K_S [mg_{COD}/L]	300 - 1400
Y [g_{VSS}/g_{COD}]	0.1 - 0.06
K_d [d^{-1}]	0.02 - 0.3

The acetogenesis degradation pathway is carried out by two different bacteria species. Each bacterial group develops according to Monod kinetics and acts specifically on the short (VFA) or long chain volatile fatty acids (LCFA). Typical model coefficient values for both groups are reported in Table 2.2.

Table 2.2 Acetogenesis model coefficients (García-Hera, 2003)

Coefficient	VFA	LCFA
μ_{max} [d^{-1}]	0.1 - 0.5	0.3 - 1.3
K_{max} [g_{COD}/g_{COD} d]	2 - 20	5 - 20
K_S [mg_{COD}/L]	100 - 4000	100 - 4000
Y [g_{VSS}/g_{COD}]	0.04 - 0.1	0.02 - 0.07
K_d [d^{-1}]	0.01	0.01 - 0.04

Although methanogenesis is considered the critical step of anaerobic process, the conversion of fatty acids into acetic acid is a fundamental phase: high concentration of fatty acids can indicate process imbalance.
Acetogenic products mainly end up in methane and carbon dioxide, which are generated during methanogenesis.
Methane production represents the end of the anaerobic process, as it is the only compound not reactive within the process itself. It can be generated through two different pathways.

In the hydrogenotrophic methanogenesis, the reaction involves carbon dioxide as electron acceptor and hydrogen as the donor, according to the following:

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$$

In the acetoclastic methanogenesis, the anaerobic degradation of acetic acid with production of methane and carbon dioxide occurs according to the following reaction:

$$CH_3COOH \rightarrow CH_4 + CO_2$$

In both cases, the bacteria growth rate can be adequately described by Monod kinetics. However, most of the methane is produced within the latter mechanism.
Both acetoclastic and hydrogenotrophic bacteria are characterized by specific values for model coefficients (Table 2.3), as they use very simple structure substrates: the former uses acetate and the latter hydrogen and carbon dioxide (García-Hera, 2003).

Table 2.3 Methanogenesis model coefficients (García-Hera, 2003)

Coefficient	Acetoclastic bacteria	Hydrogenotrophic bacteria
μ_{max} [d^{-1}]	0.1 - 0.4	1 - 4
K_{max} [g_{COD}/g_{COD} d]	2 - 7	25 - 35
K_S [mg_{COD}/L]	50 - 600	0.01 - 0.1
Y [g_{VSS}/g_{COD}]	0.02 - 0.05	0.04 - 0.1
K_d [d^{-1}]	0.02 - 0.04	0.01 - 0.04

2.2 FACTORS AFFECTING ANAEROBIC DIGESTION

Anaerobic digestion optimization depends on several factors, influencing its yields in terms of biogas production.
The presence of some factors and/or the occurrence of particular conditions can inhibit or limit both the bacteria growth and the overall conversion rate of organic substrates into methane.
Several substances have been reported to inhibit anaerobic digestion processes, but high variation in the inhibition/toxicity levels is reported

for most of them, due to the complexity of the anaerobic digestion process, where mechanisms such as antagonism, synergism, acclimation, and complexing could significantly influence the phenomenon of inhibition itself (Chen Y. et al., 2008).
As a biological process, anaerobic digestion yields are influenced by both environmental conditions and substrate characteristics, which are discussed in the following paragraphs.

2.2.1 Environmental factors

The correct development of anaerobic digestion depends on the proper occurrence of environmental conditions allowing the activity of several microbial species.
Drastic variation of the environmental conditions can negatively affect biological processes: within anaerobic treatments, this aspect is much more important as different microbial species, which do not share the same optimal environment, are involved.
Therefore, the presence of specific substances should be carefully monitored as well as process parameters.
Undesirable substances, such as heavy metals, xenobiotics and other potentially toxic substances can enter the reactor along with the substrate and reach unfavourable concentrations, thus inhibiting anaerobic digestion.
Among heavy metals, the ones of particular concern include chromium, iron, cobalt, copper, zinc, cadmium, and nickel (Jin et al., 1998).
As they are not biodegradable, heavy metals can accumulate to potentially toxic concentrations, thus inactivating enzymes. However, many heavy metals are part of the essential enzymes driving several anaerobic reactions.
Several studies confirmed that their toxicity is better correlated to the concentration of metal free ionic than to its total concentration (Chen Y. et al., 2008). This evidence suggests that the monitoring of anaerobic process environmental conditions, by means of pH and redox potential, plays a fundamental role in controlling heavy metal inhibition effects.
Chen Y. et al. (2008) also pointed out that the inhibitory level of anaerobic processes is determined by the ratio of heavy metal species: although toxicity of most mixed heavy metals was synergistic, some of them showed antagonistic inhibitory effects.

However, heavy metal inhibition is lower in anaerobic digester treating solid organics: the presence of the solid phase provides a protection effect proportional to the surface area or the amount of solids.
Additionally, toxicity can be provided by solvents (Hayward and Lau, 1989), pesticides (Khalilet al.,1991), surfactants (Feitkenhauer, 2004), phenols (Hernandez and Edyvean, 2008) and several other substances, whose release is related to human activities and can be a problem for the anaerobic digestion of some specific residual streams, such as sewage sludge or waste of industrial origin.
Table 2.4 reported the typical concentration ranges for different ions as well as synthetic compound to provide synergistic, inhibitory or toxic effects (ENEA, 2009)

Table 2.4 Typical effects of particular substances as function of their concentration (ENEA, 2009)

	Synergistic effect	Inhibitory effect	Toxic effect
Ion	**[mg/L]**	**[mg/L]**	**[mg/L]**
Sodium	100 - 200	4000	8000
Potassium	200 - 400	3500	12 000
Calcium	100 - 200	3500	8000
Magnesium	70 - 150	1200	3000
Ammonium	50 - 200	1700	3000
Sulphur	< 10	< 200	> 200
Zinc	< 1	> 1	> 160
Copper	< 1	> 1	> 170
Cadmium	< 1	> 1	> 180
Synthetic compounds	**[mg/L]**	**[mg/L]**	**[mg/L]**
Detergents	never observed	> 15	
Antibiotics	never observed	always observed	
Phenol	< 400	> 400	
Formaldehyde	< 2000	> 2000	

Conversely, municipal solid waste anaerobic digestion is more easily affected by the presence of volatile fatty acids, free ammonia and sulphur that, over certain threshold limits, can inhibit anaerobic process by shifting environmental conditions, mainly in terms of pH, beyond optimal ranges.
Their presence is related to the anaerobic degradation of the common constituents of OFMSW and they are, therefore, discussed, in the following paragraph, dealing with substrate composition.

However, inhibition is not only related to the presence of substances affecting microorganism degradation rates: a fundamental role within anaerobic processes is played by temperature.
Anaerobic biological activity has been recognized in a wide range of temperature, from 10°C (psycrophilic temperatures) to over 70°C (extreme thermophilic temperature). However, two optimal range can be identified: the mesophilic one, around 35°C and the thermophilic one, around 55°C. Typically, anaerobic digestion processes and, in particular, the ones involving solid organic wastes, are carried out in these ranges (Mata Alvarez, 2003).
Cecchi et al. (1991) highlighted that both high solid digestion of OFMSW and its co-digestion with sewage sludge were more effective under thermophilic than mesophilic conditions. The surplus achieved in biogas production could balance the additional energy consumption required to ensure the higher temperature in the digester. Although thermophilic digestion offers the advantage of higher reaction rates, 80% of the installed capacity in Europe, in 2005, was provided by mesophilic technologies (De Baere and Mattheeuws, 2008).
This data may be related to the imbalances that may occur more easily at higher temperatures, due to the complexity of biochemical reactions (Mata Alvarez, 2003).
Despite environmental factors, as already mentioned, the substrate can represent an inhibitory factor for anaerobic processes, as the relative concentration of its basic constituents can affect reaction kinetic rates.
Its characterization is, therefore, fundamental to define the optimal operating conditions during anaerobic processes.

2.2.2 Substrate characteristics

The characteristics of solid wastes can greatly influence the success of anaerobic digestion processes.
On a macroscopic scale, municipal solid waste characteristics may vary due to the method of collection, weather season and cultural habits of the community (Abdullah et el., 2008; Belgiorno et al., 2011).
However, OFMSW composition and characterization is not recorded regularly, nor methodologies implemented in different countries are uniform (Dasgupta et al., 2011), so it is extremely unpredictable.

With reference to Europe, in all the Nordic countries, biowaste mainly consists of food waste and, only for a short period throughout the year, it is enriched with fresh garden waste (Sundberg et al., 2011).
Izawa et al. (2001) reported that the main fraction of raw garbage consisted of vegetables (53.6 %); De Gioannis et al. (2007) assessed that Italian OFMSW could be modelled as composed by 10% meat, 65% fruit and vegetables, 10% bread and 15% pasta and rice (with percentages estimated by weight). Similar results were reported by Alibardi et al. (2012).
Collection systems also affect both the quantity and quality of waste going to treatment.
Often, the source-sorting of organic waste is the most suitable way to access high quality OFMSW. Cecchi et al. (2003) highlighted the differences between source sorted and mechanically separated OFMSW, whose characteristics are connected to the complexity of sorting plant.
As shown in Table 2.5, the mechanically sorted organic waste is usually characterized by a high dry solid content, due to the inert fraction, which is not completely separable with this sorting approach. This aspect is much more evident if the total volatile solid content (TVS) is considered: the average value is lower than 50%, while the common values for a source sorted organic waste ranges between 80-97% (Zhang et al., 2007).

Table 2.5 Chemical-physical characteristics of organic waste mechanically sorted in complex plants (Cecchi et al., 2003)

Parameter	average	max	min	Nr of samples	Std. Dev.
TS [g/kg]	763.0	952.0	513.1	210	81.3
TVS [%TS]	43.9	57.4	29.1	210	5.4
TCOD [%TS]	59.6	90.4	23.3	41	17.4
TOC [%TS]	19.3	34.4	7.5	187	5.3
IC [%TS]	1.3	2.7	0.3	187	0.5
TKN [%TS]	2.2	3.4	1.2	59	0.5
P [%TS]	0.11	0.22	0.05	59	0.03

As the composition changes, the presence of carbohydrates, proteins and lipids in the waste varies as well, thus affecting the anaerobic process of the whole substrate.
Both the degradability and biogas production potential from solid waste in anaerobic conditions depend on the relative amount of the main components: lipids, proteins, carbohydrates such as cellulose and hemicelluloses as well as lignin (Hartmann and Ahring, 2006).

Table 2.6 reports some hydrolysis rate constant for the basic substrates.

Table 2.6 Hydrolysis rate constant for basic substrates (APAT, 2005)

Substrate	K [d^{-1}]
Carbohydrates	0.5 - 2.0
Lipids	0.1 - 0.7
Proteins	0.25 - 0.8

Vidal et al. (2000) studied the influence of fat and protein content on anaerobic biodegradability of dairy wastewaters, founding that the anaerobic biodegradation rate of fat-rich wastewaters is slower than that of fat-poor ones, due to the slower rate of the fat hydrolysis step. However, the presence of fats in the wastewater prevents the periodic production of high concentrations of volatile fatty acids, which may adversely affect the process.
In the same study, it was pointed out that ammonia production is significant in carbohydrate-rich wastewaters, thus bringing two antagonistic effects: free ammonia causes a partial inhibition of the process but it also controls the pH drops, thus avoiding inhibition by volatile fatty acid accumulation.
Liu and Sung (2002) found that ammonia concentration below 200 mg/L are beneficial to anaerobic process, as nitrogen is an essential nutrient for anaerobic microorganisms. The threshold value for ammonia concentration to provide inhibition is, conversely, not well defined: differences are mainly related to the operating conditions adopted to perform experimental studies, in terms of substrates and inocula, as well as acclimation periods (Chen et al., 2008).
It should be pointed out that adaptation of methanogens to several inhibitory substances has been reported. It may result from internal changes in the predominant species of methanogens or from the shift of methanogenic population (Chen et al., 2008).
The composition of the substrate is, however, particularly important for the supply of the relative amounts of organic carbon and nitrogen and, consequently, the C/N ratio.
Kayhanian and Hardy (1995) found that a C/N ratio ranging between 25 and 30 can be considered optimum for anaerobic processes. If this ratio is higher, the substrate is not suitable for bacterial growth due to nitrogen lack. On the other hand, low C/N ratios indicate a relevant

nitrogen content that can result in ammonia accumulation (Hartmann and Ahring, 2006).
Nutrient content in solid organic waste is usually adequate for anaerobic digestion to develop correctly: Mata Alvarez (2003) recommend an average ratio COD/N/P of around 600/7/1 for a substrate to be anaerobically digested. The supply of micronutrients, such as sulphur, vitamins and some trace of minerals (Fe, Ni, Mg, Ca, Na, Ba, Tu, Mo, Se and Co) is also important to stimulate microbial growth (Mata Alvarez, 2003).
Some of these micronutrients, such as magnesium (mg) and calcium (Ca), are included among light metal ions along with aluminium, potassium and sodium. Their presence may be not only related to organic substrate degradation, but also to the addition of chemicals to provide pH adjustment within the digester. Beyond certain concentration, these elements can provide toxicity or severe inhibition.
Among the mentioned light metals ions, Chen et al. (2008) referred that the best documented as inhibitor of anaerobic digestion process is sodium: concentration higher than 200 mg/L were reported to be cause of anaerobic process slow down, due to its negative effects on bacteria and interference with their metabolites. However, acclimation of methanogens to high concentration of sodium over prolonged periods of time could increase the tolerance and shorten the lag phase before the beginning of methane production.
It is evident that substrate characteristics play a fundamental role within anaerobic digestion process.
Both the composition and the quality of the substrate determine different biodegradability level as well as the need for particular pretreatments in order to optimize biological process yields.

3 PRETREATMENT METHODS TO IMPROVE ANAEROBIC BIODEGRADABILITY OF ORGANIC SOLID WASTE: STATE OF THE ART

In the last decades anaerobic digestion has become a reliable technology for the treatment of organic solid waste (Cesaro et al., 2010).
The most attractive aspect of this process is the production of biogas, which can be used as a renewable energy source.
Therefore, the technical and scientific interest is directed towards the process optimisation, with the aim to maximize biogas generation from anaerobic processing of organic matter.
Among the possible systems, the use of pretreatments has raised great interest.
Pretreatments operate on the substrate in order to promote the disintegration and solubilisation of complex organic matter, thus increasing the amount of material that is readily available for the biological degradation and, consequently, improving anaerobic digestion yields.
Several pretreatment options can be considered according to:

- the input waste features;
- the technology of the anaerobic process adopted;
- quality and subsequent use of the digestate.

The study of pretreatments for anaerobic digestion have been widely performed on wastewater treatment plant residues: according to the review of Carlsson et al. (2012), most of the experimental research in the field of pretreatment application have been devoted to this kind of substrates (Figure 3.1).

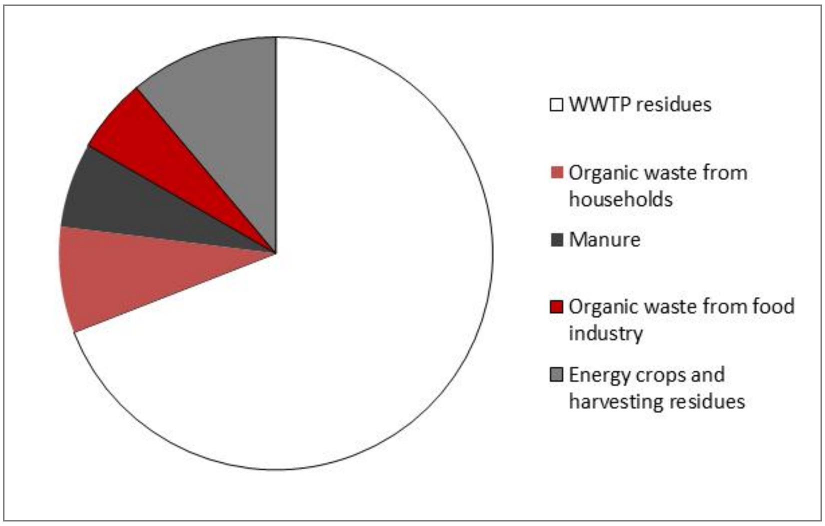


Figure 3.1 Substrates and pretreatment in literature (adapted from Carlsson et al., 2012)

The same work highlights that, focusing on household organic waste, most studies deals with physical pretreatments, with a predominance of the mechanical ones, as shown in Figure 3.2 (Carlsson et al., 2012).

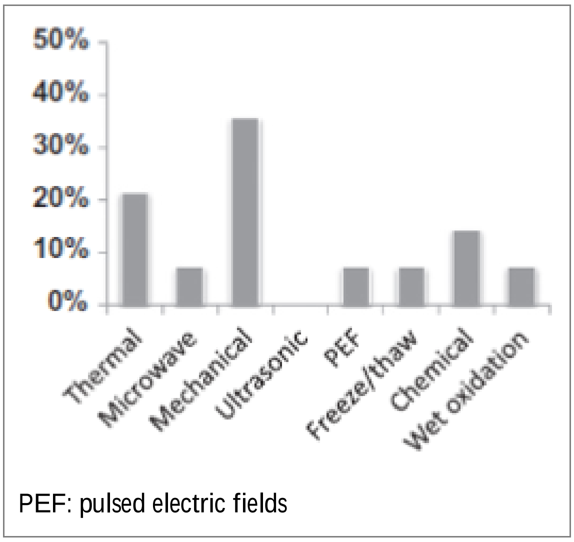


Figure 3.2 Substrates and pretreatment in literature (Carlsson et al., 2012)

Literature data reports experiences dealing with chemical, biological and physical processes, which are supposed to improve organic matter solubilisation and biodegradability (Lafitte-Trouqué and Forster, 2002), by increasing the organic substrate surface area and/or the enzymatic activity.

Among the techniques mentioned above, the use of additives has also been investigated.

In the following paragraphs, an overview of main pretreatments is reported in order to highlight the state of the art of their application to solid substrates, with particular reference to OFMSW.

3.1 CHEMICAL PROCESSES

Chemical processes are the ones relying on oxidative reactions.

Research dealing with peroxidation methods prior to biosolid anaerobic digestion was carried out, but the most studied pretreatment options are the oxidation with wet air and the ozonation.

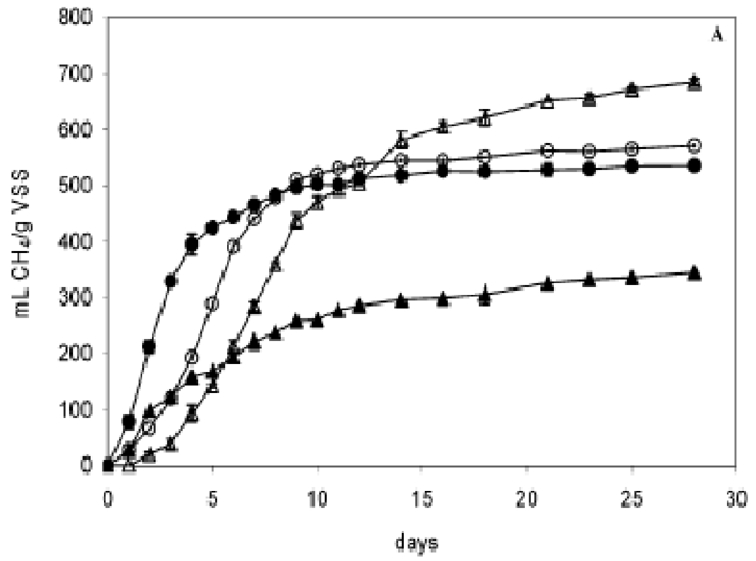
Dewil et al. (2007) compared the efficiency of the well-known Fenton process with two innovative peroxidation reactions involving peroxymonosulphate (POMS) and dimethyldioxirane (DMDO) in transforming refractory COD into readily available and soluble BOD, thus improving anaerobic treatment of wastewater treatment sludge.

Results indicate a significant increase in biogas production, which is due to the disintegration and solubilisation of organic matter during the treatment of the sludge. The innovative reactions resulted in higher release of organics into the sludge water than Fenton process. The increased solubilisation determined greater specific biogas production after POMS and DMDO treatments than Fenton ones.

In particular, the best pretreatment proved to be the one with DMDO, which determine a 2.5 fold increase in biogas production, although any variation in its composition was observed.

However, peroxidation as pretreatment of organic waste before anaerobic digestion is rarely investigated, reasonably due to the complex process handling, which requires severe controls over environmental conditions.

This kind of process is, therefore, not as referenced in literature as wet air oxidation, which should be better identified as a chemical-physical process.
Wet air oxidation can improve the contact between oxygen molecules and organic matter, thus allowing the total or partial disruption of many organic contaminants not easily biodegradable and micropollutants such as phenols, nitrogen compounds and carboxylic acids.
The basic assumption is that the reactivity of oxygen, or its capacity to oxidize, increases with temperature. On the other hand, it is necessary that the process occurs in the liquid phase, in order to support the oxygen contact with the organic matter.
Therefore, in order to maintain both conditions of high temperature and liquid phase, it is necessary to operate at high pressures, ranging between 30 and 250 bar (Bertanza and Zanabroni, 2010).
Lissens et al. (2004) compared the anaerobic biodegradability of three wet oxidized wastes (raw food waste, digested biowaste and raw yard waste) with untreated organic wastes as reference material, by measuring methane yields in batch and continuous tests.
While a doubling of the methane yield was achieved for wet oxidation yard waste compared to the reference, a minor increase (7%) was observed for raw food waste, due to the differences in ligno-cellulose composition and characteristics of the lignin fraction of both substrates (Figure 3.3).



(▲) yard waste, (●) food waste, (Δ) WO yard waste, (○) WO food waste

Figure 3.3 Cumulative methane production from untreated and wet oxidized waste (Lissens et al., 2004)

The most interesting outcome of this study is the extrapolation of the results to a full-scale plant, treating 50 000 t/year of organic waste.
On the basis of the methane yield achieved with the 3 L reactors, a theoretical gain of about 35-40% in methane production can be expected if wet oxidation (12 bar oxygen pressure) followed by a second digestion was applied on digested biowaste of the full-scale plant.
The application of this pretreatment could be suitable for substrates characterized by high ligno-cellulosic content that are favourably affected by high temperature: several studies (Hendriks and Zeeman, 2004) highlight the occurrence of solubilisation of the ligno-cellulosic biomass, firstly the hemicelluloses and shortly after that lignin, for temperature increase above 150 - 180°C.
This evidence could be confirmed by results obtained by Fox and Noike (2004), who studied wet oxidation as pretreatment of newspaper waste to be anaerobically digested, founding the highest methane conversion efficiency for newspaper pretreated at 190°C and anaerobic cellulose removals ranging between 74 and 88%.

One of the main drawbacks of this technology comes from the high pressure and high temperature, which may result in a prohibitively expensive treatment (Santos et al., 2007). Moreover, under these conditions the reaction mixture is extremely corrosive even at alkaline pH (Chamam et al., 2012).

Different changes occur in organic matter structure after ozonation. The process, which is discussed in the following chapter, was proposed in the late 1990s and it used ozone, which is known as a strong oxidant (Yasui and Shibata, 1994), to reduce biological sludge production. Subsequently, ozone was also studied as treatment prior to anaerobic digestion, but its application is still limited to liquid substrates, mainly sludge.

In the field of chemical pretreatment, also acids and alkalis have been investigated.

The latter are more compatible with anaerobic digestion process, compared to the former, since the bioconversion generally requires an adjustment of pH by increasing alkalinity (Pavlostathis and Gosset, 1985). Most of the ozonation studies report about pH decrease after the chemical treatment, so that alkaline addition is necessary before starting anaerobic digestion tests. However, acids can be effective for the anaerobic digestion of protein-rich substrates, which could bring to ammonia inhibition phenomena (Hansen et al., 1998).

Alkaline pretreatment has been examined with reference to several solid organic substrates, including OFMSW.

Zheng et al. (2009) applied different NaOH doses (2, 4 and 6%, based on substrate dry matter) to corn stover, within a wet state pretreatment. In order to reduce NaOH dose and shorten treatment time, authors applied water to thoroughly saturate corn stover and provide enough water for chemical distribution inside feedstock. This wet state alkaline pretreatment proved to be efficient in improving both biodegradability and enhancing methane production of corn stover. Results may be related to the reduction by 9.3, 18.0 and 19.1% that was achieved for lignin, cellulose and hemicellulose, respectively, after pretreatment, as shown in Figure 3.4.

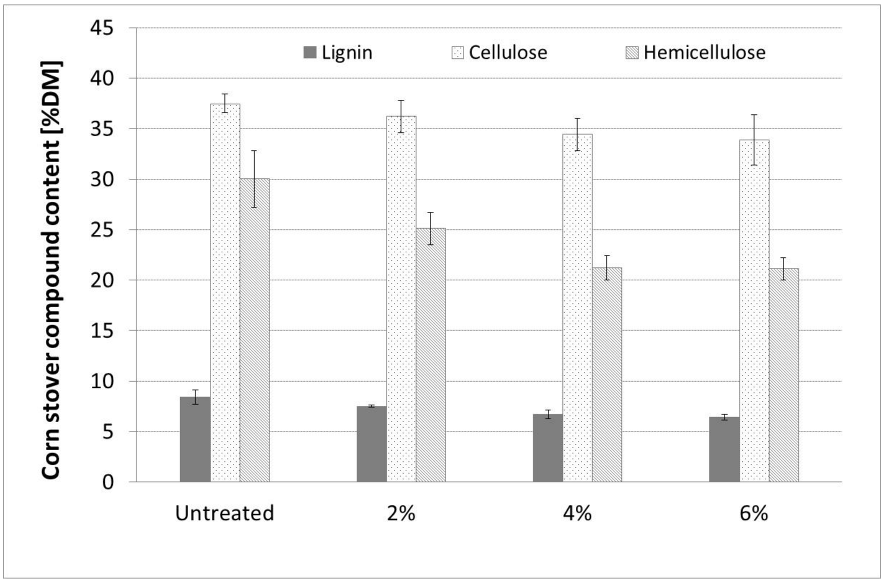


Figure 3.4 Content of lignin, cellulose and hemicellulose after NaOH wet state pretreatment of corn stover

López Torres and Espinosa Llórens (2008) demonstrated the improved OFMSW anaerobic digestion efficiency after an alkaline pretreatment with $Ca(OH)_2$.

The addition 62.0 mEq $Ca(OH)_2/L$ and 6 hours provided a 11.5% increase in the soluble COD out of the total COD. As shown in Figure 3.5, further addition of the reagent determined a decrease in solubilisation, which authors attributed to the formation of complex, non-soluble compounds.

In the same study, alkaline pretreatment, under the best conditions, allowed a methane yield from pretreated waste anaerobic digestion of 0.15 m^3_{CH4}/kg_{VS}, which corresponded to 172.0% of the control.

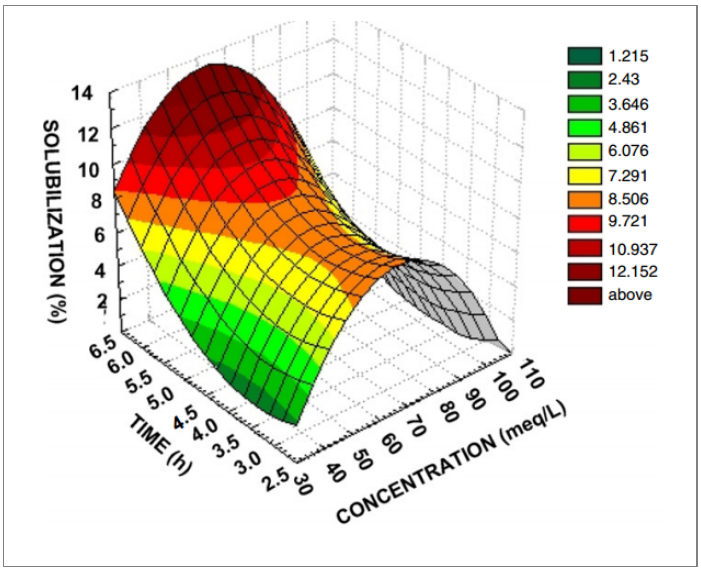


Figure 3.5 Effect of $Ca(OH)_2$ on OFMSW solubilisation (López Torres and Espinosa Llórens, 2008)

However, alkali treatments are not without problems.

In continuous reactors fed with alkaline-treated samples, whose initial concentration of sodium or potassium was 0.21 mol/L, acetate and glucose degradation rates were found to fall to 5% and 50%, respectively, due to toxic compounds generated during the saponification reaction (Ward et al., 2008).

Moreover, as alkaline pretreatment mainly improve anaerobic digestion yields by favouring the stabilization of anaerobic process itself, more suitable solutions can be adopted, such as the implementation of anaerobic co-digestion.

Neves et al. (2006) found that both an alkaline hydrolysis pretreatment and co-digestion with kitchen waste were beneficial to enhance the methane production of barley waste.

According to these results, alkali or acid addition could be suitable options to face temporary inhibitory conditions in anaerobic digesters, related to the accumulation of volatile fatty acids, free ammonia or other substances that can affect anaerobic process by varying environmental conditions in terms of pH. Table 3.1 summarizes the results of the main works previously discussed.

Table 3.1 Main chemical pretreatments studied as anaerobic digestion pretreatment

Substrate	Treatment conditions	Anaerobic digestion conditions	Main results	Reference
Biowaste	Wet oxidation: 185-220 °C; 0-12 bar; 15 min	3L semicontuous reactors, 55°C	35-40% biogas production increase expected in full-scale plant	Lissens et al., 2004
Newspaper waste	Wet oxidation: 170°C-31 kg/cm^2, 190°C - 40,9 kg/cm^2, 210°C-51,1 kg/cm^2	Batch, 35°C	Maximum solubilization ratio of 29% at 210°C	Fox and Noike, 2004
Corn stover	2, 4, 6% NaOH	Batch, 35°C	Increased CH_4 yield, reduction of digestion time	Zheng et al., 2009
OFMSW	40-100 meq/L, 1-6 h	Fill and drawn mode	11,5% COD increase, 172% CH_4 increase	Lόpez Torres and Espinosa Llórens, 2005
Barley waste	0.3 g_{NaOH}/g_{TS}, at 25°C	Batch, 37°C	Doubled VS reduction over the control after alkali pretreatment	Neves et al., 2006

3.2 PHYSICAL PROCESSES

Among the physical pretreatments, both mechanical and thermal ones have to be listed but other emerging technologies are being implemented at both research and industrial level, such as high pressuring machines, focused-pulse technologies and ultrasonic devices.
Research experiences in the field of ultrasound application as pretreatment of organic matter before anaerobic digestion are discussed in the next chapter.
As a relatively novel option, focused-pulsed technologies have demonstrated their effectiveness at full-scale, when used to enhance anaerobic digestion yields.
The basic mechanism is cell disruption occurring by exposing cell membranes to a series of short, high-voltage pulse (20 to 30 kV, at 2000 to 3000 Hz). Although treatment time lasts less than one second, the required voltage is very high (Lee and Rittmann, 2011), thus increasing risks associated to incorrect process management.

3.2.1 Mechanical pretreatments

Mechanical pretreatment is used to reduce the particle size and crystallinity of lignocellulosic materials as well, in order to increase the specific surface area and reduce the degree of polymerization. This effect can be obtained by a combination of chipping, grinding or milling, depending on the final particle size of the material (Sun and Cheng, 2002).
In anaerobic conditions, cell-associated enzymes prevail on free enzymes (Hobson, 1987). Therefore, particle size reduction provides a greater area for enzymatic attack (Palmowski and Müller, 2000; Hartmann and Ahring, 2006), thus favouring hydrolysis.
Mshandete (2006) reported that by reducing the size to 2 mm, the potential methane production of sisal fiber waste would improve to more than 20% and the total fiber degradation increased from 31% to 70% compared to the untreated fibers. The degradation of fibers, obviously resulted in the improvement of methane production, which

was found to be proportional to the particle size of the substrate, as reported in Figure 3.6.

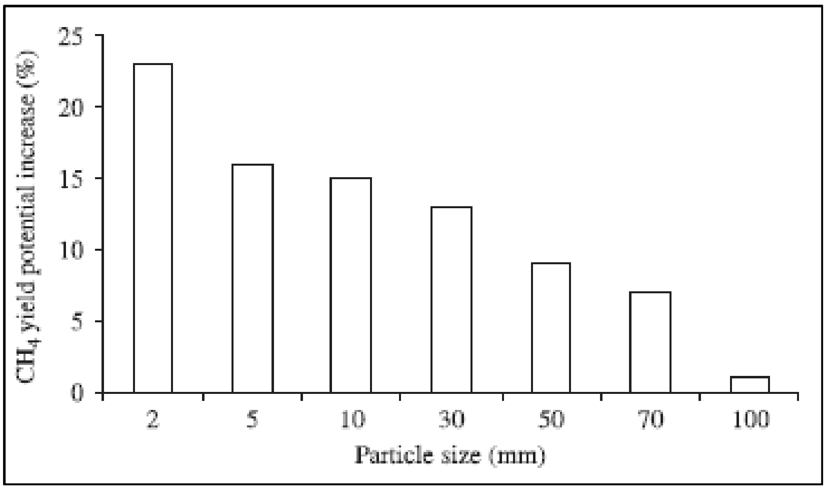


Figure 3.6 Methane content as function of substrate particle size (Mshandete et al., 2006)

On the other hand, particle size reduction may accelerate the hydrolysis and acidogenesis steps as well as the production of soluble organic material such as volatile fatty acids (VFAs), resulting in excessively high organic loading in the anaerobic digestion reactor.

Izumi et al. (2010) demonstrated that grinding pretreatment by bead milling resulted in 30% higher solubilisation over the control (Figure 3.7), but excessive size reduction caused VFA accumulation, resulting in decreased methane production and decreased solubility in the anaerobic digestion process.

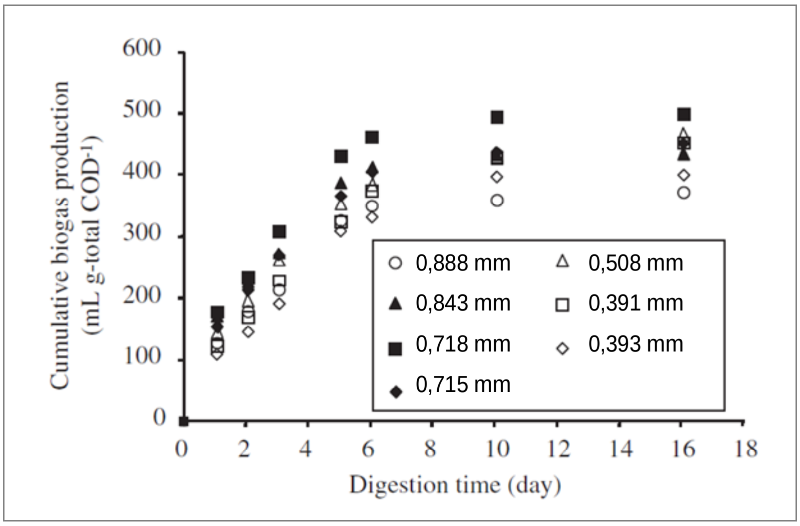


Figure 3.7 Cumulative biogas production of differently milled food waste (Izumi et al., 2010)

This aspect could be an advantage if the anaerobic digestion plant capacity is not adequate to treat the received amount of waste: in this case, by intensifying pretreatment step, hydrolysis is accelerated and digestion time can be lowered, thus allowing the treatment of greater amount of waste.
The best operating conditions must be defined according to specific energy balances: for mechanical pretreatment devices, the power requirement is relatively high depending on the final particle size and the biomass characteristics. Particularly, the strong structure of green waste makes its size reduction very energy intensive and conducting some kind of chemical pretreatment prior to wood size reduction is appearing as an alternative (Zhu et al., 2010).
More often, in full-scale plants, the particle size of the waste is homogenized through sieving systems, with the further objective to separate not biodegradable materials, such as plastic bags, from the organic stream destined to the biological treatment.
Mechanical pretreatments also include the use of high pressure gradients. Several studies dealing with sludge (Onyeche, 2004) have been performed in order to achieve anaerobic digestion improvement from the disruption of sludge cell walls of microorganisms.

Nah et al. (2000) examined the effects of this kind of mechanical pretreatment on a thickened waste activated sludge, which was jetted to a collision plate at different pump pressure values.

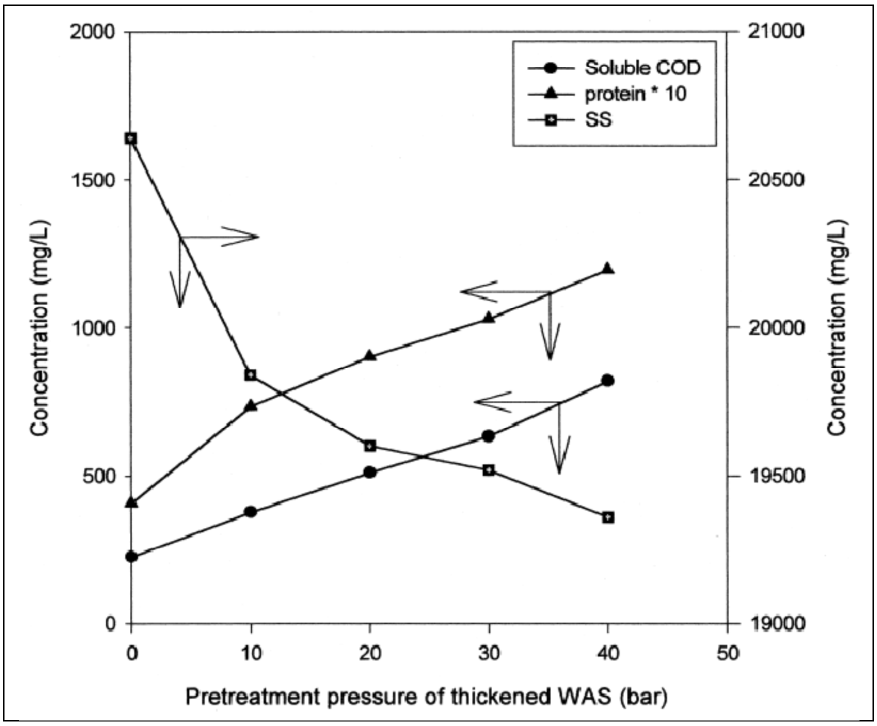


Figure 3.8 Variation in sludge characteristics for increasing pressure values (Nah et al., 2000)

Figure 3.8 highlights that for increasing pressure values, suspended solid content decreased, while soluble COD and protein increased as a consequence of microbial cell disruption. These change in sludge characteristics allowed the reduction of retention time in anaerobic digester, without affecting anaerobic digestion yields.

Nowadays, OFMSW to be treated anaerobically in full-scale plants is often pretreated in press-extruders, waste pressuring machines designed to physically separate waste into two fractions: an organic wet fraction and a solid dry one.

The process consists in squeezing the waste with very high pressure into an extrusion chamber, so that the wet organic fraction squeezed out from the extrusion holes can be used for energy production in anaerobic

digestion plants (Gonnella, 2011) and the solid one can be used within aerobic stabilization processes.
In the extrusion chamber, waste volume reduces following the increasing of the pressure. Once reached a certain pressure value, which can vary according to both the characteristics of the material and the diameter of the extrusion holes, the fluidification of the organic fraction starts and wet material is pushed out the extrusion holes. During this phase the pressure inside the chamber is almost constant. At the end of the extrusion step, the material is further compressed, until the maximum pressure defined by the design of the machine is reached.
In Figure 3.9, volume reduction as function of pressure increase is plotted. For pressure higher than 700 bar, volume reduction depends on the size of the extrusion holes: the smaller is the size, the higher is the pressure level required in the extrusion chamber.

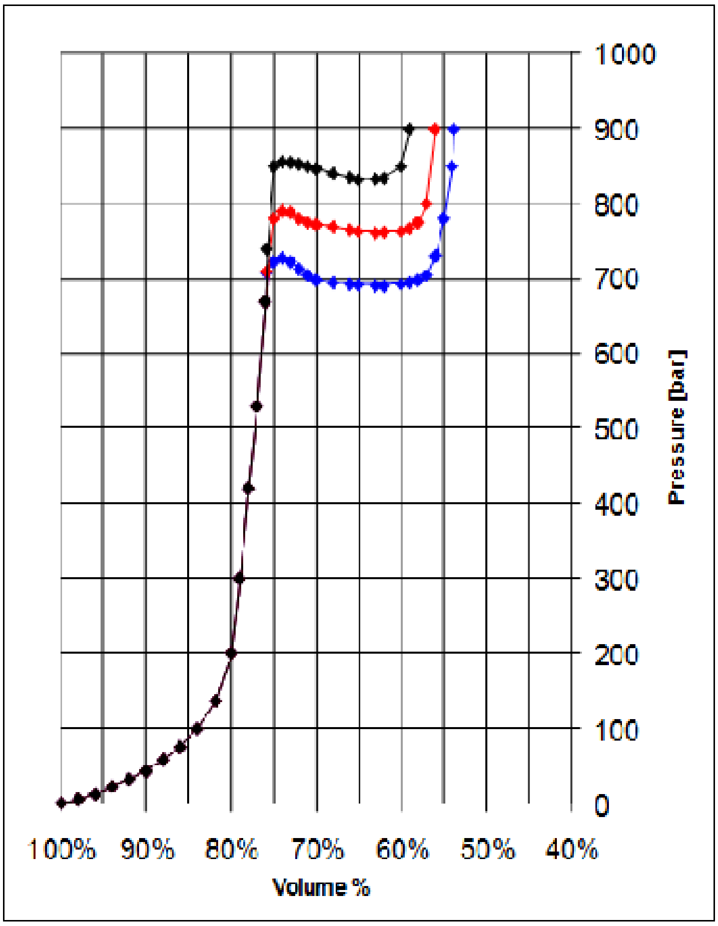


Figure 3.9 Press-extrusion diagram (Gonella, 2011)

This pretreatment is implemented in the integrated anaerobic/aerobic plant in Salerno, treating OFMSW coming from separate collection.
The main advantage of these pressuring machines lays in the possibility to obtain a solid fraction, which is characterized by an organic content lower than the rough OFMSW and requires a lower energy consumption during the aerobic stabilization phase (Scaltriti, 2007).
At the same time, the organic fraction going to anaerobic digestion processes is characterized by a total solid content of 20-30%, thus reducing the need for dilution as well as energy required for mixing the substrate to be digested and allowing the simplification of mechanical

systems (i.e. pumping systems) within anaerobic digesters, with a consequent decrease in capital and operating costs.
However, these devices require high quality OFMSW as feeding substrates: if this condition is not fulfilled, great amounts of impurities end up in the solid phase, affecting its further treatment and possible utilization.

3.2.2 Thermal pretreatments

Thermal treatment has been largely used as a conditioning process for raw or digested sludge (Bougrier et al., 2007) as it improves the dewaterability properties of such waste.
Furthermore, heat treatment alters the structure of the insoluble fraction to make it more amenable to biodegradability.
Kuo and Cheng (2007) studied thermal pretreatment of kitchen waste under various temperatures (37, 50 and 60°C), in order to evaluate the effect on hydrolysis. The overall performance of anaerobic digestion process with thermal pretreatment was more stable and the removal efficiencies of inhibitory compounds, added to solid waste, were higher than those of the system without pretreatment. In particular, authors found that thermal pretreatment helped the hydrolysis of kitchen waste into soluble form, increasing the reaction rate in the methanogenic step: therefore, a 2.2-fold increase in biogas production was achieved as well a 48% improvement in methane content.
Low temperature thermal pretreatment was also studied by Appels et al. (2010) in order to verify the occurrence of solubilisation, evaluated not only in terms of soluble COD, but also as the release of carbohydrates and proteins that are primary carbon and nitrogen source for the anaerobic micro-organisms, respectively.
Approximately 10% of the total protein content was released by applying temperature of 80 and 90°C for 60 min. The same trend was observed for the carbohydrate release. These results suggest that both decomposition of extracellular polymer substances (EPS) and cell lysis take place during the treatment.
Heat effects on the physical and chemical properties differ according to both the operating temperature and the kind of biomass.
Thermal pretreatment at 175 C and 60 min significantly decreases viscosity and increases soluble chemical oxygen demand, soluble sugar

and soluble proteins of kitchen waste, vegetable/fruit residue and waste activated sludge (Liu et al., 2012).

Notwithstanding, for kitchen waste and vegetable and fruit residues, a 7.9% and 11.7% methane decrease was observed, respectively. The authors attributed this evidence to the formation of melanoidin, a co-polymer which is well-known to be difficult to degrade.

The main disadvantage of thermal pretreatment lays in the high energy consumption that can be overcome through microwave-based pretreatment.

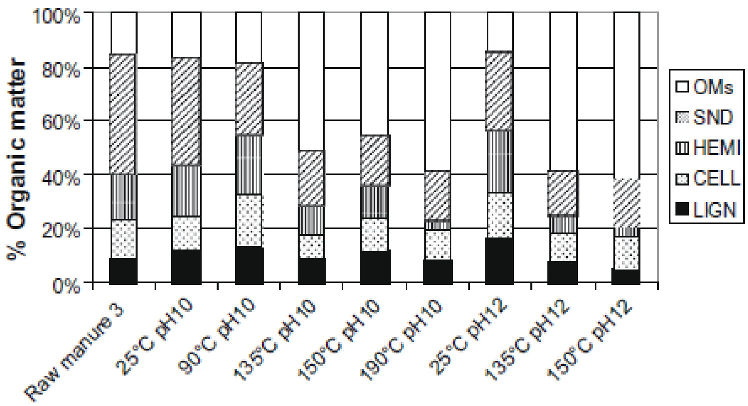
Compared to conduction/convection heating, which is based on supercritical heat transfer, microwave uses the ability of direct interaction between a heated object and an applied electromagnetic field to increase heat (Hu and Wen, 2008), thus combining both thermal and non-thermal effects generated in aqueous environment. The movement of ions and the vibration of polar molecules give rise to heat and extensive intermolecular collisions which accelerate chemical, physical, and biological processes. When microwave is used to pretreat ligno-cellulose, it selectively heats the more polar part and this unique heating feature results in an improved disruption of the recalcitrant structures of ligno-cellulose. Regarding non-thermal effects, the electromagnetic field helps to accelerate the destruction of crystalline structures and changes the super molecular structure of ligno-cellulosic material improving its reactivity. The short length of the process as well as the low inhibitor production is reflected in high cost effectiveness.

Microwave pretreatments were carried out at first by immersing the biomass in water, but recently the potential of different chemical reagents has been studied, with successful results (Tomas-Pejo et al., 2011). The feasibility of the combined process is, however, still not spread due to the high costs related to both microwave irradiation and chemical use.

Also thermal pretreatments were studied in combination with alkaline ones.

Battimelli et al. (2009) studied the use of 0.3 wt % sodium hydroxide at 60°C for 30 min to enhance biogas production from fatty slaughterhouse wastes. Anaerobic batch test results showed that the pretreatment led to a slight improvement in the initial reaction kinetics, indicating enhanced bio-availability provided by chemical hydrolysis, but any relevant conclusion could be drawn with reference to hydrothermal pretreatment effects on long chain fatty acids inhibition.

Conversely, Carrère et al. (2009) reported the effects of several hydrothermal pretreatment conditions on the fractionating of the organic matter content of pig manure.



OMs: soluble organic matter; SND: soluble in naturant detergent fraction; HEMI: hemicellulose; CELL: cellulose; LIGN: lignin

Figure 3.10 soluble and fractionated particulate organic matter under investigated pretreatment conditions (Carrère et al., 2009)

As showed in Figure 3.10, most pretreatments allowed both the transfer of organic matter from the fraction to the supernatant as well as a slight reduction of the hemicellulose-like fraction that favoured anaerobic degradation.

Thermo-chemical pretreatment have been also investigated on solid substrates. Hao et al. (2009) studied the effects of temperature, reaction time and alkali dose on the organic solubilisation and anaerobic biodegradability of mechanically sorted MSW, leaves and model kitchen garbage. To this end, experiments were run at temperature of 90-190°C for 60 minutes; subsequently, the waste was pretreated with 1-4 g NaOH /100 g solid, at 130-170°C, with a retention time ranging between 30 and 90 minutes.

Authors found that hydrothermal alkaline pretreatment can shorten digestion time of both kitchen waste and green residues, but optimal temperature conditions differ for different wastes, reasonably due to the lower ligno-cellulosic content of kitchen waste, which contributes most to the methane production.

Despite the improvement that thermo-chemical processes can provide if applied as anaerobic digestion pretreatment, they were found to present some problems, such as:

- the possibility of producing compounds which can inhibit subsequent anaerobic digestion processes;
- solubilisation of molecules characterized by a structure which is difficult to degrade;
- use of chemicals, that can led to toxicity problems.

3.3 BIOLOGICAL PROCESSES

Hydrolysis step can also be improved through the increase in the microbial activity per unit of surface area. This effect can be achieved not only by substrate inoculation, but also by the use of enzymes.
Therefore, biological pretreatments include both the use of microorganisms with high ability in degrading a substrate and the addition of enzymes that support biological reactions within anaerobic digesters.
Fdez-Guelfo et al. (2011a) examined the addition of mature compost to industrial OFMSW to enhance organic matter solubilisation and, hence, the biogas and methane production and the organic matter removal during the anaerobic digestion. They found that the organic matter removal percentage, measured in terms of eliminated DOC and VS, was improved up to 61.2% and 35.3% over the control without pretreatment and, as a consequence, the biogas and methane production increased up to 60.0% and 73.3% over the control (Figure 3.11).

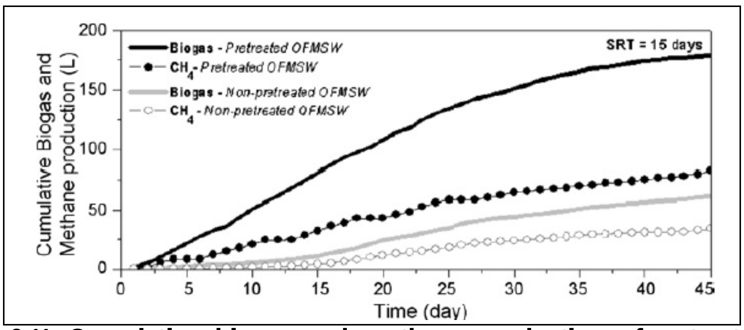


Figure 3.11 Cumulative biogas and methane production of untreated and pretreated industrial OFMSW (Fdez-Guelfo et al., 2011a)

Subsequent work proved that mature compost, in comparison to the fungus A. awamori and waste activated sludge, was the best hydrolytic agent (Fdez-Guelfo et al., 2011b), as highlighted in Figure 3.12.

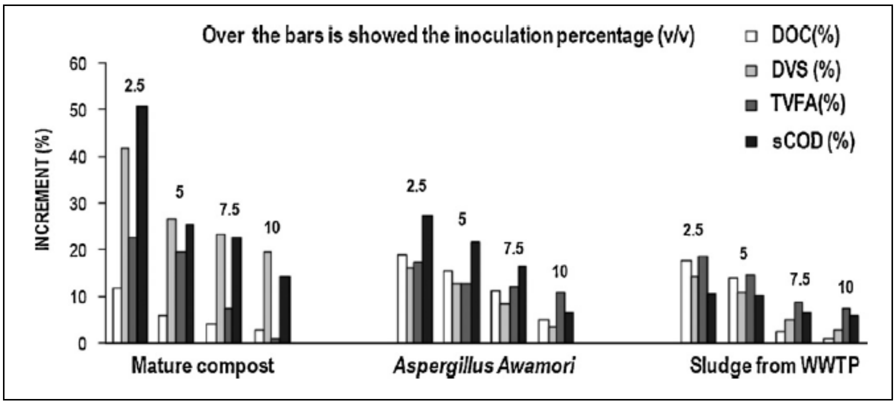


Figure 3.12 Comparison between different biological pretreatments (Fdez-Guelfo et al., 2011b)

White rot fungi with selectivity to lignin degradation over cellulose can be successfully applied in microbial pretreatments. Several studies (Keller et al., 2003; Kumar and Wyman, 2009; Shi et al., 2009) reports the high delignification efficiency of different rot fungi on various ligno-cellulosic biomass.

On the basis of his study, Keller et al. (2003) came to the conclusion that fungal pretreatment of ligno-cellulose can be regarded as an environment-friendly process, due to its several advantages, including no use of chemicals, reduced energy input, no requirement for pressurized and corrosion-resistant reactors, no waste stream generated and minimal inhibitors productions.

Most of these advantages can also be related to hydrolytic enzymes, which have to be appropriate selected if intended to increase significantly anaerobic digestion yields (Mallick et al., 2010).

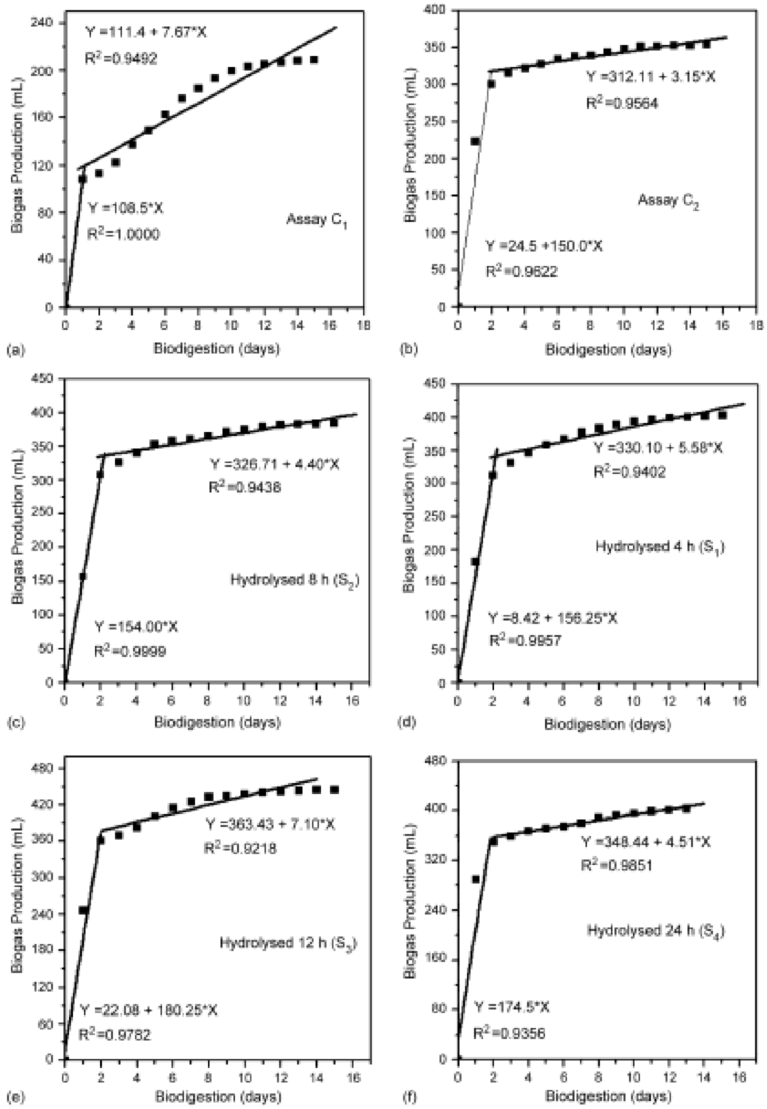
Several studies refer to enzyme use on both liquid and solid fat-dominated substrates.

Hydrolysis of oil and grease in dairy wastewater by a solid preparation of lipase was firstly proposed by Cammarota et al. (2001). The results of that work showed that hydrolysis supported by enzymes improved the treatment of dairy wastewater in a bench-scale UASB reactor. A similar work was carried out by Leal et al. (2002), using a liquid preparation of enzymes and batch operated anaerobic reactors. The highest level of oil and grease investigated by these authors was 1200 mg/l and the removal attained in reactors fed with the hydrolyzed stream was significantly higher than that reached in the control reactor without pre-treatment.

Dairy wastewater was also investigated in the study of Mendes et al. (2006).

Authors proved that the use a low-cost lipase preparation can increase the liquefaction of lipids, whose hydrolysis can be considered as a limiting step for the biogas generation and organic matter removal (COD) in the anaerobic digestion process. Enhanced lipid liquefaction resulted in increased bioavailability for anaerobic microorganisms, which was reflected in higher biogas production from untreated substrates.

However, as shown in Figure 3.13, biogas increase is not as noticeable as the one provided by other kind of pretreatments nor increasing incubation periods provided significant improvement in biogas production.



[(a)]rough wastewater; [(b)]wasteater supplemented with lipasese [(c)]8 h incubation ; [(d)] 4 h incubation ; [(e)] 16 h incubation ; [(f)] 24 h incubation ;

Figure 3.13 Biogas production for different enzymatic pretreatment conditions (Mendes et al., 2006)

Masse et al. (2003) and Valladão et al. (2007) studied the effects of enzymatic pretreatment respectively on fat degradation during slaughterhouse wastewater anaerobic digestion and poultry slaughterhouse effluent anaerobic treatment.
The former found a small overall effect on fact particle digestion at 25°C, marked by a decrease of about 5% in digestion time to achieve 80% reduction in long chain fatty acid concentration. The latter pointed out the enhancement of raw effluent anaerobic treatment efficiency when a 0.1% concentration of enzymatic pool was used in the pre-hydrolysis stage with 1200 mg/L oil and grease.
Using solid-state fermentation technology at elevated temperatures, Forbes et al. (2009) produced thermo-stable enzymes and applied them to carbohydrate-rich wastes prior to anaerobic digestion. Rapid reactor start up and subsequent successful mesophilic and thermophilic anaerobic treatment of the thermo-enzyme hydrolysates of wastes was achieved. The comparable performance at 55°C to that of the control reactor at 37°C strongly suggests that thermophilic treatment is a positive option, especially considering that enzymes were produced at high temperatures (60-80°C).
In general, such processes offer advantages such as low capital cost, low energy, no chemicals requirement, mild environmental conditions and no inhibitory compounds formation.
However, if complex substrates, such as OFMSW, are considered, the definition of the enzymatic pool is particularly difficult. Commercial products are available and often their composition is based on the specific characteristics of the substrate that is intended to be treated. The variability of these characteristics can result in highly changeable pretreatment performances.
However, the main drawback to develop biological methods can be recognized in the low hydrolysis rate obtained in most biological processes compared to other technologies (Wan and Li, 2010).

3.4 THE USE OF ADDITIVES

It has been already pointed out that pretreatments operate on substrate in order to promote the hydrolysis of complex organic matter to soluble compounds.

However, in order to ensure proper hydrolysis development, it is fundamental to keep a kinetic balance between each of the steps in the process. To this end, one of the most important factors is the production of intermediates and the monitoring of their formation, which can be achieved by means of adequate additives.
The addition of these substances to the substrate fed to the digester can be ascribed among pretreatment. Although additives do not affect directly substrate composition and its degradation mechanisms, their presence in the reactor contributes to keep the proper environmental conditions to correctly promote hydrolysis, mainly by means of the adsorption of inhibitory compounds generated during organic matter anaerobic degradation.
Scientific literature reports several studies dealing with the use of different additives. Among the most common, zeolites have been largely investigated (Milan et al., 2001; Yadvika et al., 2004; Montalvo et al., 2006).
Zeolites are micro-porous crystalline solids with well-defined structures. Generally they contain silicon, aluminium and oxygen in their chemical structure and cations, water and/or other molecules within their pores. Many zeolites occur naturally as minerals and are extensively mined throughout the world. Others are synthetic and prepared for specific commercial and scientific purposes. Because of their unique porous properties, zeolites are used in a variety of applications with a global market of several million tonnes per annum.
However, the aspect of interest with reference to anaerobic digestion processes is their capacity to adsorb ammonia (Chen et al., 2008).
One of the most interesting studies performed the comparative assessment of different kinds of inorganic additives, zeolites, clay, minerals and manganese oxides, in order to clarify the factors that improve the methane production during anaerobic digestion of NH_4^+-rich organic sludge (Tada et al., 2005).
Authors found that the increase in methane production (Figure 3.14), which occurred in all reactors with additives, was related to the reduction in free ammonia (Figure 3.15) provided by the additive.

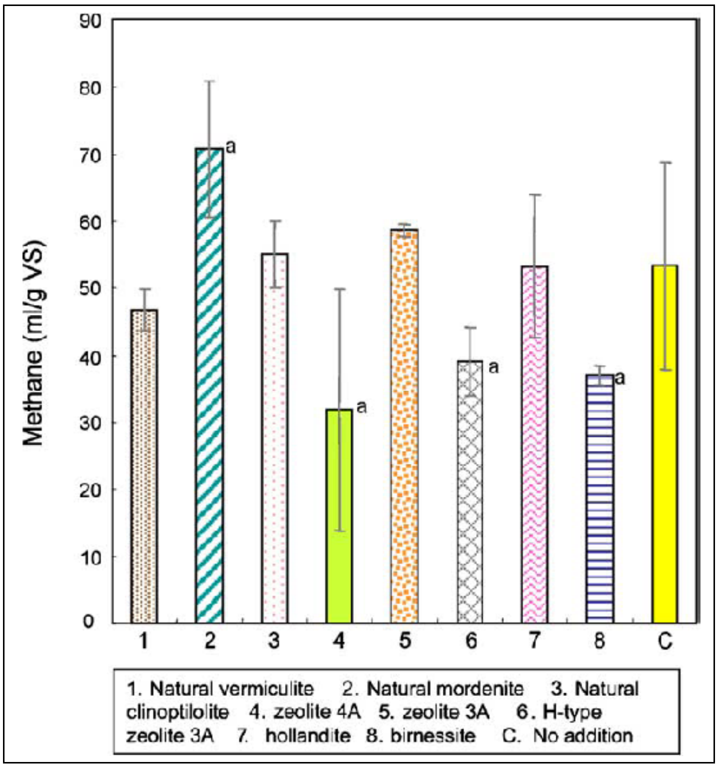


Figure 3.14 Methane increases after the addition of inorganic additives (Tada et al., 2005)

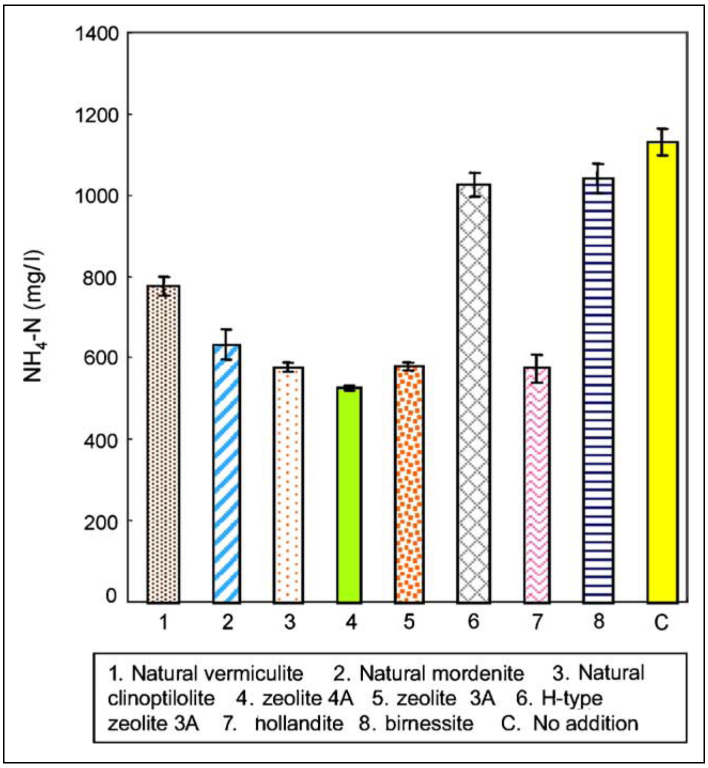


Figure 3.15 Ammonia reduction after the addition of inorganic additives (Tada et al., 2005)

The improvement of additives performances have been also studied, by introducing specific modification to the substances under investigation.
In the study of Nguyen-Thanh et al. (2005), sodium-rich montmorillonite was modified with iron in order to introduce active centres for hydrogen sulphide adsorption.
Three different configurations were investigated:

- in the first modification, interlayer sodium cations were exchanged with iron;
- in the second, iron cations were introduced to the clay surface;
- the last one, modification was based on doping of iron within the interlayer space of aluminium-pillared clay.

Iron-doped samples showed a significant improvement in the capacity for H_2S removal, despite of a relevant decrease in micro-porosity compared to the initial pillared clay.

According to the results of their experimentation, authors came to the conclusion that better results can be obtained by having iron cations evenly dispersed within the interlayer space, so that a high pore volume would be ensured where the products of surface reactions could be stored.
Additive utilization was studied also with reference to solid substrates.
Li et al. (2009) investigated the effects of porphyritic andesite on the hydrolysis and acidogenesis of solid organic wastes by batch and continuous experiments using a rotational drum fermentation system.
Results show that the proper dose of the studied additive can adsorb volatile acids, thus providing elevate local pH levels and accelerating VS degradation within anaerobic process.
According to the experience reported in literature, differently from the pretreatments, the addition of these elements is useful when particular substrates are fed to the digester or the presence of specific substance must be avoided.
Conversely, when the anaerobic process is expected to evolve properly, any significant increase in biogas production can be provided by additives.

3.5 DESIGN SOLUTIONS AND TECHNOLOGIES FOR PRETREATMENT

The interest in the study of pretreatments to enhance solubilisation and anaerobic biodegradability of solid substrates is mainly related to the possible implications of the experimental results in the field of the solid waste management systems.
Since OFMSW anaerobic digestion is a relatively recent treatment solution, most pretreatment technologies discussed in the previous paragraphs have been applied only at bench scale, as shown in Table 3.2.
Some of these technologies have been performed on a full scale on sewage sludge, often through the application of patented processes.

Table 3.2 Application scale of OFMSW pretreatments previously discussed

Pretreatment	Technology	Application to OFMSW	Reference
Chemical	Wet air oxidation	Lab-scale	Lissens et al., 2004
	Alkaline/acid treatment	Lab-scale	López Torres and Espinosa Llórens (2008
Physical	Shredding	Full-scale	Pavan et al., 2007
	High pressure systems	Full-scale	Gonella, 2011
	Thermal	Bench-scale	Kuo and Cheng, 2007
Biological	Enzyme preparations	Bench-scale*	Fdez-Güelfo et al., 2011a

* Enzyme pretreatment is applied on industrial scale anaerobic digesters treating industrial food waste

Among patents, the well-known Sonix™ is a US technology that uses ultrasound energy in fluids. In the standard reactor by Sonix™, stacks are mounted adjacent to one another, perpendicular to the direction of flow, within a 10 bar (g) rated stainless steel reactor. The fluid to be sonicated is pumped through the reactor flowing through the centre of the radial horns where it is exposed to the intense cavitation energy. Higher throughputs catered for by multiple units.

In Avounmouth Wessex water (UK) installed an ultrasound system for treating the domestic and industrial mixed sludge (i.e., population equivalent of 1,200,000). The TS and VS reduction of the untreated sludge in the digesters was 40% and 50%, respectively, and that of sonicated sludge was 60% and 70%, respectively. Similarly, Sonix™ system was installed in many plants in UK, US and Australia (Hogan et al., 2004).

Another US patented equipment is the one provided by Ultrawaves GmbH (Hamburg, Germany), a device normally fitted with five oscillating units, which can be supplied with up to 2 kW of power each. The transformation of electrical energy into mechanical-acoustic energy is performed by air-cooled piezo-ceramic transducers. In order to produce a high degree of efficiency by rupturing the organic matter suspended in the fluid treated, the reactor space in the Ultrawaves reactor has been optimized in order to obtain cavitation uniformly throughout the flow area.

Ultrawaves technology has been applied also for the improvement of full-scale farmland biogas plants.

As regards ozonation, full-scale trials were performed at a municipal wastewater treatment plant to evaluate the effectiveness of ozone treatment of the return activated sludge. Excess sludge generation data showed an overall 19-35% reduction in sludge production by exposing sludge to 0.05 kg_{O3}/kg_{TSS} (Sievers et al., 2004). Moreover, a patented full scale process was developed in Japan. The ozone is produced from air, requiring about 15 to 20 kWh/kg_{O3}. The process works by withdrawing sludge from the aeration tank or secondary clarifier and passing it through an ozone contactor before recirculating it to the aeration tank.

About 0.05 to 0.15 kg_{O3}/kg_{DS} is required depending on the type of chemicals used elsewhere in the treatment process.

However, the operational uptake of pretreatments techniques has been relatively slow despite over ten years of research, much of which has shown the potential large benefits related to biogas production and solids reduction from all the main technologies.

High capital costs and long pay back periods, great consumption of energy and/or chemicals, significant operating problems as well as scaling up issues have slowed or stopped commercial development.

The effectiveness of a pretreatment should not only be evaluated according to the improvement in anaerobic digestion yields, but also considering if the energy spent matches the one produced, in order to achieve economic benefits. As a consequence, energy balances play a fundamental role.

Several works suggest that the highest profits can be achieved by the less energy intensive methods, as found by Ma et al. (2011), but according to the specific operational conditions, the greatest financial yield can be extremely changeable.

Therefore, specific techno-economic analysis should be drawn up in order to prove the feasibility of a pretreatment implementation within an anaerobic digestion plant scheme.

Full-scale testing of most techniques has been limited and operational installations are largely restricted to few techniques (Zábranská et al 2006), often patented ones.

However, physical pretreatment, such as shredding and pulping, always occur in industrial OFMSW anaerobic digestion facilities.

European technologies all use extensive pre-processing units, regardless of the waste source or anaerobic technology adopted (California Integrated Management Board, 2008).
A typical sorting line includes the following components:

- receiving, which can include some visual (manual or robotic) sorting and removal of bulky or potentially harmful items, in order to prevent clogging of the pumps and to reduce the amount of reactor volume occupied by inert material;
- particle size reduction, that can be mechanical and/or biological, according to the ease of reducing the particle size of the organic fraction;
- separation, based on magnetism, density, and size.

Some receiving areas use robotics to minimize human contact with the waste; others incorporate a sorting line for workers to manually remove the most obvious inorganic materials. Once the OFMSW has been loaded into the mechanical separation system, human contact is minimal as biological and mechanical processes prepare the MSW for density and/or size separation.
Density separation requires waste wetting; therefore, it is more commonly applied when using low-solids digesters. Organic material breaks into smaller particles more easily than inorganic material, so a mechanical macerator or agitator is often employed prior to screening. In addition, some aerobic treatment can help breaking down the organic matter. This may also be accompanied by a loss of digestible organic matter, so that short retention times are used: average values ranging between several hours and one or two days is typical for rotating drums, or "biomixers," which combine agitation with aerobic treatment.
In the largest Dranco facilities, built in Brecht (Belgium), the source separated organic wastes are first sent to slowly rotating drums, with a retention time of 4 to 6 hours. The material is screened and the organics passing a sieve of 40 mm are sent to the digester after ferrous metals are removed by means of an overband magnet.
The pretreatment line in Bassano del Grappa (Italy) plant is quite similar and mainly consists of a bag breaker equipment, a radial sieve machine (80 and 50 mm) and a magnetic separator. In Camposampiero (Italy) plant, using wet digestion technology, a hydropulper is also used to provide the suspension for the digesters (Pavan et al., 2007).
As already mentioned, in the last years, several OFMSW anaerobic digestion plants, implementing wet technologies include high pressure

pretreatments. In Salerno (Italy), this kind of technology has been installed to treat source separated organic waste prior to anaerobic digestion.
The equipment presses the solid organic waste, which is converted into a suspension with approximately 15% of dry matter. Very little information can be found about the performances of these systems, because they are usually patented and since their application is quite recent, full-scale experience reports are not available.
Therefore, research can still provide an important contribution in the evaluation of the feasibility of pretreatment application, with reference to both technical and economic aspects.

4 SONOLYSIS AND OZONATION AS PRETREATMENT OF ORGANIC SOLID WASTE FOR ANAEROBIC DIGESTION

Sonolysis and ozonation, which have been traditionally used as wastewater advanced oxidation processes (Sangave et al., 2007), have been widely investigated with reference to liquid substrates, particularly sewage sludge.
In this contest, aim of the pretreatment was the reduction of excess sludge from wastewater treatment or the improvement of both aerobic and anaerobic digestion.
In this chapter, the basic assumptions of both processes are pointed out, in order to highlight the different action mechanisms, with particular reference to the reactions with organic matter.

4.1 PRINCIPLE OF ULTRASOUND PROCESSES

Sonolysis is a chemical-physical process that relies on the use of ultrasound (US).
Ultrasound is sound waves in a frequency range between 20 kHz and 10 MHz: the conventional power ultrasound, for industrial processing and sonochemistry, ranges between 20 kHz and 2 MHz (Mason and Peters, 2002).
Sound waves are transmitted through a medium by inducing vibrational motion of the molecules constituting the medium itself, so that the structure of molecules is, alternatively, subjected to compression and rarefaction waves.
The average distance between the molecules varies as the molecules oscillate about their mean position. In a liquid, if a sufficiently large negative pressure is applied, the distance between molecules can exceed the critical molecular distance to hold the liquid intact so that liquid molecules are pulled apart and tiny micro-bubbles (cavities) are created. These bubbles grow in successive cycles up to a critical size and then

violently collapse. This process, which is known as cavitation (Figure 4.1), brings both physical and chemical effects (Mason, 2002; Mohajerani et al. 2010), related to the occurrence of both a great energy release and the sudden creation of drastic localised conditions.
According to the hot-spot theory, temperature and pressure inside the collapsing bubble can raise, respectively, up to 5000 K and 3000 atm (Mason and Peters, 2002).

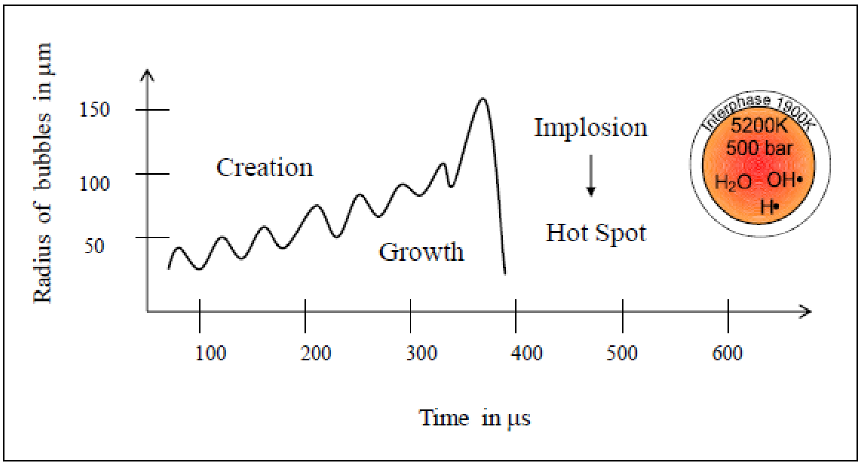


Figure 4.1 Acoustic cavitation

As the bubble collapse, the motion of the imploding bubble walls reaches the speed of sound. Subsequently, during the collapse, the trapped gas inside the bubble suddenly decelerates the wall motion, resulting in the release of a strong pressure wave.
Moreover, the sudden bubble collapse produces shear forces in the liquid medium, whereas the strongly oscillating cavitation bubbles as well as the collapsing transient bubbles promote radiation forces on the particles. The latter phenomenon is known as micro-streaming (Mason and Peters, 2002).
At the same time, the extreme conditions inside the bubble stimulate the generation of free reactive radicals that can act as oxidant agents.
If low-frequency US is applied physical effects such as increase in temperature and pressure gradients, turbulence and high shear forces are predominant (Mason et al., 2002), as highlighted in Figure 4.2.

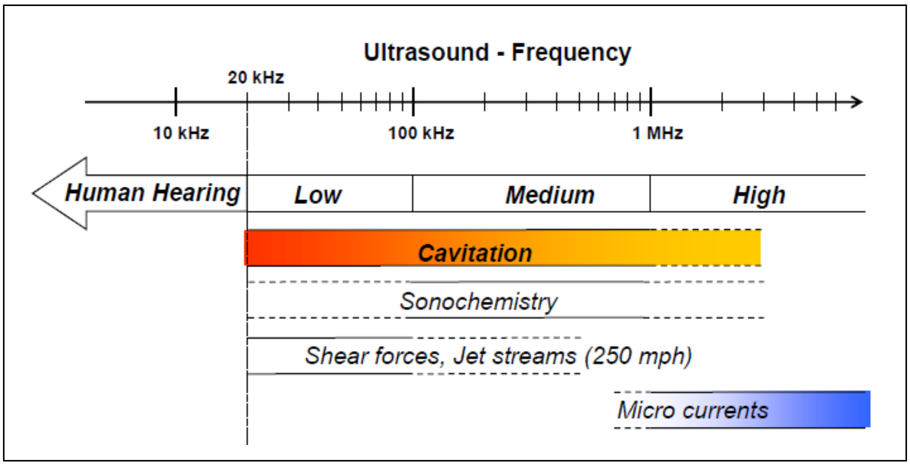


Figure 4.2 Ultrasound frequency fields and predominant effects

4.2 PRINCIPLE OF OZONE PROCESSES

Both solubilisation and increase of biological availability of organic matter can be achieved also by ozonation, a process relying on the use of ozone, which is a very strong oxidant.

At room temperature ozone exists as a colourless gas, with a pungent odour readily detectable at concentrations as low as 0.02 to 0.05 ppm (by volume), which is below concentrations of health concern. Additionally, ozone gas is highly corrosive and toxic as well as sparingly soluble in water: at 20°C, the solubility of 100% ozone is 570 mg/L (Kinman, 1975).

Research carried out on sludge ozonation proved that, mechanistically, ozone reacts with polysaccharides, proteins and lipids, which are components of cell membranes, transforming them into smaller molecular-weight compounds.

Therefore, the cellular membrane is ruptured and the cytoplasm of microbial cell is spilled. If the ozone dose is sufficiently high, mineralization of the released cellular compounds can also occur (Elliott and Mahmood, 2007).

Basic chemistry research (Hoigné and Bader, 1983a; Hoigné and Bader, 1983b) showed that ozone decomposes spontaneously in a liquid medium by a complex mechanism that involves the generation of hydroxyl free radicals. Although these radicals are among the most reactive oxidizing agents in water, their half-life of is on the order of microseconds.

In aqueous environment, ozone can react by either or both modes (Hoigné and Bader, 1977):

- direct oxidation of compounds by molecular ozone ($O_{3(aq)}$);
- oxidation of compounds by hydroxyl free radicals produced during the ozone decomposition.

The direct oxidation is slower than the hydroxyl free radical oxidation, but, under normal ozonation conditions, the concentration of aqueous ozone is higher than the one of hydroxyl radicals.

Hoigné and Bader (1977) found that under acidic conditions, the direct oxidation with molecular ozone is the prevailing action mechanisms, while the hydroxyl oxidation starts to dominate, when environmental conditions promote their production (i.e high pH, exposure to UV, or addition of hydrogen peroxide).

4.3 ULTRASOUND AND OZONE ENHANCEMENT OF BIOLOGICAL PROCESSES

The use of sonolysis and ozonation to enhance biological processing of organic matter has been extensively studied on liquid substrates and, in particular, on sewage sludge.

Results obtained by different authors (Thiem et al., 2001; El-Hadj et al., 2007; Naddeo et al., 2009a) show that sonolysis can significantly improve the COD solubilisation and the anaerobic biodegradability of sludge. Kim et al. (2003a, 2003b) found that methane production increased by 34% with ultrasonic pretreated sludge compared to untreated one. Similarly, Elbeshbishy et al. (2011a) applied US pretreatment to hog manure batch anaerobic digestion tests, finding out a 28% increase in methane production.

Depending on the treatment conditions, these effects can be related to organic matter physical disintegration as well as to the enhancement of microbial activity (Kwiatowska, 2011).

The application of US to sewage sludge is well known, so that its application developed at larger scale, resulting in three times more sludge being assimilated in the anaerobic digester, with a 20-30% increase in gas production (Hogan et al., 2004).

Therefore, most recent works deal with particular aspects of sonicated sludge digestion (Köksoy et al., 2010) and, to a small extent, to sonolysis application to solid substrates.

Chen L. et al. (2008) investigated ultrasound effects on hydrolysis and acidogenesis of a fermentation broth from solid organic wastes.

Authors found a volatile solid degradation 53% higher than the control (Figure 4.3), due to volatile acid desorption from substrate particle surfaces and direct particle disruption provided by ultrasound.

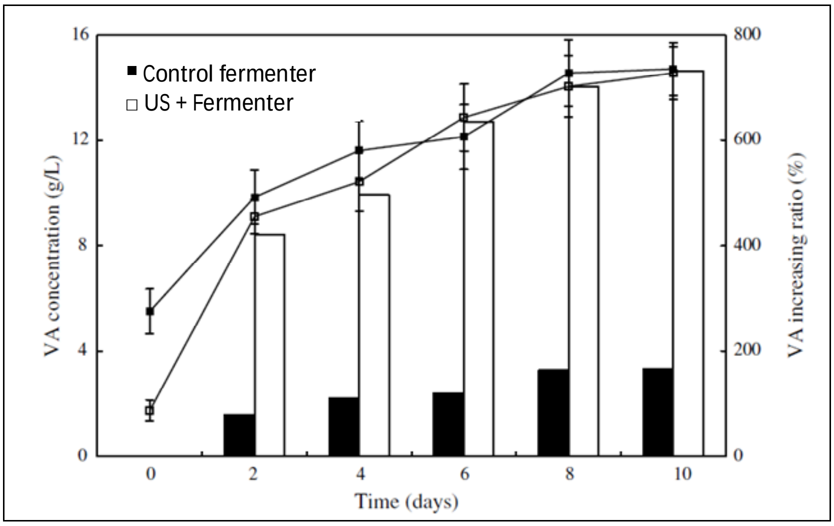
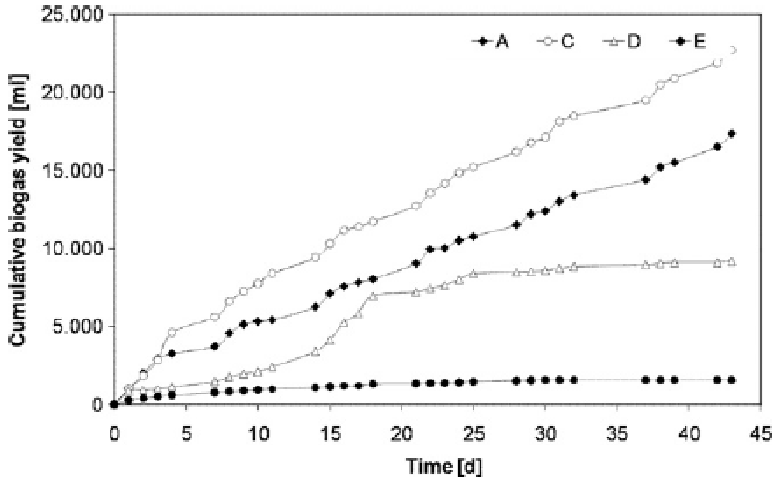


Figure 4.3 Volatile acid concentrations increasing ratios in the subsequent acidogenesis of the broth with and without sonolysis (Chen et al., 2008)

Elbeshbishy et al. (2011b) achieved a 27% increase in hydrogen production from a CSTR fed with sonicated OFMSW in comparison to the one with untreated substrate.

Similar results, in terms of process performance improvement, were also obtained treating with ultrasound a mixture of mechanically sorted organic fraction of municipal solid waste and sewage sludge, prior to anaerobic digestion: Cesaro et al. (2012) found a 24% increase in biogas production from sonicated mixture with respect to the untreated one (Figure 4.4).



A: untreated mixture; C: partially sonicated mixture; D: OFMSW; E: sewage sludge

Figure 4.4 Cumulative biogas production of investigated substrates in the study of Cesaro et al. (2012)

The state of art for ozone application is quite different and mainly focused on sludge reduction. However, the potential of ozonation in improving sludge anaerobic digestion performances have also been studied and, to some extent, applied at industrial scale.

Experimental results show that ozone dose of 3.4 g/L increased biogas production and anaerobic biodegradability by 44% and 60% respectively over the control (Delgenès et al., 2003).

Weemaes et al. (1999) found that ozone dose of approximately 1.5 g/L resulted in 29% solubilisation and 38% oxidation, thus bringing a 67% change in the volatile solid content of ozonated sludge.

The same pretreatment determined the enhancement of methane production (Figure 4.5) as well as the acceleration of digestion rate, suggesting the possibility of lowering digestion retention times.

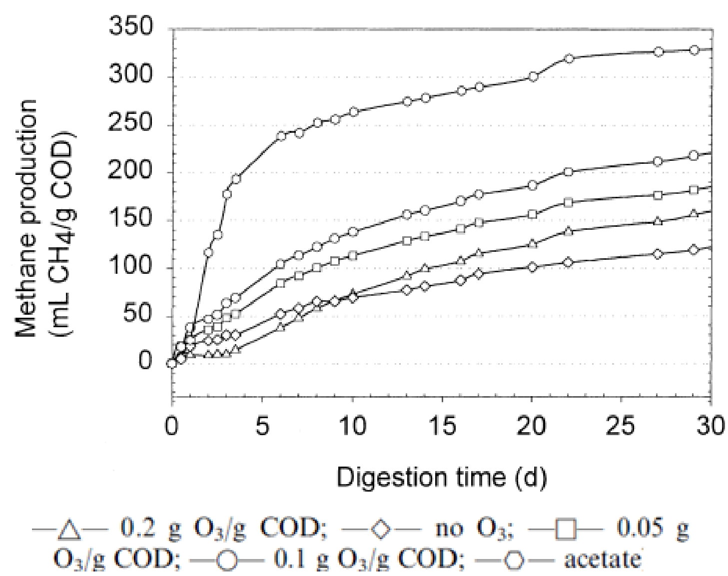


Figure 4.5 Methane yields of ozonated substrates (Weemaes et al., 1999)

It should be pointed out that, although in most cases comparable results can be achieved by applying the same pretreatment, several factors can influence the efficiency of the process in terms of solubilisation and biodegradability enhancement as well as the effectiveness of its combination with anaerobic process.
In the following paragraphs, not considering the obvious influence of the substrate, main factors affecting sonolysis and ozonation efficiency are discussed, in order to highlight the relation between process operating conditions and effects.

4.4 FACTORS AFFECTING SONOLYSIS

Sonolysis effects are related to the occurrence of cavitational phenomena, which are influenced by several parameters.
It is possible to classify these factors in two categories:

- acoustic factors, such as frequency and intensity;
- external factors, including environmental parameters and solvent characteristics.

Moreover, as the effectiveness of cavitation tightly depends on the propagation of sound waves in the medium, the design of the ultrasonic device plays a fundamental role. The optimal condition is the one promoting evenly distribution of cavitational bubbles in the whole medium (Ragaini et al., 2001).
In the studies dealing with sonolysis application as treatment prior to anaerobic digestion, the parameters that are considered as factors affecting process performance are frequency, intensity and treatment time.
Therefore, in the following paragraphs, an overview of the variation of sonolysis effectiveness as function of these parameters is provided.

4.4.1 The influence of frequency

Conventional ultrasound applications in the field of environmental engineering are carried out in a frequency range between 20 kHz and 100 kHz. However, it has been proved that the use of low and high frequencies can differently influence sonolysis effects, in terms of disintegration and solubilisation of organic matter.
The frequency directly affects the rarefaction phase: increasing ultrasound frequencies shorten the rarefaction phase, so that if the same cavitational effects have to be maintained, more power is needed at higher frequencies. Conversely, at lower frequencies, the rarefaction cycle lasts enough to pull molecules apart and create larger cavities in the liquid medium (Mason and Peters, 2002). Their subsequent collapse is thus more violent than the one obtained at high frequencies and, consequently, the disintegration effect is more remarkable.
It has been proved that the lower ultrasonic frequency (20 kHz) provides higher sludge disintegration efficiency (Thiem et al., 2001) in comparison to high frequency US, resulting in solubilisation and digestibility enhancement (Kim et al., 2003a; Naddeo et al., 2009b).
Neis et al (2001) investigated the effects of ultrasound with varying frequencies up to 3000 kHz on sludge disintegration and found that the degree of disintegration decreased for increasing frequencies, as shown in Figure 4.6.
The highest disintegration degree, expressed as COD, was found to be 40% and it was achieved for the lower frequency value (41 kHz).

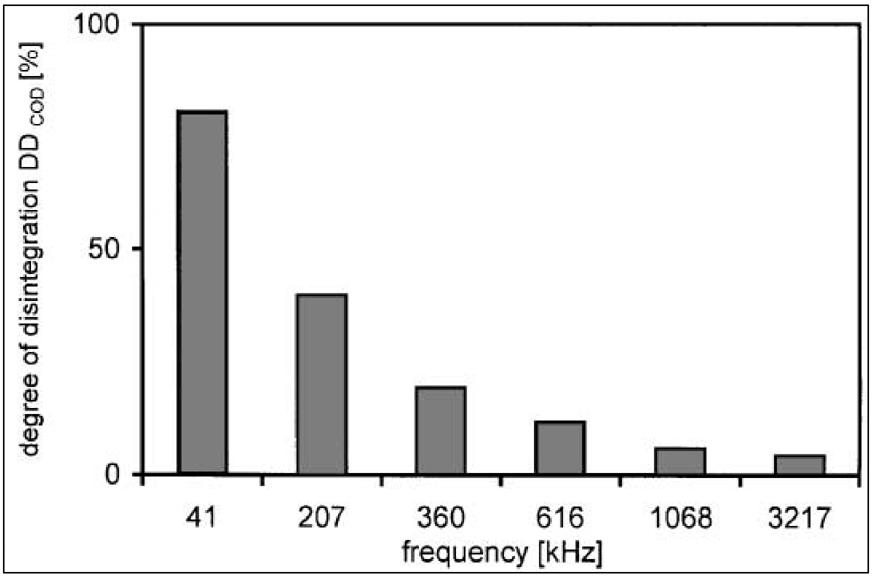


Figure 4.6 Effect of ultrasonic frequencies on sludge disintegration (Neis et al., 2001)

The lower is the frequency, the better are sonolysis effects in terms of organic matter disintegration. However, within the range of low frequency, any relevant difference can be observed for different specific frequency values, as highlighted by Zhang et al. (2006).
They studied the effects of three different low frequencies (30, 62 and 83 kHz) on sludge sedimentation and, although the lower investigated value gave the best result, any significant difference could be observed by applying the various frequencies (Figure 4.7).

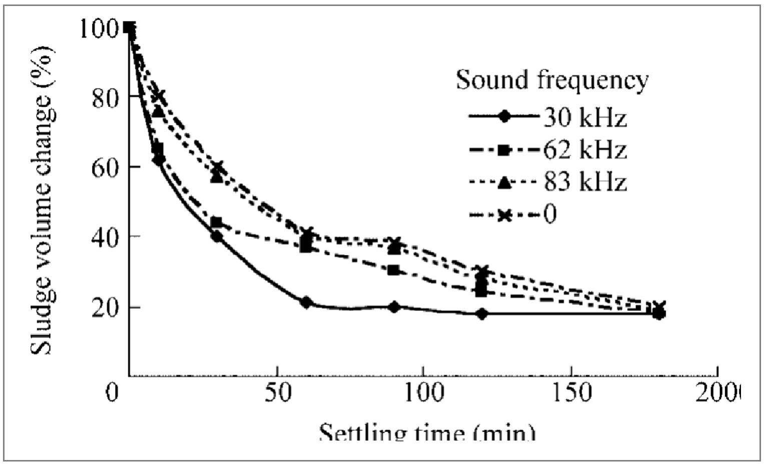


Figure 4.7 Effect of different low frequency values on sludge settling properties (Zhang et al., 2006)

Most studies dealing with organic matter disintegration have thus been successfully performed using low-frequency ultrasonic devices, in order to investigate the effects of different ultrasonic intensity values as well as the impact of treatment duration.
However, more recent studies consider the application of high frequency ultrasound as pretreatment of sludge destined to anaerobic processing.
At higher frequency bubble collapse is less violent since the rarefaction cycle is extremely short, so free radical action is prevailing on physical effects. Therefore, previous studies have reported the application of high frequency ultrasound mainly as oxidant destruction process for the treatment of hazardous contaminants in water and wastewaters (Abu-Dayeh Matouq and Al Anber, 2007; Thangavadivel et al., 2009).
Braguglia et al. (2012) used an ultrasound equipment operating at 200 kHz (average power 90 - 100 W) to assess sonolysis effectiveness on sludge floc disintegration and anaerobic biodegradability.
Authors came to the conclusion that 200 kHz can be considered a valuable alternative to the low frequency pretreatment of sludge, as it provides up to 40% increase in biogas production.
The same frequency value allowed the removal of organic contaminants from sludge. Gallipoli and Braguglia (2012) reported removal percentages of linear alkylbenzenesulphonates, an organic pollutant, in the range 17 to 42%. In their study, authors also compared the disintegration degree, in terms of COD, obtained at both low and high

frequencies, as function of the specific energy supplied. For energy values higher than 30 000 kJ/kg_{TS}, higher frequency ultrasound application provided greater disintegration degree than low frequency sonolysis (Figure 4.8).

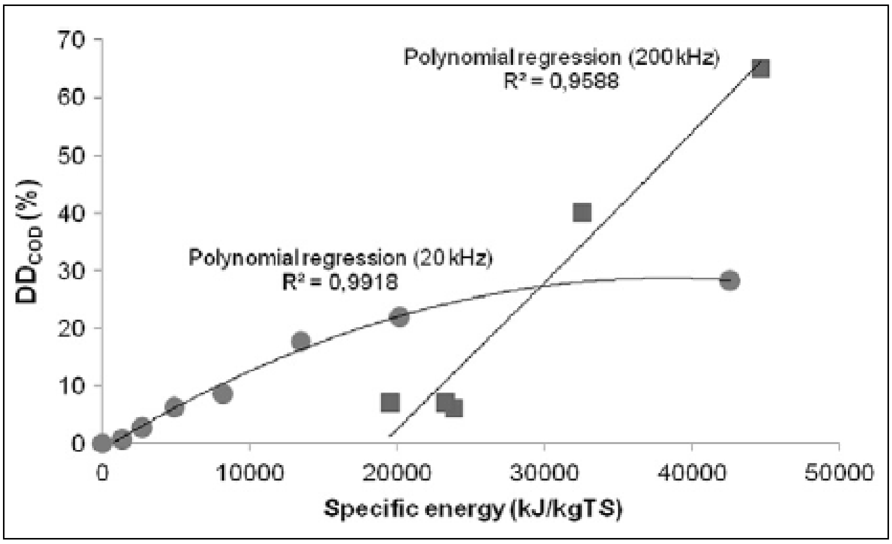


Figure 4.8 Effects of high and low frequency sonolysis for different specific energy values (Gallipoli and Braguglia, 2012)

If the treatment is intended to disintegrate organic matter, for low energy inputs, low frequency ultrasound is more advisable.
On the other hand, high frequency ultrasound allows the degradation, via oxidation, of organic pollutants, that can affect digestate quality and, as a consequence, reduce its possible application on soil.
It should be also pointed out that low frequency US were found to improve microbial activity more effectively than slightly higher frequency.
In the study of Zhang et al. (2008), a frequency of 25 kHz, power density of 0.2 W/mL for 30 seconds determined a 28% increase in sludge oxygen utilisation rate and a 12.5% of biomass growth rate. These positive effects were a result of ultrasound improving mixing, phase transfer and cell membrane permeability, providing an acceleration of growth of the microorganisms in the sludge.

According to Lin and Wu (2002), the increase in biological activity may be a consequence of self-defence mechanisms as a result of ultrasonic mechanical stress.

4.4.2 The influence of intensity

The acoustic intensity is related to the vibration of the ultrasonic source and, of course, to the input power.
It should overcome a threshold value in order to induce cavitation: at low frequencies, the intensity required is smaller (Mason and Peters, 2002).
Effects of ultrasonic intensity have been studied both on organic matter disintegration and enzyme activity.
It has been proved that low power ultrasonic treatment increases microbial activity. In particular, Huan et al. (2009) found that low density and long-duration sonication was more efficient than the high density and short-duration for the improvement of microbial activity as well as dewaterability.
Narbalatz et al. (2010) demonstrated that power intensity of 3.9 W/cm^2 was enough to extract the maximal amount of protease and lipase, finding that a further increase in the ultrasonic power did not determine any significant variation in the amount of enzymatic activity recovered (Figure 4.9).

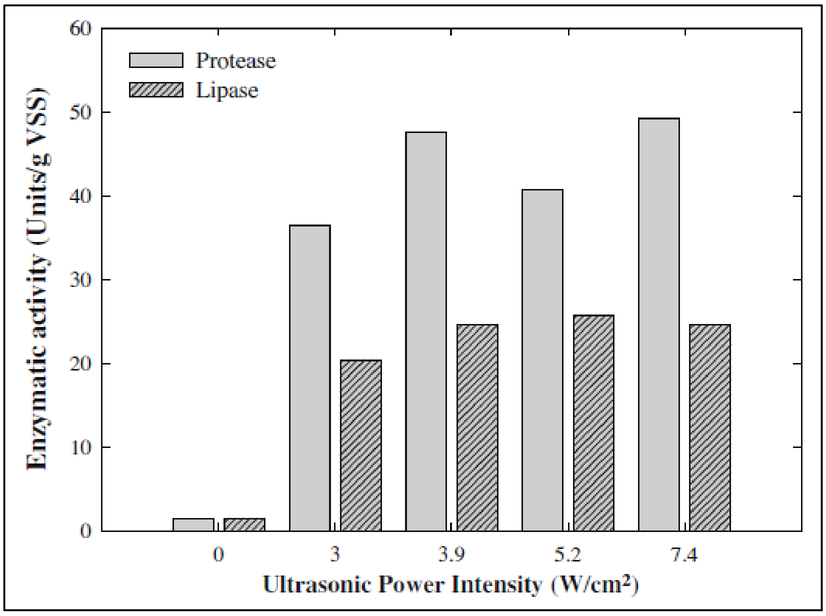


Figure 4.9 Effects of ultrasonic power intensity on enzymatic activity (Narbalatz et al., 2010)

Conversely, when high ultrasound power of 500 W was used, enzyme deactivation occurred (Rokhina et al., 2009), likely due to enzyme aggregation and subsequent obstruction of active sites as well as to the role of shear forces generated by ultrasound.

The effect of input power on sludge disintegration was evaluated by Chu et al. (2001) and it was observed that with an increase in power level from 0.11 W/mL to 0.33 W/mL at 120 min sonication, the total COD solubilised was 2% and 20% in the supernatant, respectively. At the highest investigated power level of 0.33 W/mL, the ratio BOD/TCOD increased up to 80% over the control.

When dealing with sludge disintegration, the influence of ultrasonic power is often expressed as the amount of energy consumed related to the sludge dry solid content, which affects disintegration efficiency.

Dewil et al. (2006) demonstrated that higher solubilisation was achieved for increasing dry solid (DS) content as well as for higher specific energy values (Figure 4.10). DS acts as nuclei, enhancing cavitation and, due to their high concentration, particles are more easily affected by cavitation itself.

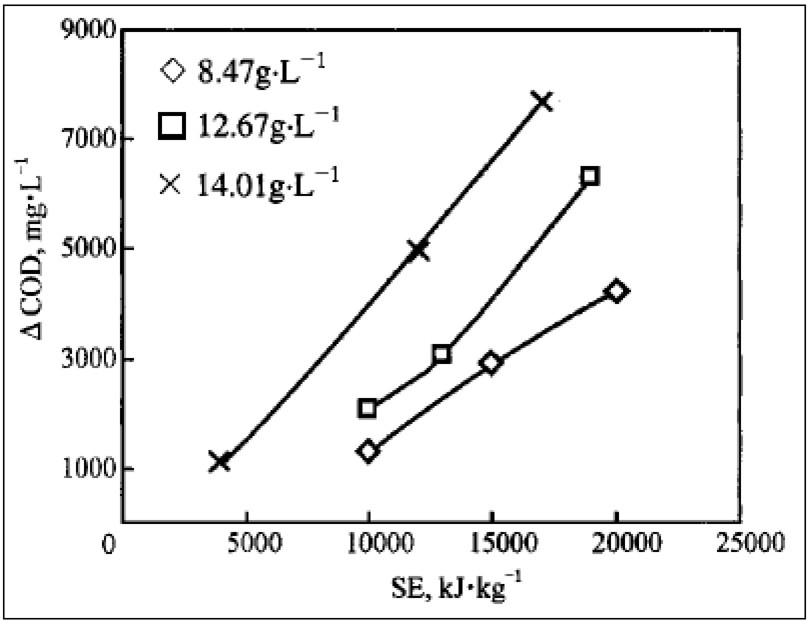


Figure 4.10 Effects of DS content on soluble COD increase as function of ultrasonic energy input (adapted from Dewil et al., 2006)

4.4.3 The influence of treatment duration

Among the operating conditions affecting sonolysis, duration treatment can also influence cavitation extent and, consequently, its performances.
The effect of sonication time in the range 7.5 to 150 minutes, was evaluated by Tiehm et al. (2001), who found that at the shortest sonication time, any variation in soluble COD of treated samples was achieved. Rising sonication time to 30, 60 and 150 minutes, disintegration degree was found to be 4.7, 13.1 and 23.7% were achieved respectively. This effect resulted in higher biogas recovery from sonicated substrates (Figure 4.11): at sonication time of 150 minutes, a 8.59% increase was obtained over the control.

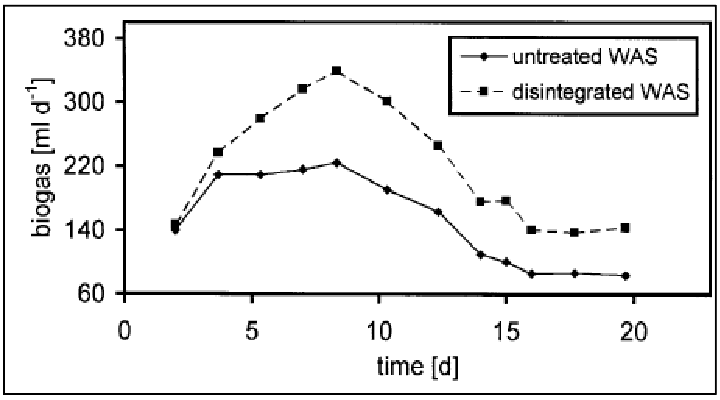


Figure 4.11 Enhanced biogas production from sonicated sludge (Tiehm et al., 2001)

The effect of sonication time on the sludge disintegration and the subsequent anaerobic digestion batch test was also evaluated by Wang et al. (1999).

Methane content in biogas increased by 12%, 31%, 64% and 69%, corresponding to sonication times of 10, 20, 30 and 40 minutes, respectively. Therefore, the optimum pretreatment time for enhancing anaerobic digestion yields of waste activated sludge should be approximately 30 min.

4.5 FACTORS AFFECTING OZONATION

Ozonation efficiency depends on the nature of compounds to be oxidized, on the ozone dose and on the pH reaction.

The use of ozone for sludge treatment before anaerobic digestion has been largely investigated (Delgenès et al., 2003) and the enhancement of ozonation effects has been studied as well (Chu et al., 2008).

As already highlighted, the pH mainly affects ozone action mechanism by favouring free radical attack rather than direct ozone oxidation.

Delgenès et al. (2003) reported that the fraction of destroyed insoluble organic carbon is higher at very acid pH, when ozone molecular form is prevailing on free radical one. Oxidation action of molecular ozone is more aggressive towards suspended than soluble solids. Conversely, in the basic pH range, ozonation products are more biodegradable and less

inhibitory than those formed under the same ozone dose and lower pH conditions.
In the following paragraphs, the influence of ozone dose as well as treatment time on the treatment of organic substrate to be biologically treated is discussed, pointing out main research outcomes in this field.

4.5.1 The influence of ozone dose

The effectiveness of ozonation as organic matter treatment prior to anaerobic digestion is mainly related to the enhanced biodegradability provided by ozone oxidation reactions.
Biodegradability improves with increasing ozone doses as well as with increasing extent of ozonation (Wang, 1990). Usually, large ozone doses are required to obtain biodegradable products, while low ozone doses may reduce the effect of toxic compounds on methanogenic cultures (Delgenès et al., 2003).
In scientific literature, ozone dose is usually expressed as the ratio between ozone mass transferred to the sample and the dry solid content of the sample.
Bougrier et al. (2006) investigated the anaerobic biodegradability of sludge treated by ozone doses of 0.1 and 0.16 g_{O3}/g_{TS}, finding an increase in biogas volumes of 1.08 and 1.25 times, respectively. The results were consistent with the ones achieved in terms of solubilisation, evaluated as disintegration degree, showing an enhancement in soluble COD for increasing ozone doses. However, in a previous work, a 2.58-fold gain in biogas volume from sludge treated by an ozone dose of 0.16 g_{O3}/g_{TS} had been obtained. This difference was explained through the occurrence of inhibitory conditions or formation of refractory compounds.
Similarly, Braguglia et al. (2012) found a 17% increase in biogas production from ozonated substrate (0.07 g_{O3}/g_{TS}) with respect to the untreated one, while lower ozone dose of 0.05 g_{O3}/g_{TS} resulted in the reduction of anaerobic biodegradability.
On the other hand, Zhang et al. (2009) proposed the dose of 0.05 g_{O3}/g_{TS} as the optimal one for sludge treatment prior to its biological processing. In this study, higher ozone doses proved to be responsible for a 45% reduction in volatile solid content, suggesting that severe ozone treatment can result in mineralization phenomena.
As in both studies, sludge was used as substrate, Braguglia and co-authors came to the conclusion that the reduction in biogas production

after 0.05 g_{O3}/g_{TS} supplied could be related to a not-well adapted inoculum.
The effect of ozone doses below 0.05 g_{O3}/g_{MLSS} on sludge was studied by Zhao et al. (2007): authors found both a reduction in particle size (Figure 4.12) and an enhancement in solubilisation (Figure 4.13) for ozone doses higher than 0.04 g_{O3}/g_{MLSS}.

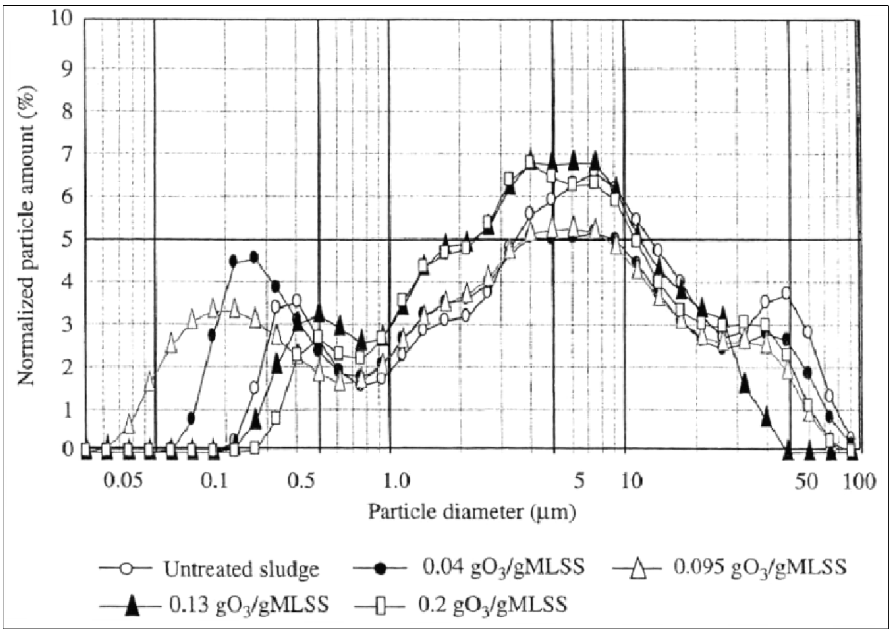


Figure 4.12 Effect of ozone doses on particle size distribution (Zhao et al., 2007)

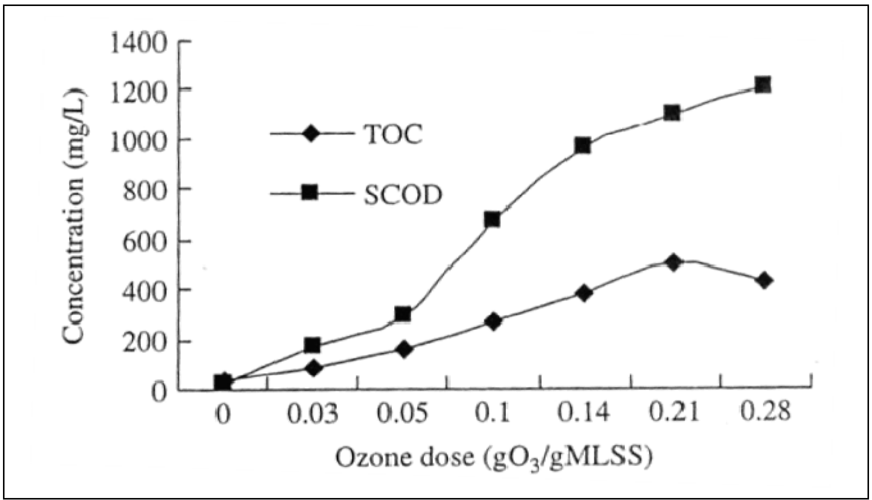


Figure 4.13 Soluble COD and TOC trend in the supernatant of ozonated sludge (Zhao et al., 2007)

Furthermore, results plotted in Figure 4.13 show that ozone dose increases above 0.2 g_{O3}/g_{MLSS} resulted in any significant solubilisation improvement. A similar trend was observed for soluble carbohydrate concentration, which decreased for ozone higher than 0.2 g_{O3}/g_{MLSS}, as reported in Figure 4.14.

This evidence suggested that extensive ozonation treatment determined the oxidation to CO_2 of a fraction of the organic carbon constituting carbohydrates.

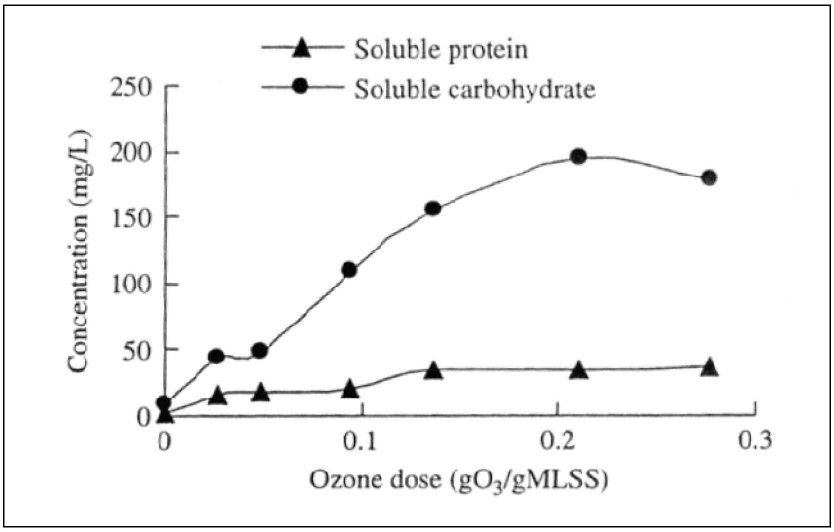
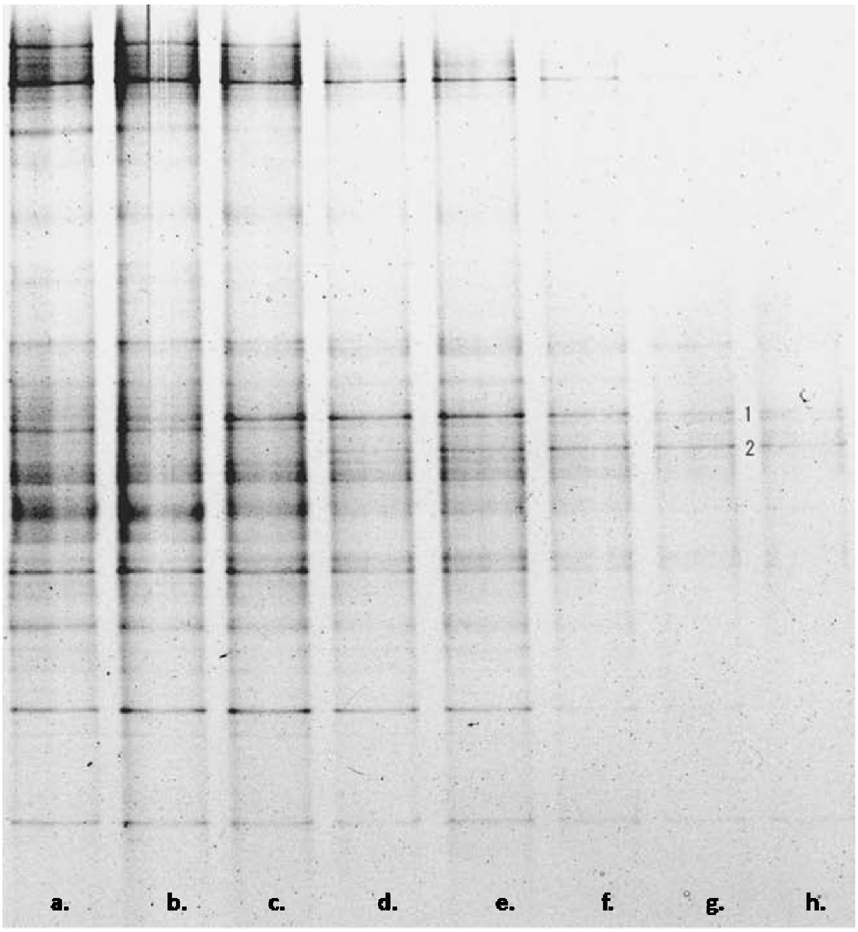


Figure 4.14 Soluble Carbohydrates and proteins trend in the supernatant of ozonated sludge (Zhao et al., 2007)

Ozone dose can also affect biomass activity.
Yan et al. (2009) analyzed by PCR-DGGE the alteration in bacterial DNA after sludge treatment at ozone doses ranging between 0 and 0.08 g_{O3}/g_{TS}. Increasing ozone doses led to the reduction of bands in DGGE fingerprint (Figure 4.15), indicating bacteria breakdown and their consequence DNA release: at the highest ozone value, DNA could not be amplified anymore by PCR.



a. 0 g_{O3}/g_{TSS}; **b.** 0.005 g_{O3}/g_{TSS}; **c.** 0.01 g_{O3}/g_{TSS}; **d.** 0.02 g_{O3}/g_{TSS}; **e.** 0.03 g_{O3}/g_{TSS}; **f.** 0.04 g_{O3}/g_{TSS}; **g.** 0.06 g_{O3}/g_{TSS}; **h.** 0.08 g_{O3}/g_{TSS}.

Figure 4.15 Microbial DNA changes in ozonated sludge (Yan et al., 2009)

More recently, the effectiveness of the process and the influence on ozone dose on anaerobic biodegradability have been investigated on liquid substrates different from sludge. Increase in digestibility after ozone treatment was found for palm oil mill effluent (Chaiprapat and Laklam, 2011) as well as for vinasse coming from ethanol production (Siles et al., 2011).

In particular, the latter work proved that the increase in anaerobic biodegradability of vinasse was not related to solubility enhancement, but

to the reduction in phenol concentration, which increased for reaching a mean value of 39% after 15 minutes pretreatment.
Further increase in treatment time up to 60 minutes determined the enhancement of both phenol removal and biodegradability, evaluated as BOD_5/COD ratio, up to 65% (Figure 4.16).

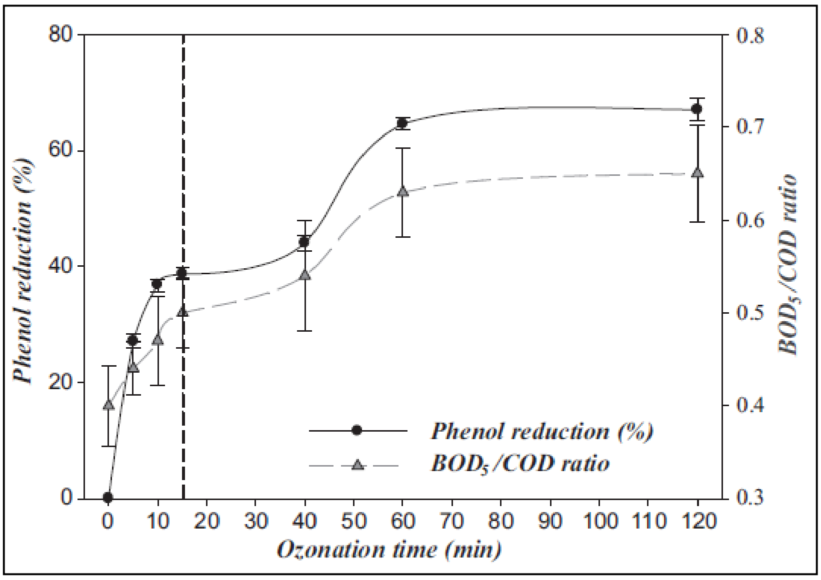


Figure 4.16 Phenol reduction as function of ozone dose (Siles et al., 2011)

It is evident that, while the kind of substrate influences the specific action mechanism, increasing ozone doses result in overall enhanced anaerobic biodegradability.
However, since ozone action is not selective, drastic oxidation process can determine the occurrence of mineralization phenomena, which are not desirable as they involve an organic matter degradation pathway alternative to its conversion to biogas.

5 RESEARCH EXPERIMENTAL ACTIVITY

The enhancement of biogas production from organic solid waste is particularly interesting, from both a scientific and technical point of view.

The possibility to increase the amount of methane recovered from anaerobic processing of solid waste is consistent with legislative directives in the field of solid waste management and renewable energy production.

In order to improve anaerobic digestion yields and, as a consequence, enhance methane production and recovery, proper pretreatments can be applied. Scientific literature analysis highlights that several technologies can be applied and their combination is also reported (Jian et al., 2008; Xu et al., 2010).

Pretreatment objective is to obtain an extension or an acceleration of the anaerobic process, in order to achieve an increase in biogas production as well as in volatile solid reduction or the decreasing of digestion time. Therefore, especially when considering solid substrates, such as the organic fraction of municipal solid waste, pretreatments mainly aim to promote the accessibility of hydrolytic microorganisms to the solid matter and hydrolysis of complex polymeric components (Delgenès et al., 2003).

Ozonation and sonolysis have been widely investigated as sludge pretreatment for anaerobic digestion. In scientific literature, any study deals with solid waste ozonation, whereas first attempts in the field of solid waste ultrasonic treatment have been documented only during the last year.

Therefore, both ozone and sonolysis application represent innovative pretreatment options for solid organic substrates to be treated by means of anaerobic digestion.

In this study, they were considered as OFMSW treatment options in order to compare their effectiveness on anaerobic digestion yields as well as to evaluate the technical and economic feasibility of the combined process providing the best performances.

Moreover, following recent research developments (Pilli et al., 2011), the relation between sonication pretreatment effects and organic matter composition was assessed, in order to in-depth analyse the factors affecting the variability of methane production enhancement after ultrasonic treatment.

5.1 INVESTIGATION PLAN

The overall aim of this research is the optimization of OFMSW anaerobic digestion by the application of advanced technologies, such as ozonation and sonolysis, useful to overcome obstacles related to hydrolysis of complex organic matter, thus achieving the increase of biogas production and the possibility of higher energy recovery as well.
To this end, experimental activity was structured in two main steps:

- the first one was focused on the comparative assessment of ozonation and sonolysis effectiveness in promoting solubilisation and anaerobic biodegradability enhancement of OFMSW;
- the second part, performed on the basis of the results of the previous phase, was focused on sonolysis effects. In particular, experimental activity was addressed towards the definition of main parameters, in order to assess the technical and economic feasibility of the full scale US pretreatment.

Therefore, the research first step pursued the following objectives:

- the definition of both ozonation and sonolysis optimal operating conditions;
- the evaluation of ozonation and sonolysis in improving solubilisation and anaerobic biodegradability of OFMSW samples.

In order to state the optimal operating conditions for ozonation, ozone doses ranging between 0 and 1.6 g_{O3}/g_{TS} were applied to OFMSW samples.
Following the hypothesis that increasing ozone doses provided higher solubilisation until the occurrence of mineralization phenomena, the threshold ozone dose was determined according to ozone demand as well as the variation in volatile solid content of ozonated samples.

For the identified optimal ozone doses, the variation in soluble COD was assessed, in order to verify the relation between increasing ozone doses and the correspondent solubilisation variation.
On the other hand, the optimal ultrasonic specific energy input was assessed as the one providing the highest solubilisation as well as the lowest reduction in volatile solid content.
Sonolysis tests were performed under different operating conditions, as described in details in the following chapter. Specific energy values ranging between 15000 and 40000 kJ/kg_{TS} were studied by setting the instrument amplitude to 40%. Further trials were performed by setting specific energy inputs to 15000 and 40000 kJ/kg_{TS} and varying amplitude in the range 20 - 60%.
According to the results of both ozonation and sonolysis solubilisation tests, anaerobic biodegradability trials were performed in order to quantify biogas production from differently pretreated OFMSW samples, thus assessing a comparison between ozonation and sonolysis effectiveness as anaerobic digestion pretreatment.
Moreover, the evaluation of the biodegradability of the soluble fraction of pretreated OFMSW samples was also estimated through the ratio BOD_5/COD, as proposed by Cossu and Raga (2008). This analysis was useful to achieve qualitative information about the composition of the soluble compounds generated after both ultrasound and ozone application.
The comparison of results, as reported in Chapter 7, addressed the research towards the in-depth analysis of sonolysis mechanisms on organic solid matter destined to anaerobic digestion.
The second part of the research was, therefore, focused on sonolysis in order to assess the technical and economic feasibility of an ultrasonic installation as pretreatment step within OFMSW anaerobic digestion facilities.
To this end, the basic unit of the ultrasonic device commercialized by Ultrawaves GmbH was used and, although the comparative assessment of ozonation and sonolysis had been carried out on rough OFMSW, this second part of the research was performed using OFMSW digestate. As discussed in details in Chapter 7, the need to shift from the rough to the anaerobically digested OFMSW was necessary mainly to reduce energy consumption.
The experimental activity, which was carried out in the second part of the research, pursued the following objectives:

- the evaluation of main chemical and physical effects promoted by the basic unit of a commercialized ultrasound device, operating under different conditions;
- the assessment of the anaerobic biodegradability enhancement of sonicated samples under the investigated operating conditions;
- the identification of the proper parameters to assess the economic feasibility of an ultrasonic installation within OFMSW anaerobic digestion facilities.

Further analytical determination included:

- scanning microscopy, which was performed in order to visualize the treatment disrupting effect;
- microbiological trials, in order to verify ultrasonic disintegration mechanisms toward the natural microbial population of treated samples.

Following recent scientific literature developments, the definition of the relation between sonolysis effects and substrate composition was stated as an additional objective of the second part of this research, necessary to better define ultrasound action mechanisms.

As OFMSW digestate is recognized as a mainly ligno-cellulosic material, additional tests were performed using a protein-rich substrate, as discussed in the following paragraphs.

6 MATERIALS AND METHODS

This chapter describes both experimental set up and analytical procedures adopted to carry out the research.
To this end, it is divided in two main sections:

- the first one is focused on the materials and methods used for the comparative evaluation of sonolysis and ozonation effectiveness as pretreatment of OFMSW to be anaerobically digested;
- the second section describes materials, devices and methods that allowed the in-depth study of sonolysis mechanisms.

6.1 OZONATION AND SONOLYSIS

In order to compare ozonation and sonolysis effectiveness as OFMSW treatment prior to anaerobic digestion, the variation in both solubilisation and anaerobic biodegradability was evaluated.
In particular, experimental activity first focused on the definition of the optimal pretreatment characteristics, according to the variation in organic matter solubilisation, which was evaluated in terms of soluble Chemical Oxygen Demand (sCOD).
Subsequently, anaerobic digestion tests were performed at laboratory scale in order to evaluate biogas production from sonicated and ozonated substrates in comparison to untreated ones.
Biodegradability tests were also performed, through the evaluation of the ratio BOD_5/COD (Cossu and Raga, 2008).
All tests were performed in triplicate and set of tests were repeated at least twice, so that average values could be considered for discussion.

6.1.1 Substrate

The first part of the experimental work was performed using organic fraction of municipal solid waste coming from separate collection (SS-OFMSW).

For each experimental run, approximately 2 kg of OFMSW were ground by a bench scale shredder model M20 - Universal IKA to reduce particle size in order to improve matter solubilisation (Izumi et al., 2010) and stored at 4°C until use. Storage period was not longer than one day.

Before treatment application, the substrate was diluted with distilled water, in order to facilitate solubilisation. The dilution ratio was identified by iterating from 1:1 down to 1:3 (w/w). The latter was chosen for the experimental work, thus allowing the treatment of a substrate characterized by a total solid content lower than $10\%_{FM}$.

With particular reference to sonolysis application, although the presence of solids amplifies cavitational phenomena effects (Mason and Peters, 2002; Dewil 2006), total solid concentrations higher than $10\%_{FM}$ determined operational problems to the ultrasonic device. Similarly, high solid concentrations reduced ozone transfer into the sample.

The substrate was characterized with reference to the main chemical and physical parameters, as shown in Table 6.1.

pH was measured by pHmeter model HI 99121 (Hanna Instruments). Both total solid (TS) and volatile solid (VS) contents were evaluated according to the procedures of DIVAPRA (1998); soluble COD was determined according to Standard Methods (AWWA-APHA-WEF, 1998), whose test was carried out on each sample after centrifugation (14000 rpm, 15 minutes) and filtration (<0.45 µm).

The elemental characterization of SS-OFMSW was performed by the Elemental Analyzer OEA Flash 2000 (Thermo Finnigan), which also allowed protein content determination.

Carbohydrates were determined according to the Wendee Method (Kasprzak and Rzedzicki, 2008); lipid content was estimated through the Sozhlet Method (ISS, 1996). The procedure involves lipid extraction with petroleum ether, which is removed before the drying and weighing the residue.

The characterization of OFMSW in terms of carbohydrates was performed in order to evaluate the structural carbohydrates, including all the fibrous components, cellulose, hemicellulose and lignin, which constitute the fruit peel.

Results are consistent with the ones obtained in other works (Frunzo, 2011) and reflect the composition of waste, which was mainly composed of fruit, vegetables and bread; lower amount of pasta and meat-based wastes were found.

Table 6.1 Characterization of OFMSW samples used in the investigation

Parameter	Value
pH [-]	6.5 ± 0.1
ST [%]	26.3 ± 7.7
SV [%ST]	88.7 ± 4.9
CODs [mg/g]	35 ± 10
C [% $_{SS}$]	48.1 ± 5.5
H [% $_{SS}$]	6.9 ± 0.9
N [% $_{SS}$]	2.7 ± 0.5
Carbohydrate content [% $_{SS}$]	26.9 ± 5.2
Protein content [% $_{SS}$]	17.1 ± 1.3
Lipid [% $_{SS}$]	27.9 ± 3.2

6.1.2 Inoculum

The assessment of anaerobic biodegradability of pretreated substrates was performed through batch anaerobic digestion tests. To this end, digested sludge was used as inoculum.
It was sampled at the municipal wastewater treatment plant in Salerno and stored at the test temperature (35°C) until use.
The storage period lasted 5 days and it allowed the development of anaerobic processes in order to reduce sufficiently sludge own gas production by means of a hunger phase. Inoculum characterization is reported in Table 6.2.

Table 6.2 Characterization of digested sludge used as anaerobic test inoculum

Parameter	Value
pH [-]	7.1 ± 0.1
ST [%]	2.4 ± 0.5
SV [%ST]	49.4 ± 4.4
CODs [mg/L]	70 ± 15
C [% $_{SS}$]	17.6 ± 2.9
H [% $_{SS}$]	2.6 ± 0.4
N [% $_{SS}$]	2.2 ± 0.3

6.1.3 Ozonation experimental set up

In this experimental activity, ozone was provided by a UV generator system (model Ozone - Procom srl). The equipment produced ozone from the ultraviolet application on the air flow provided by a compressor (Figure 6.1).
Air stream containing ozone was introduced into a glass reactor through a Teflon tube. Exhaust gas stream was forced to flow from the top of the reactor into a Drechsel trap, filled with 200 mL of 2% KI solution, in order to capture residual ozone and determine the ozone demand.
Although the energy consumption per ozone volume produced is higher than oxygen fed devices, air fed system is a commonly used and reliable technology, suitable for both small and large systems.

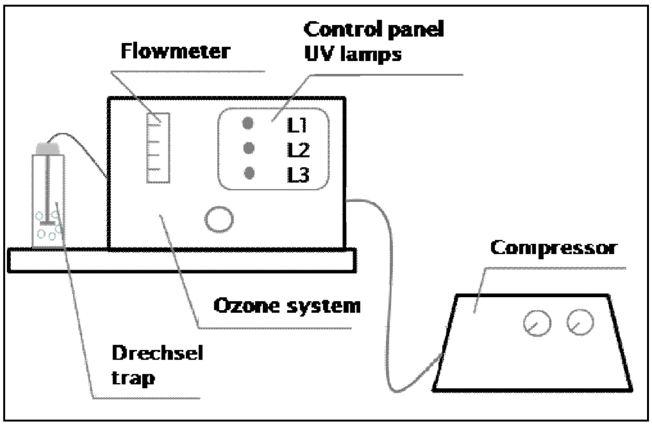


Figure 6.1 Scheme of the ozone generator

Two kinds of contact reactors were used. The first one consisted of a Drechsel trap, as suggested in the study of Chaiprapat et al. (2011): in this case, both the inlet and outlet of ozone stream were on the top of the reactor itself.
In the second configuration, a bubble column was used: in this case, ozone was introduced from the bottom of the reactor and forced to flow out from the top of the reactor itself.
Figure 6.2 shows the bubble column connected to the Drechsel trap, which was filled with KI (2%) for the determination of residual ozone.

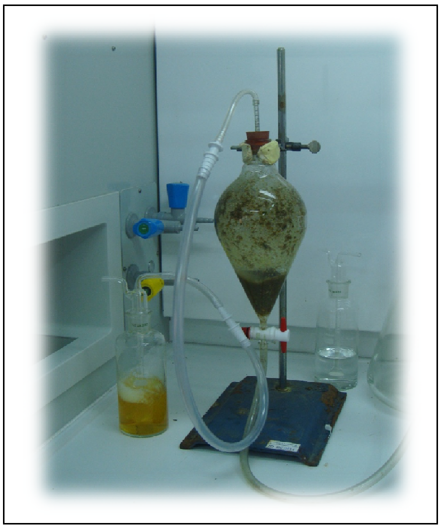


Figure 6.2 Bubble column reactor connected to the Drechsel trap

6.1.3.1 Definition of ozone concentration in the air flow

In the system used to generate ozone, the reagent concentration in the gaseous stream can vary according to:

- the air flow provided by the compressor;
- the number of UV lamps providing their action on the air flow.

The device used is equipped with a fluximeter for the measurement of the air flow and with three UV lamps, working independently to generate ozone in the flow air provided by the compressor.

In order to define ozonation operating conditions, preliminary experiments were performed using air flows ranging between 1 and 10 L/min and a different number of UV lamps.
Increasing both the air flow and the number of working UV lamps, the mass ozone produced in 10 minutes improved, as shown in Table 6.3.
Moreover, as plotted in Figure 6.3, a direct correlation was found between ozone produced and air flow, for fixed number of working lamps as well as between ozone mass and number of UV lamps working on a fixed air flow.
Standard deviation values highlights very slight variations for each investigated conditions, with the exception of the ones involving an air flow of 5 L/min. This result was expected since, during the test period, air flow value was highly fluctuating.

Table 6.3 Ozone produced under different operating conditions (ozonation time: 10 minutes)

Air flow [L/min]	Working UV lamps [-]	Ozone mass [g_{O3}/min]
1	1	0.28 ± 0.06
3	1	0.86 ± 0.07
3	2	1.59 ± 0.01
3	3	2.07 ± 0.05
5	1	1.30 ± 0.20
5	2	2.01 ± 0.46
5	3	3.00 ± 0.66
10	1	2.15 ± 0.05
10	2	3.98 ± 0.06
10	3	5.35 ± 0.17

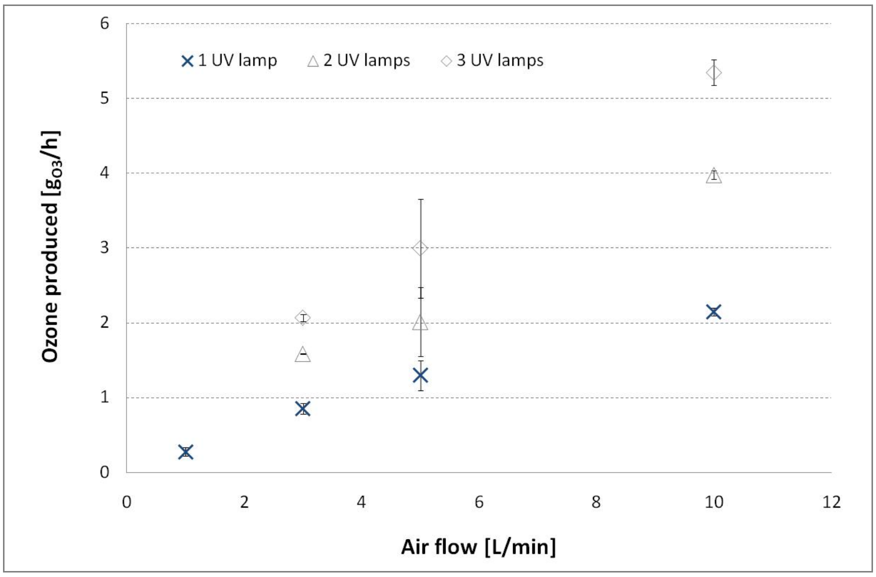


Figure 6.3 Ozone generated for increasing air flow and working UV lamps

In order to shorten treatment duration, the air flow was set on 10 L/min and the maximum number of UV lamps was used. Treatment time was, thus, varied so that ozone doses ranging between 0 and 1.6 g_{O3}/g_{TS} were investigated.

6.1.4 Sonolysis experimental set up

Sonolysis application relied on a low-frequency (20 kHz) bench sonicator, model VCX 750 (Sonics, USA), ranging from 0 to 750 Watt. The device consists of a generator, a transducer and an ultrasonic probe ending in a replaceable 13 mm titanium tip, as shown in the scheme of Figure 6.4.

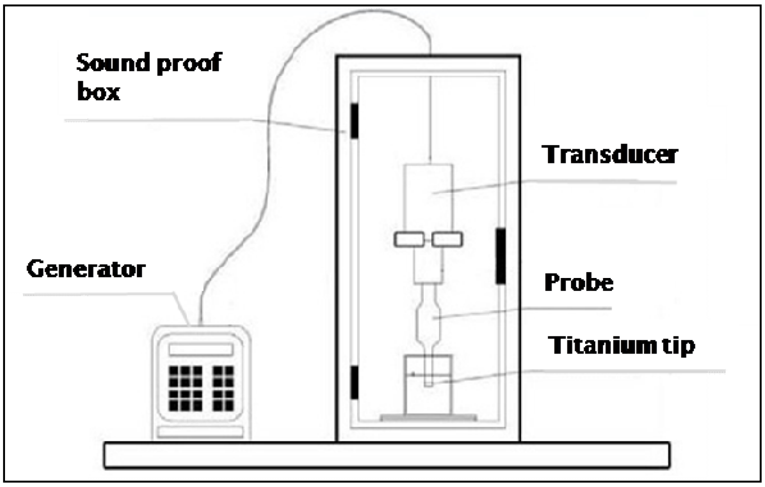


Figure 6.4 Scheme of the bench sonicator, model VCX 750 (Sonics, USA)

The generator is the source of alternating frequency (20 kHz) that supplies the transducer, an oscillatory system able to transform electrical energy into sound waves. In particular, the low vibratory motion of the transducer is amplified by the upper and fixed part of the horn, which is connected to the detachable horn, transferring the ultrasonic energy into the sample.
To ensure reproducibility, the ultrasonic processor is designed to deliver constant amplitude, which is the excursion of the tip. As the resistance to the movement of the probe increases, additional power will be delivered by the power supply to ensure that the excursion at the probe tip remains as selected. The device is equipped with an amplitude control system, which allows the excursion of the tip to be set to any desired level, up to 100%. Therefore, if the process at 100% amplitude has the probe moving for the maximum designed distance, at 50% the probe excursion is halved.
Both the vibration amplitude and the electrical power to the transducer are visualized, during a run, by digital display on the front panel of the generator assembly.
Therefore, the power transferred to the sample can be calculated as the difference between the electrical power supplied to the transducer when the probe is placed into the sample and the one displayed when the probe is not immersed into the sample. However, as this difference is not significant (Naddeo, 2006), it was assumed that the amount of

energy, expressed in Joules, which was being delivered to the probe, was the one transferred to the sample.
The mixture to be treated was put in a cylindrical Pyrex beaker with the ultrasonic probe placed in the centre and immersed up to 2 cm. The mixture was continuously mixed by a magnetic stirrer in order to avoid solid settling at the bottom of the beaker.
Temperature control in the sample was provided by ice bath.
As energy input can be highly variable according to the nature of studied organic matter, most studies dealing with sonolysis (Bougrier et al., 2006; Braguglia et al., 2012) are focused on the effect of increasing specific energy inputs, expressed as the ratio between the energy released to the sample, which was visualized on the equipment display and the dry matter content of the sample itself, according to the following expression:

$$E_S = \frac{P \cdot t}{V \cdot TS}$$

where:

- E_S is the specific energy provided;
- P is the ultrasonic intensity as power;
- t is the treatment time;
- V is the volume of the sample;
- TS is total solid content of the sample.

According to previous works, specific energy values ranging between 15000 and 40000 kJ/kg_{TS} were studied.
To this end, amplitude was set to 40% and different energy inputs were provided by varying treatment time.
Additional tests were carried out applying specific energy inputs of 15000 and 40000 kJ/kg_{TS} and varying amplitude in the range 20 - 60%.

6.1.5 Anaerobic biodegradability test experimental set up

Anaerobic digestion tests were carried out in 2 L glass reactors, immersed into a water bath in order to ensure mesophilic conditions (35-40°C).

After the feedstock addition, pH was measured; when it was lower than 6, approximately 20 mL of NaOH solution (2 N) was added until the pH reached values ranging between 7 and 8.
Headspaces of the reactors were flushed with nitrogen gas for about 2 min and then the bottles were sealed with rubber septa and gas collection system connected.
Figure 6.5 shows the scheme of the system used to assess anaerobic biodegradability of investigated substrates according to the adopted analytical procedure.

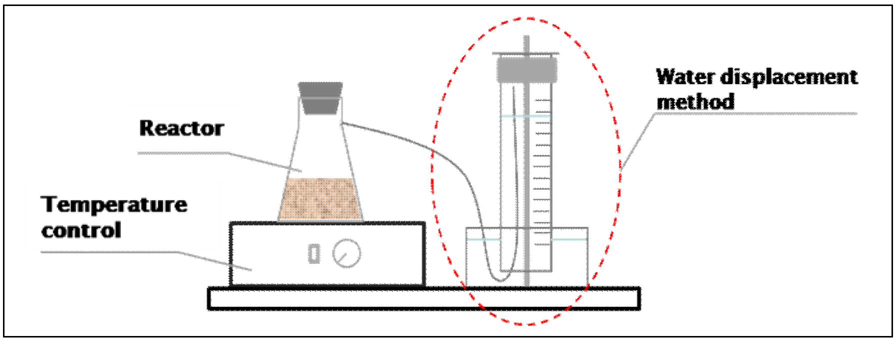


Figure 6.5 Scheme of anaerobic digestion test system

6.1.6 Analytical set up

In order to perform the correct monitoring of ozonation treatment, the determination of ozone dose supplied to the sample as well as the one involved in oxidative reactions was fundamental.
To this end, the analytical procedure suggested by US-EPA (Standard Method 2350 E - Ozone Demand/Requirement - Semi-Batch Method) was used. Therefore, residual ozone was forced to pass a Drechsel trap filled with 2% KI (Carlo Erba 472737). The volume of KI solution was 200 mL.
Ozonation and sonolysis effects were monitored in terms of solubilisation and anaerobic biodegradability variation.
Solubilisation was assessed through the evaluation of soluble COD (sCOD), before and after the treatment. Analyses were carried out according to Standard Methods (AWWA–APHA–WEF, 1998).

Anaerobic biodegradability was assessed by the procedure described by Luste et al. (2009).
Daily biogas production was settled by a water displacement method: gas was recovered directly from inverted 1000 ml measuring cylinder (filled under vacuum with water) used for water displacement through a tube connected to the top of the apparatus (Castillo et al., 2006).
Methane (CH_4) and carbon dioxide (CO_2) content in biogas were determined twice a week, using a portable infrared gas analyser instrument (GA 2000, Geotech).
Moreover, the occurrence of mineralization phenomena was assessed by the variation of volatile solid (VS) content, estimated according to Standard Methods (DIVAPRA, 1998).
The evaluation of BOD_5, for biodegradability assessment was performed on the soluble fraction of both treated and blank samples according to Standard Methods (AWWA–APHA–WEF, 1998).

6.2 SONOLYSIS FOCUSED STUDIES

The second part of the research activity was focused on sonolysis as pretreatment of solid organic waste destined to anaerobic digestion.
In particular, experimental activity was addressed towards the definition of main parameters, in order to assess the technical and economic feasibility of US application as pretreatment step within OFMSW anaerobic digestion facilities.
Moreover, the same parameters were used to compare the effects of sonolysis on different organic substrates, in order to highlight any correlation between disintegration mechanisms and organic matter composition.
To this end, the monitoring of chemical-physical and biological effects included:

- the evaluation of total solid (TS) and volatile solid (VS) content, which allowed the evaluation of mineralization phenomena;
- the analysis of the soluble fraction of both sonicated and untreated samples, in terms of soluble COD, dissolved organic carbon (DOC) and nitrogen concentration;
- the evaluation of sample biodegradability under anaerobic conditions.

Moreover, ultrasonic disintegration assessment relied on scanning microscopy, with the aim of visualizing the treatment disrupting effect.
Microbiological effects were also investigated, in order to verify ultrasonic disintegration mechanisms towards the natural microbial population of treated samples.

6.2.1 Substrates

The experimental activity was carried out using the following substrates:

- organic fraction of municipal solid waste (OFMSW) digestate, sampled at the anaerobic digestion plant in Luebeck (Germany);
- Protigrain®, a dried and pelletized stillage, produced from the Südzucker bioethanol plant in Zeitz (Saxony-Anhalt). It is a certified animal feed for cattle, pigs and poultry and a valuable protein and energy source, whose nutritional composition is reported in Table 6.4.
- dried distilled grains with solubles (DDGS) digestate, withdrawn from a bench scale anaerobic fermenter, using Protigrain® solution as feeding material. The fermenter organic loading rate was 5 g_{VS}/L d and retention time was 7 days.

Table 6.4 Nutritional composition of Protigrain®

Constituent	Value [$\%_{SS}$]
Fat	9.1
Protein	31.4
Starch and sugar	7.8
Fibre	7.5
Ash	6.0

DDGS is a protein-rich substrate. In this investigation, it was used both in its rough and partially degraded form in order to assess ultrasound effects on a substrate characterised by a different composition.
DDGS is one of the main by-products of ethanol fermentation processes. Ethanol fermentation is a biological process, developing in four steps: liquefaction, fermentation, distillation and purification. During the process, organic material is converted by microorganisms to simpler compounds, such as sugars, which are then fermented by microorganisms to produce ethanol and CO_2. The distillation stage

results in the production of a stillage, containing about a quarter of the feedstock dry solids.
The production of DDGS develops from stillage drying, which is a very energy intensive process and can account for up to 35% of the total parasitic thermal demand of the ethanol process (Murphy and Power, 2008).
In 2010, ethanol bio-refineries produced approximately 32.5 million metric tons of DDGS. Almost all of the DDGS produced is currently utilized as low-value animal feed (RFA, 2011) and only a minor part can be properly considered as waste. However, DDGS inclusion in animal feed is generally limited due to the fiber content, so that this material could be valuably used in different ways, such as within anaerobic digestion process.
The average total solid (TS) contents were 2.5% (± 0.01), 93.0% and 1.7% (± 0.01) respectively for OFMSW digestate, DDGS and DDGS digestate.
In order to ensure a proper application of ultrasonic treatment, the dry matter content of the substrates needed to range around 5%. To these end, for both OFMSW and DDGS digestates, the adjustment of the higher dry matter contents was achieved by separating the liquid phase in a centrifuge (Varifuge F, Heraeus, Dargatz), while DDGS was milled and used to prepare a 5% TS solution.
All the substrates were stored at 4°C until use.

6.2.2 Inoculum

Thickened digested sludge, was used as inoculum for biological tests. The sludge, which had an average total solid (TS) content of 3.1% (± 0.16) and a volatile solid (VS) content of 62.1% TS (± 3.2), was sampled at the wastewater treatment plant in Hamburg (Germany) and incubated at 35°C for 5 days, in order to reduce sufficiently sludge own gas production by means of a hunger phase.

6.2.3 Experimental set up

6.2.3.1 Sonolysis

Ultrasonic pretreatment was carried out by low frequency (20 kHz) sonication unit.

It consists of a sonotrode (Figure 6.6), with a 50 mm diameter tip, which is the basic unit of the device (Figure 6.7) commercialized by Ultrawaves GmbH (Hamburg, Germany).

Figure 6.6 Basic unit of Ultrawaves GmbH ultrasonic device

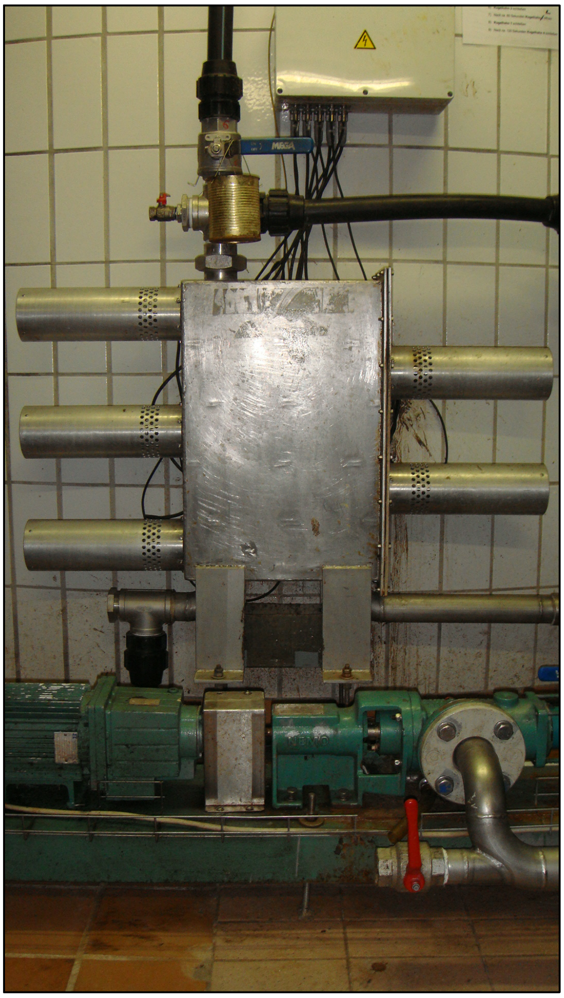


Figure 6.7 Ultrasonic device by Ultrawaves GmbH (Hamburg, Germany)

The latter device consists of a steel reactor, with a 29 L volume, equipped with five sonotrodes. Feeding material is pumped upflow through the reactor channels, designed to suits the cavitation field geometry, as shown in Figure 6.8.

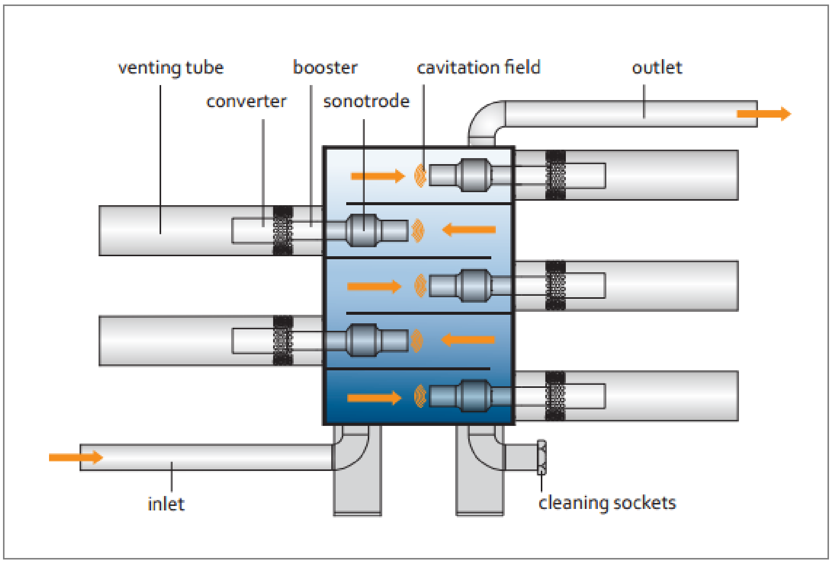


Figure 6.8 Schematic representation of the reactor designed by Ultrawaves GmbH (Hamburg, Germany)

The nominal given power to the sonotrode was 1 kW (which can provide ultrasonic intensity in the range 25-50 W/cm^2); the power released to the sample was displayed on an energy counter attached to the power source and it was recorded every 15 seconds, in order to calculate energy input.
Instrument amplitude was set to 100%, according to company recommendation and sonication time of 5, 10 and 15 minutes were used. The volume of substrate treated in each test was 1 L. Therefore, average energy inputs of 30, 60 and 90 W/h L were provided.
The correspondent specific energy values varied according to the kind of substrate that was being investigated, as reported in Table 6.5.

Table 6.5 Experimental ultrasonic conditions for different investigated substrates

Energy input [Wh/L]	Specific energy [kJ/kg$_{TS}$]		
	OFMSW digestate	DDGS	DDGS digestate
30	2102 ± 21	2835 ± 63	2264 ± 4
60	4219 ± 40	5630 ± 122	4581 ± 59
90	6291 ± 95	8493 ± 252	6858 ± 46

The substrate to be treated was put in a cylindrical beaker with the US sonotrode placed in the centre and immersed up to 2 cm.
During ultrasound application, the substrate was continuously mixed by a magnetic stirrer in order to avoid precipitation of solids at the bottom of the beaker.
The degree of disintegration was dependent on the specific energy supplied, which was calculated as the ratio between the energy supplied and the initial TS content of the mixture (Braguglia et al., 2010).

6.2.3.2 Anaerobic biodegradability

Biological tests were performed trough the Eudiometer system, schematically shown in Figure 6.9.

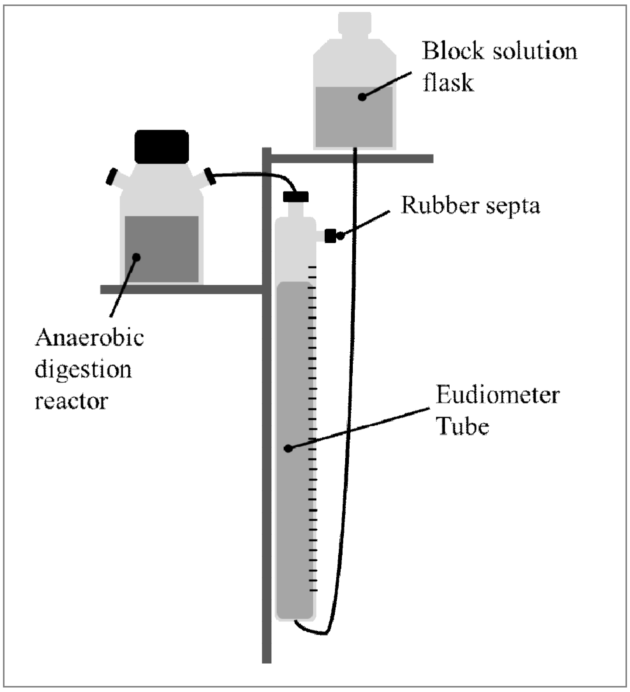


Figure 6.9 Schematic representation of the Eudiometer system

It consists of 500 mL reactors, connected to Eudiometer tubes, which were filled with a block solution.
Biogas produced in each reactor flows in the tube, thus allowing the measure of its volume through the displacement of the solution.

Rubber septa allowed the sampling of biogas, for the analysis of its composition.
Anaerobic biodegradability tests were performed in a climate chamber, in order to ensure mesophilic conditions (35-40°C).

6.2.4 Analytical set up

TS and VS were evaluated according to Standard Methods (DIVAPRA, 1998), while sCOD was assessed by AWWA-APHA-WEF Standard Methods (1998).
Disintegration degree was calculated as:

$$DD_{COD} = \frac{COD_{US} - COD_0}{COD_{NaOH} - COD_0} * 100$$

where:

- COD_{US} is the soluble COD of the sonicated sample;
- COD_0 is the soluble COD of untreated sample;
- COD_{NaOH} is the soluble COD in the supernatant of the reference sample obtained with 1 M NaOH digestion (Muller, 2001).

The sample soluble fractions, obtained after centrifugation (14000 rpm, 15 minutes) and filtration (< 0.45 μm) were also analyzed in terms of nitrogen content, determined by Kjeldahl Method and DOC, evaluated by Multi N/C 2000 (Analytikjena, Jena, Germany).
Anaerobic digestion tests were carried out according to the German guideline VDI 4630. Therefore, pH was measured, before and after anaerobic digestion tests, by pHmeter (WTW, Weilheim, Germany). Daily biogas production was settled by the water displacement method, while biogas composition, in terms of methane, carbon dioxide, nitrogen and oxygen, was analysed by a GC-TCD, model HP 6890 (Hewlett Packard, Waldbronn, Germany).

6.2.4.1 Microscopy

In order to achieve a further characterization of investigated samples, their analysis was performed by means of a light microscope as well as a scanning electronic microscope (SEM).

In the former case, samples in the native state were examined at different magnification and images recorded by a camera, which could be coupled to the microscope.
In the latter, samples were dried and sputtered with gold (Sputter SCD 0050 - BAL-TEL) before undergoing SEM (Leo 1530) analysis.
Figure 6.10 and Figure 6.11 show the preparation with gold and the microscope respectively.

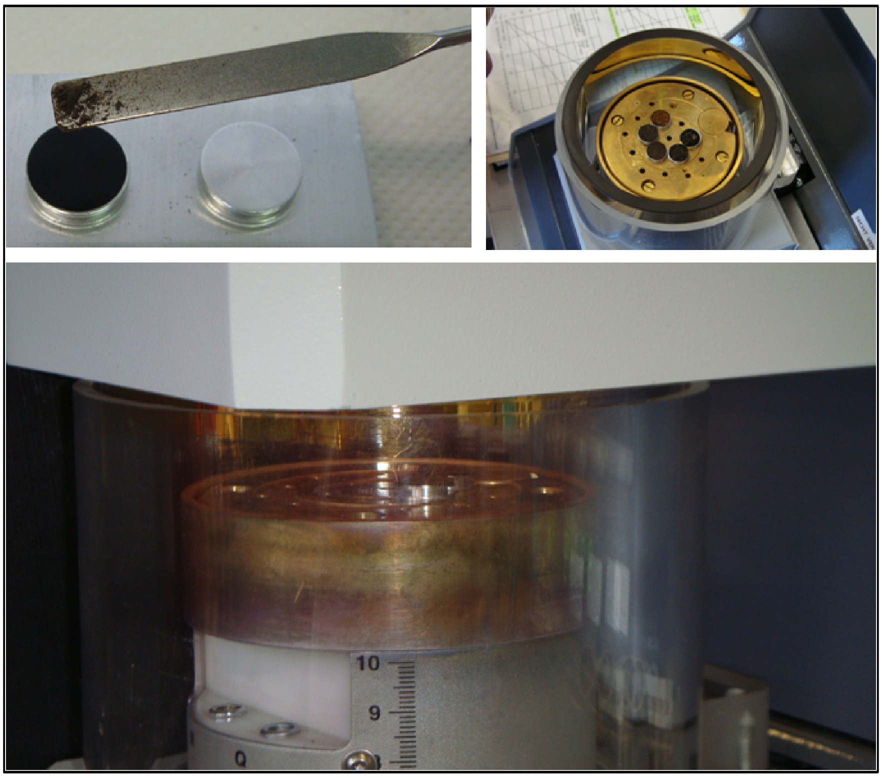


Figure 6.10 Sample preparation for SEM analysis

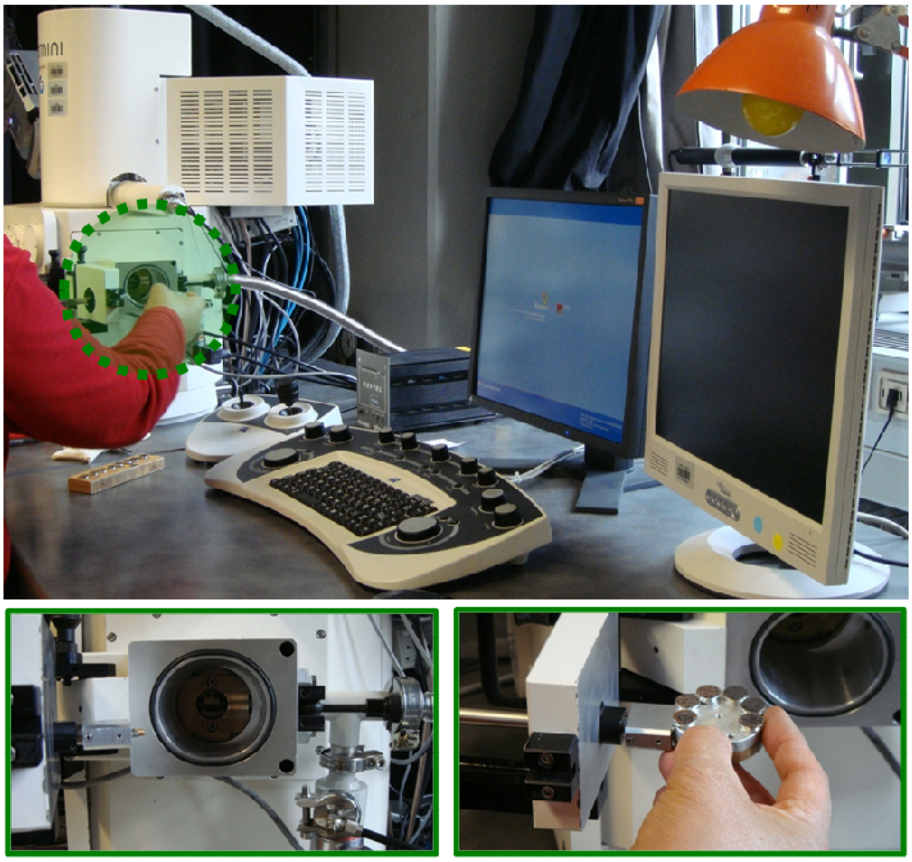


Figure 6.11 Scanning Electronic Microscope

6.2.4.2 PCR-DGGE test

PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis) is an analytic procedure useful to study genetic diversity in complex microbial ecosystems by means of a fingerprinting approach.
Therefore, this method allowed visually profiling and monitoring of changes that occur in microbial communities after ultrasonic treatments.
After isolating DNA in both untreated and sonicated samples, by PowerSoil DNA Isolation KIT (MO BIO Laboratories Inc., Carlsbad, USA), the method relied on repeated heating and cooling for DNA melting and enzymatic replication of the DNA itself. Short DNA fragments containing sequences complementary to the target region

along with a DNA polymerase enabled selective and repeated amplification.
Subsequently, a gel created to form a denaturing gradient was poured and run to separate similarly sized PCR products by electrophoresis.
During this phase, the gel was stained and band patterns were visualized revealing the presence of different microbial communities.

6.2.4.3 DAPI staining and fluorescence microscopy

DAPI (4', 6-diamidino-2-phenylindole) is a DNA stain, which is used extensively in fluorescence microscopy. DAPI can pass through an intact cell membrane, making it fluorescent. This technique favour live cells counting by means of fluorescent microscopy, highlighting any affection of ultrasonic pretreatment to treated sample microbial communities.
The sample was treated with a 4% (w/v) paraformaldehyde (PFA) solution, shaken for 30 minutes at 4°C and exposed to centrifugation/suspension cycles in appropriate solutions.
The suspension was, subsequently, added to the DAPI solution, mixed and incubated for 5 minutes on ice.
The DAPI-stained sample was filtrated, washed with filter-sterilized H_2O and 80% ethanol and the membrane filter air-dried, before being used for microscopy, at -20°C, in the dark.

7 RESULTS AND DISCUSSION

In the following paragraphs, the discussion of the experimental activity results is provided.

To this end, this chapter is divided in three main sections:

- the first one is focused on the results of the comparative evaluation of sonolysis and ozonation effectiveness as pretreatment of OFMSW to be anaerobically digested;
- the second section deals with the results of sonolysis focused studies;
- the third part discusses sonolysis scale up in order to highlight the technical and economic feasibility of the combined ultrasound/anaerobic digestion process for both the ligno-cellulosic and protein-rich investigated substrates.

7.1 COMPARATIVE ASSESSMENT OF OZONATION AND SONOLYSIS

The results of this first experimental activity, which have been recently published, were fundamental to address the second part of the research, as they allowed:

- the definition of process operating conditions for both ozone and sonolysis;
- the comparison between ozonation and sonolysis performances in terms of organic matter solubilisation and anaerobic biodegradability variation.

7.1.1 Ozonation tests

Ozonation operating conditions were defined with reference to the occurrence of both solubilisation and mineralization phenomena as well as to the estimation of residual ozone. Due to the high costs associated

to ozone generation, it was necessary to provide ozone amount as high as the one effectively required for oxidative reactions.
Chu et al. (2009) demonstrated a direct relation between ozone doses and organic matter solubilisation, which stands until the occurrence of mineralization phenomena. Therefore, the effect of different ozone doses on both solubilisation and mineralization of OFMSW samples was considered along with the correspondent residual ozone amount.
Figure 7.1 plots results obtained by using the Drechsel trap as reactor. It is evident that increasing ozone doses above 0.2 g_{O3}/g_{TS}, residual ozone enhanced more than the ozone involved in oxidative reactions. For an ozone dose of 1.6 g_{O3}/g_{TS}, residual ozone was almost 60% of the total amount of ozone introduced in the reactor.

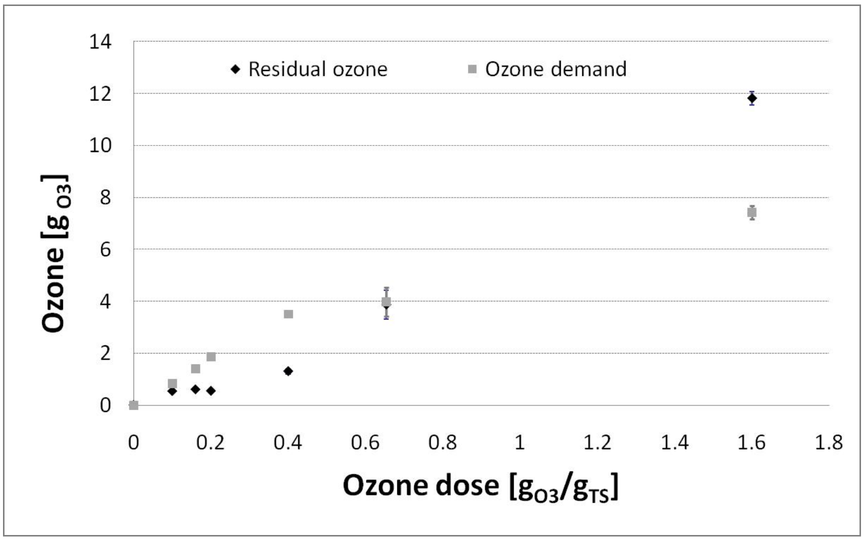


Figure 7.1 Residual ozone variation as function of ozone dose - Drechsel trap as reactor (Cesaro and Belgiorno, 2012)

Differently from previous studies, this evidence could not be related to the occurrence of mineralization phenomena, as the volatile solid content of sample did not vary significantly after the supply of different ozone doses (Figure 7.2).
After ozonation treatment, VS content was stable at an average value of 96.3%TS (with a standard deviation of 0.14).
Moreover, any variation in the ozone stream had been provided, so that the results was ascribed to the poor contact between ozone and

substrate, determined by the change in the substrate dewaterability properties, as found for the sludge by Zhao et al. (2007).

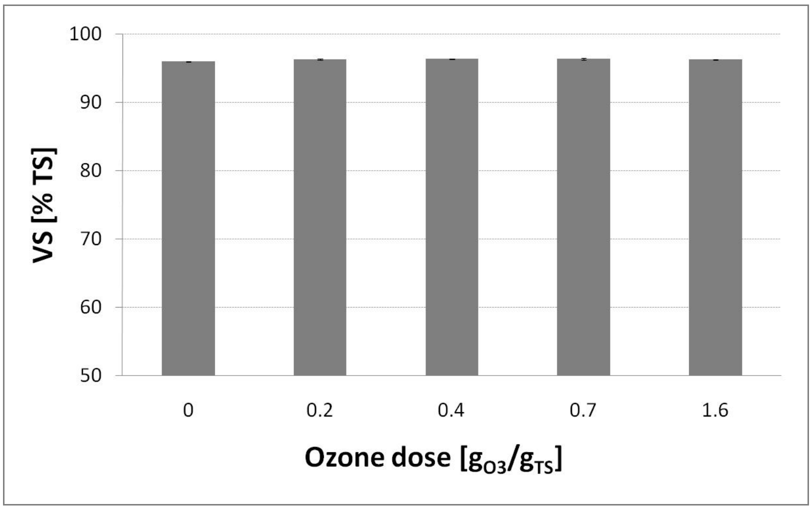


Figure 7.2 VS content of OFMSW samples for increasing ozone doses

In order to promote the contact between the ozone flow and the substrate, the same tests were repeated using a bubble column. This system, as already explained, is characterized by ozone inlet at the bottom and exhaust stream outlet at the top of the reactor. It allows, therefore, the ozone flow to be forced through the whole substrate, avoiding possible short-circuiting.
Figure 7.3 shows the comparison in ozone demand, expressed as the amount of reacting ozone out of the total ozone mass introduced in both Drechsel trap and bubble column reactors.

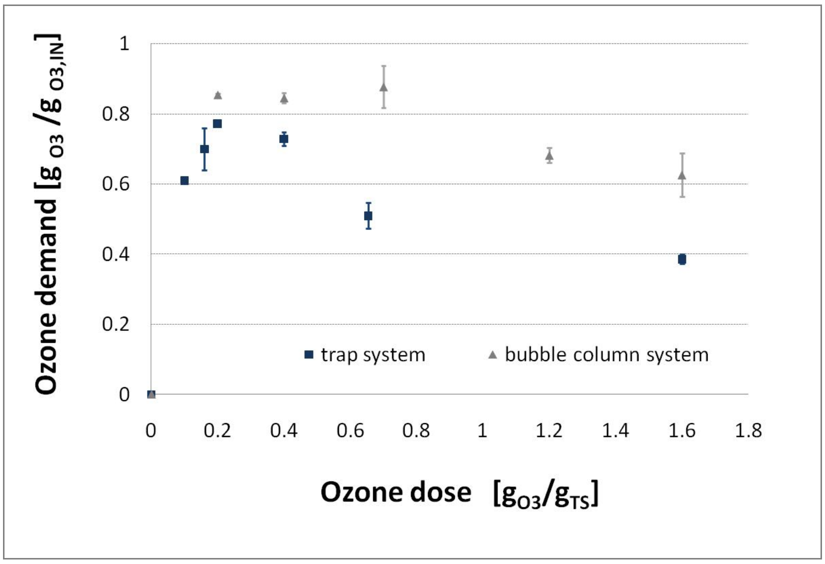


Figure 7.3 Variation of ozone demand for increasing ozone doses provided in both Drechsel trap and bubble column reactors (Cesaro and Belgiorno, 2012)

As plotted in Figure 7.3, at an ozone dose higher than 0.7 g_{O3}/g_{TS}, in both systems a reduction in the ozone mass involved in the chemical reactions occurred.

As any variation in VS content was observed and ozone flow was kept constant, this evidence was related to the occurrence of changes in the substrate structure that limited oxidative reactions. Therefore, ozone doses of 0.4, 0.7 and 1.2 $g_{O3}/g_{TS,}$ were chosen to perform solubilisation tests. Additionally, a dose of 0.16 g_{O3}/g_{TS} was considered, in order to establish a comparison with results obtained on sewage sludge by Bougrier et al. (2006).

Figure 7.4 plots the results of soluble COD tests performed on ozonated samples.

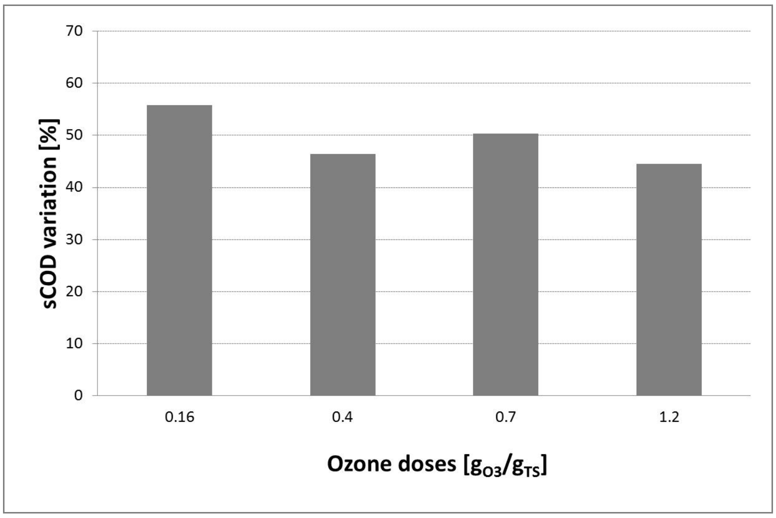


Figure 7.4 Variation of soluble COD for increasing ozone doses investigated (Cesaro and Belgiorno, 2012)

Results show that there is not a linear trend in soluble COD variation for increasing ozone doses supplied. Soluble COD increases after ozonation in all samples, ranging between 45 and 56%. The highest value was achieved for the lower ozone dose.
Several studies dealing with sludge ozonation report of higher solubilisation increases after the use of ozone doses much lower than the ones investigated in this study (Braguglia et al., 2012; Zhang et al., 2009). The difference lays in the substrate characteristics, both in terms of heterogeneity and molecular structure complexity.
However, similar consideration also arises if the application of other chemical treatment to solid organic waste is considered. Hao et al. (2009) found that the mean concentration of sorted municipal solid waste COD increased from 5931 to 12 007 mg/L moving from 1 to 4 g_{NaOH}/100 g solid.
According to the results of solubilisation tests, any relevant change in biogas production was expected for increasing ozone doses provided to OFMSW before anaerobic digestion tests. Nevertheless, ozone doses of 0.16, 0.4 and 1.2 g_{O3}/g_{TS} were chosen as pretreatment of OFMSW for anaerobic digestion, in order to evaluate if any change in the substrate biodegradability occurred as consequence of the different ozone doses provided.

7.1.2 Sonolysis tests

Sonolysis, as ozonation, provides solubilisation improvement for increasing extent of the ultrasonic energy input, until mineralization phenomena occur. Therefore, the identification of ultrasonic operating conditions was carried out by evaluating the effects of different treatments on the variation of soluble COD and VS content, whose reduction indicates the occurrence of mineralization.

Figure 7.5 reports the variation in soluble COD and VS content, with reference to the untreated substrate, for specific energy values ranging between 15000 and 40000 kJ/kg_{TS}. These energy inputs were provided, for a fixed amplitude value of 40%, by varying sonication time.

An overall increase in COD solubilisation was observed for increasing specific energy inputs, corresponding to increasing sonication time. However, the reduction of volatile solid content was also observed, suggesting the occurrence of mineralization.

According to these results, sonolysis provided both solubilisation and mineralization of organic matter and the extent of both effect enhanced for increasing sonication time. Although solubilisation phenomena prevails on mineralization ones, as shown in Figure 7.5, part of the energy provided to the sample is spent to promote the conversion of organic matter into inorganic compounds, thus reducing the amount of substance readily available for the subsequent anaerobic process.

Therefore, it is reasonably possible that the supply of the same amount of energy in a different period of time could induce different results.

To verify this hypothesis, further tests were carried out according to the operating conditions summarized in Table 7.1, with the aim to monitor the influence of amplitude on the extent of sonolysis effects.

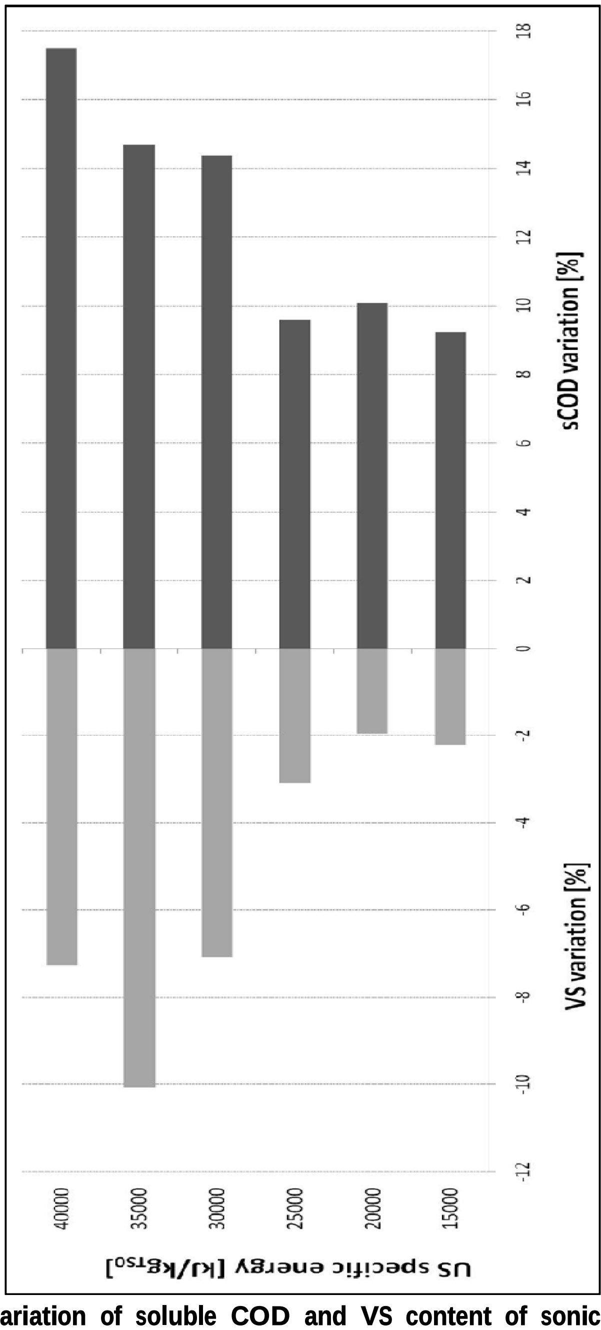


Figure 7.5 Variation of soluble COD and VS content of sonicated samples (adapted from Cesaro and Belgiorno, 2012)

Table 7.1 Ultrasonic operating conditions for the evaluation of amplitude influence on sonolysis effects

Specific energy [kJ/kg$_{TS}$]	Amplitude [%]	Sonication time [min]
15 000	20	220.5 ± 3.5
	40	76.2± 1.5
	60	39.6 ± 1.5
40 000	20	535.0 ± 1.4
	40	186.0 ± 8.5
	60	109.5 ± 3.5

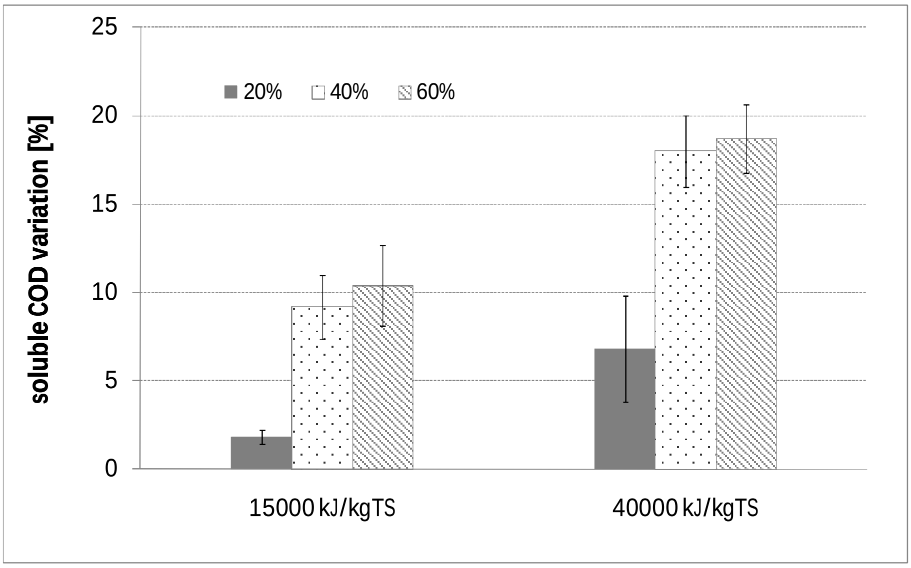


Figure 7.6 Soluble COD variation for set specific energy values and varying amplitude

Results plotted in Figure 7.6 show that, in both cases (15 000 and 40 000 kJ/kg$_{TS}$) solubilisation enhanced for increasing amplitude. Remarkable improvements were observed when amplitude was shifted from 20 to 40%: this variation corresponded to a significant reduction of sonication time, which resulted in the supply of a certain energy amount with a higher power.

For the lower specific energy value provided to organic samples with an amplitude of 20%, the variation in soluble COD is negligible, as well as the reduction of volatile solid content. This evidence suggested that,

under that operating condition, cavitation mainly provided a mild mixing of the sample, but not disintegration phenomena.
Mineralization phenomena increased for improved amplitude, following a trend similar to the one already discussed for solubilisation. In particular, at 20% amplitude, VS reduction was minor for both 15000 and 40000 kJ/kg_{TS}; when amplitude raised, VS reduction improved, reaching comparable values for both 40 and 60% amplitudes (approximately 7 ± 1.2 %).
According to these results, the application of specific energy values higher than 15000 kJ/kg_{TS} did not provide significant solubilisation effects along with negligible mineralization phenomena.
As the biodegradable fraction of municipal waste is characterized by an energetic potential of approximately 20000 kJ/kg_{TS} corresponding to the anaerobic conversion of approximately 50% VS, any biogas increase is expected to be so noticeable to justify the application of specific energy values higher than 15000 kJ/kg_{TS}.
Therefore, US treatment effect on anaerobic biodegradability was evaluated for the specific energy value of 15000 kJ/kg_{TS} that resulted in an average increase in soluble COD of approximately 9%.

7.1.3 Anaerobic biodegradability tests

The effectiveness of both sonolysis (15000 kJ/kg_{TS}) and ozonation (0.16, 0.4 and 1.2 g_{O3}/g_{TS}) on OFMSW anaerobic biodegradability was assessed via batch tests.

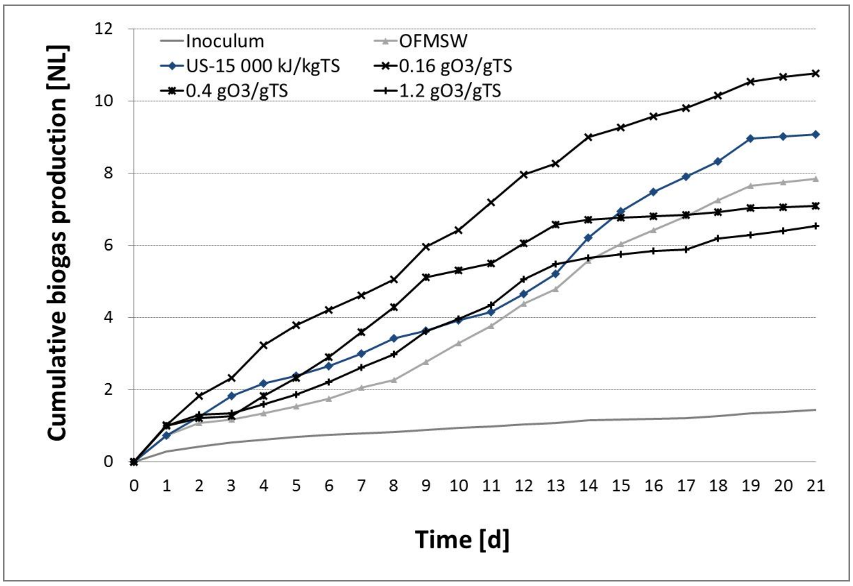


Figure 7.7 Cumulative biogas production in 21 days (adapted from Cesaro and Belgiorno, 2012)

Figure 7.7 plots cumulative biogas production from both untreated OFMSW and differently pretreated substrates. During anaerobic digestion tests, methane content was analysed, but its percentage in biogas was almost constant (58% average highest value, with 4.9 standard deviation).

During the first ten days, as expected, both sonicated and ozonated samples produced higher biogas volumes than rough OFMSW. Both ozonation and sonolysis had promoted an increase in the concentration of soluble compounds, thus allowing the anaerobic degradation of greater organic matter amounts.

From the tenth day, in the reactor fed with untreated substrate, the hydrolysis of complex organic matter determined an increase of soluble monomers, so that biogas production became faster and higher biogas volumes were produced. As a result, the slope of the curve changed and developed similarly to the ones from pretreated substrates.

However, as anaerobic tests proceeded, the improvement of biogas volumes produced after the application of higher ozone doses (0.4 and 1.2 g_{O3}/g_{TS}) started to reduce and, after thirteen days, it was definitely stopped. As shown in Figure 7.7, at the end of the tests, ozonation at doses of 0.4 and 1.2 g_{O3}/g_{TS} determined the production of biogas

volumes lower than untreated OFMSW, despite the solubilisation achieved under both pretreatment conditions.
Conversely, ozone dose of 0.16 g_{O3}/g_{TS} and ultrasonic specific energy of 15000 kJ/kg_{TS} proved to be effective in enhancing biogas production, leading to an increase of 37% and 16%, respectively.
The relatively lower biogas increase from sonicated substrate reflects the minor solubilisation provided by sonolysis in comparison to the one achieved by the application of the lower ozone dose.
These results suggested, for ozonation, the absence of a direct correlation between biogas production and soluble compound concentration. Similar results were obtained by Ma et al. (2011), who studied the effects of different chemical and physical pretreatments of kitchen waste for anaerobic digestion. In their study, authors found that the highest cumulative biogas production was observed in the less effective pre-treatment in terms of solubilisation and, conversely, the highest solubilisation pre-treatment (thermo-acid) resulted in less biogas production than the control.
This discrepancy between the solubilisation effect and the anaerobic biodegradability was not related to the occurrence of mineralization phenomena, as stated for sewage sludge by Bougrier et al. (2006), but it was probably explained by the formation of inhibitory or refractory compounds generated during the pretreatments.
This hypothesis was consistent with the outcome of the study performed by Carballa et al. (2009), who discussed the formation of inhibitory or refractory compounds during chemical pretreatments of anaerobically digested sludge.
In order to verify the limited availability of easily biodegradable compounds, in the present study, biodegradability tests were performed and results are plotted in Figure 7.8.

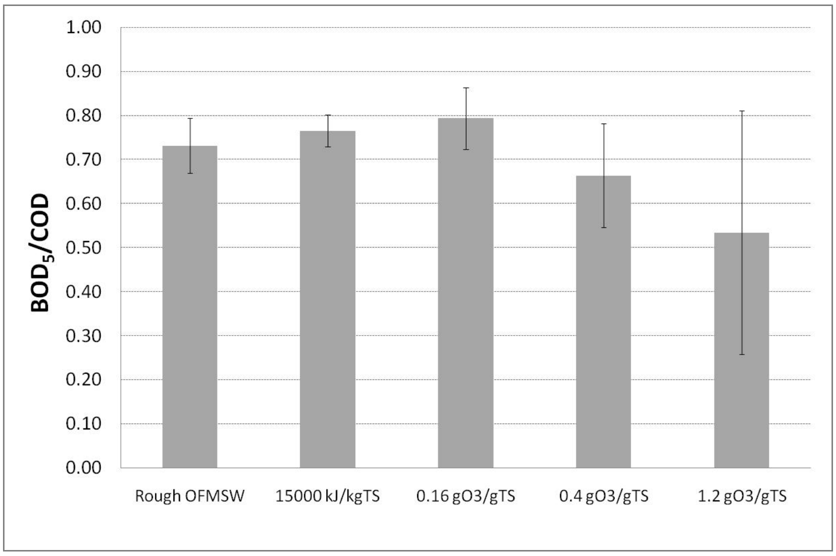


Figure 7.8 Biodegradability test results (adapted from Cesaro and Belgiorno, 2012)

Results highlight that the higher BOD_5/COD ratio was obtained from those substrates producing higher biogas volumes; in particular, when 0.4 and 1.2 g_{O3}/g_{TS} were applied, the ratio resulted in a lower value than the one from rough OFMSW. Therefore, biogas production from ozonated substrates is in direct correlation with the biodegradability of substrate itself.

OFMSW ozonation can determine a reduction in organic matter biodegradability as already found for sewage sludge (Weemaes et al., 1999), making ozonation less competitive than sonolysis.

7.1.4 Ozonation and sonolysis comparison: conclusive remarks

According to the experimental result, in order to exploit the potential of ozone application as pretreatment of solid organic substrates, very low ozone concentration must be kept, thus forcing the maintenance of very strict operating conditions as well as the implementation of precise monitoring systems.

Any increase in ozone concentration can provide the formation of compounds, even intermediates, which are less biodegradable than rough

substrate and can promote undesirable effects in terms of biogas production and energy recovery. Although the formation of refractory compounds is a common aspect of chemical processes, the delicate and costly ozone generation does not justify the interest in this treatment.
Conversely, sonolysis application led to increase in both solubilisation and biodegradability of complex organic matter, which resulted in increased biogas production from sonicated substrates.
Additional advantages of ultrasonic application had already been pointed out by Chen et al. (2008), who studied sonolysis effects on an organic solid waste fermentation broth and demonstrated that ultrasound can also help acid desorption from organic substrate, thus reducing the chances of anaerobic digestion failure by acid accumulation.
Therefore, sonolysis proved to be a versatile technology, which can be effectively used to enhance OFMSW anaerobic digestion yields. The main drawback could be related to the consumption of energy that is required to run an ultrasonic device.
Mass and energy balances performed on full-scale applications to sewage sludge demonstrated that energy produced is more than the one consumed: 1 kW of ultrasound used will generate 7 kW of electrical energy after losses (Barber, 2005).
This kind of analysis had guided the application of several patented device for the treatment of sludge before anaerobic digestion and addressed towards the in-depth analysis of sonolysis application as pretreatment of OFMSW for anaerobic digestion.

7.2 SONOLYSIS FOCUSED STUDIES

In this session, results dealing with sonolysis application are summarized and discussed in order to highlight:

- main disintegration effects of ultrasound on the digested organic fraction of municipal solid waste;
- the relation between ultrasonic action and organic matter composition.

Although the comparative assessment of ozonation and sonolysis was carried out on rough OFMSW, the second part of the research was carried out using OFMSW digestate.

Several studies shown that digestate can still contain a high biogas potential, mainly as a consequence of residual and undigested volatile solids (Menardo et al., 2011a). Its composition is less heterogeneous than the one of rough OFMSW and its organic matter content and quality mainly depend on the operating conditions of the anaerobic digestion plant: when high organic loading rate and short hydraulic retention time are applied, the digestate-produced biogas collected during its storage could be economically attractive (Menardo et al., 2011b). However, OFMSW digestate is mainly a ligno-cellulosic material (Hartmann et al., 2000), which is minimally digestible by bacteria. Its pretreatment, therefore, would be convenient.
Previous studies carried out with the basic unit of Ultrawaves device showed that comparable solubilisation and biogas production can be achieved by energy inputs up to eight-fold lower than the ones required to treat rough substrate (Vural, 2011). Therefore, the need to shift from the rough to the anaerobically digested OFMSW was necessary to reduce energy consumption.
Moreover, the treatment of the digestate would allow several operational advantages for sonolysis scale up. This substrate is characterized by a total solid content adequate for the direct application of ultrasound. Therefore, any dilution phase is required to provide cavitational phenomena and a further reduction of digestate amount can be ensured.

7.2.1 Effects of US on OFMSW digestate

Sonolysis of the OFMSW digestate determined an increase in soluble COD (Figure 7.9), which resulted in a disintegration degree improvement (Figure 7.10) ranging between 22 and 53%, for increasing energy inputs.

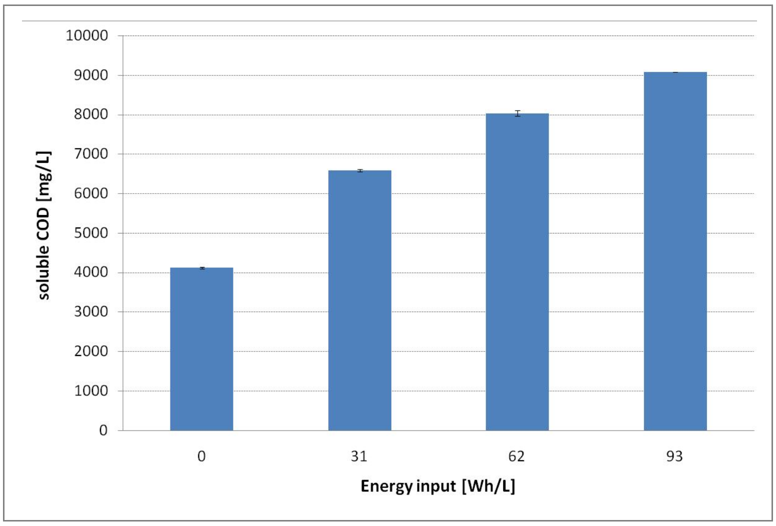


Figure 7.9 Soluble COD variation for increasing energy inputs

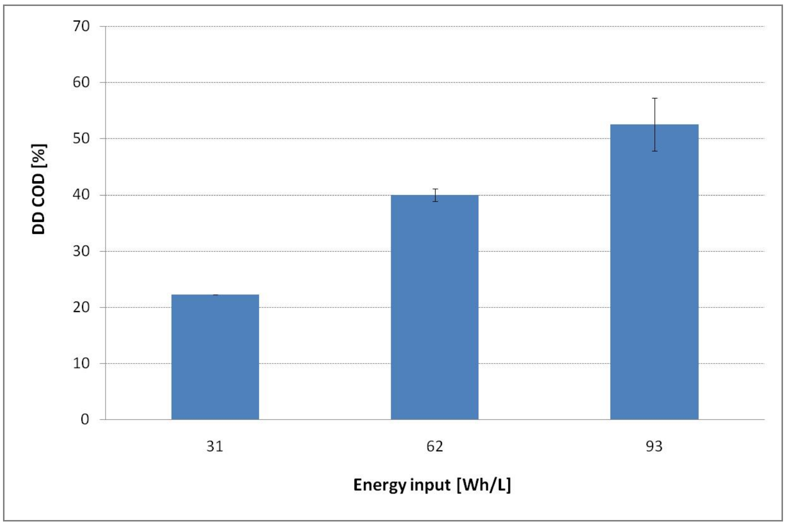


Figure 7.10 Disintegration degree in terms of soluble COD

Moreover, the absence of mineralization phenomena was observed, as proved by the constancy of VS content, which is plotted in Figure 7.11, before and after US application.

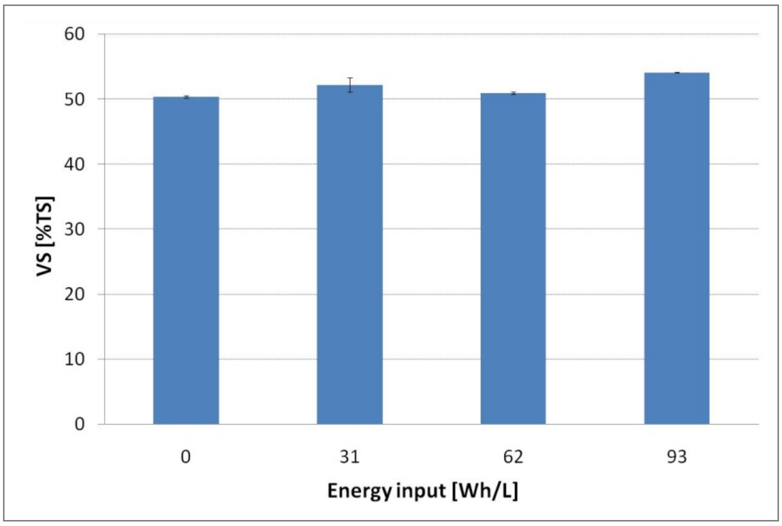


Figure 7.11 VS content variation after the supply of different energy inputs

These results are slightly different from the ones obtained for the rough OFMSW. Although lower energy inputs (2102 - 6291 kJ/kg$_{TS}$) were provided, higher solubilisation was achieved and the volatile solid content remained almost constant, suggesting that the amount of organic matter in the sample was not affected by ultrasound but remained available for its conversion in biogas.

This evidence should be related not only to the composition of the substrate, but also to the different ultrasonic device used.

The wider tip and the design of the sonotrode allowed a better propagation of sound waves in the sample. As a consequence, a more homogeneous collapse of cavitation bubbles occurred and ultrasonic effects were adequately distributed into the whole sample.

The energy provided was, thus, used to disintegrate both ligno-cellulosic material and bacteria cells, which are the main components of OFMSW digestate.

As shown in Figure 7.12 and Figure 7.13, the increased soluble COD was consistent with the enhanced concentration of carbon and nitrogen. The former was related to the simplification of undigested organic matter as well as to the disruption of microbial cells, which also determined the release of nitrogen, as found in several studies dealing with ultrasonic sludge pretreatment (Bougrier et al., 2005).

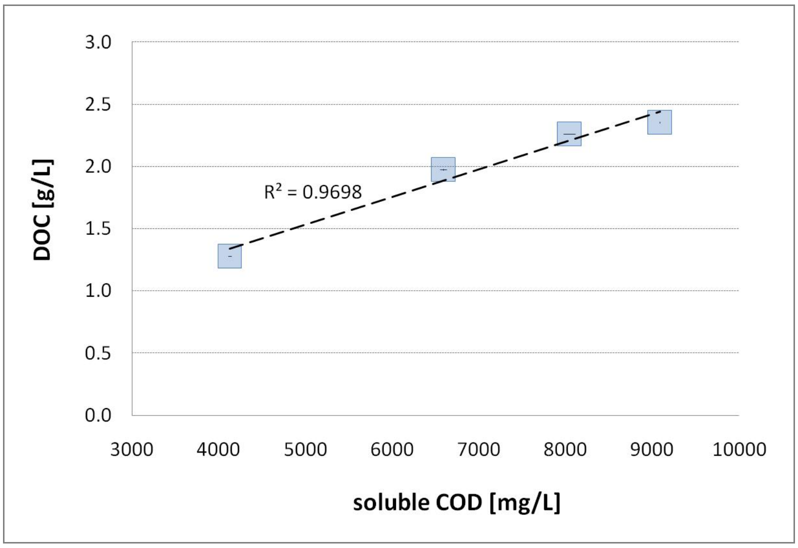


Figure 7.12 Correlation between soluble COD and DOC

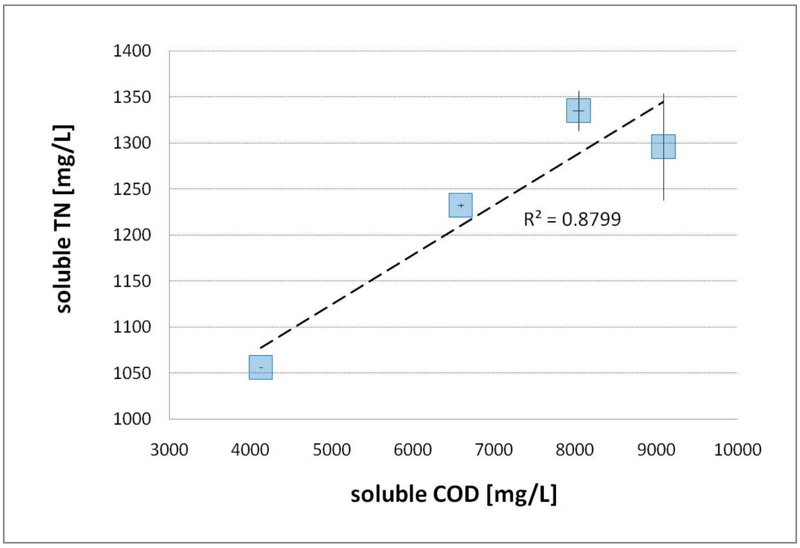


Figure 7.13 Correlation between soluble COD and nitrogen in the soluble fraction of the samples

Due to the enhanced solubilisation of organic matter, as expected, the anaerobic biodegradability of pretreated OFMSW digestate samples increased, as shown in Figure 7.14. At the end of the tests, the improvement in specific biogas volumes was 46, 60 and 71% for specific energy inputs of approximately 2100, 4200 and 6300 kJ/kg_{TS}, respectively.

Braguglia et al. (2012) applying a specific energy input of 2500 kJ/kg_{TS} on waste activated sludge, found a 26% increase in biogas production after almost 65 days.

The difference lays in the higher concentration of organic matter in OFMSW digestate that can be involved in biological degradation mechanisms.

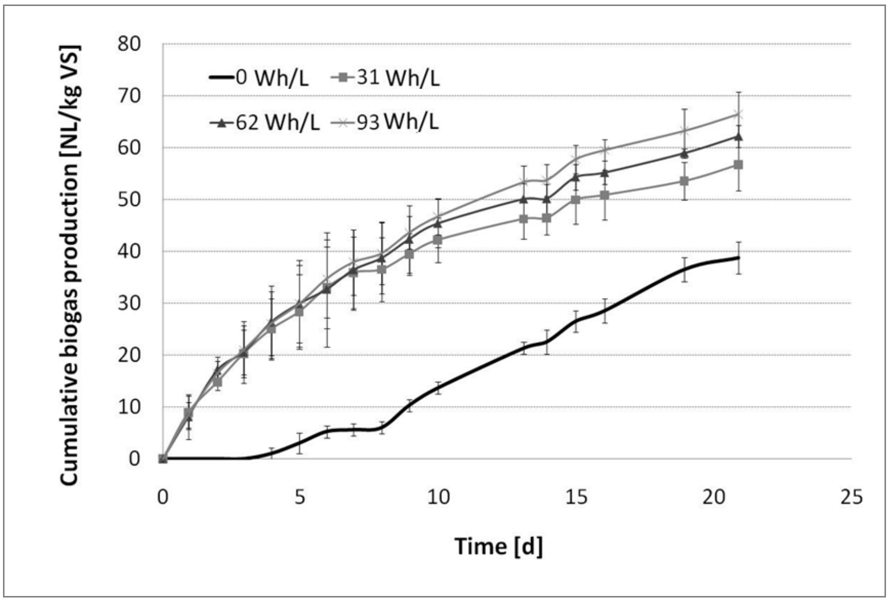


Figure 7.14 Specific cumulative biogas production for both untreated and sonicated OFMSW digestate samples

Moreover, the analysis of biogas composition highlighted that methane content in biogas increased with ultrasonic energy inputs and during digestion time, as shown in Figure 7.15.

Similar results were also obtained by ultrasonic pretreatment of both mixed sludge (Chu et al., 2002) and waste activated sludge (Wang et al., 1999)

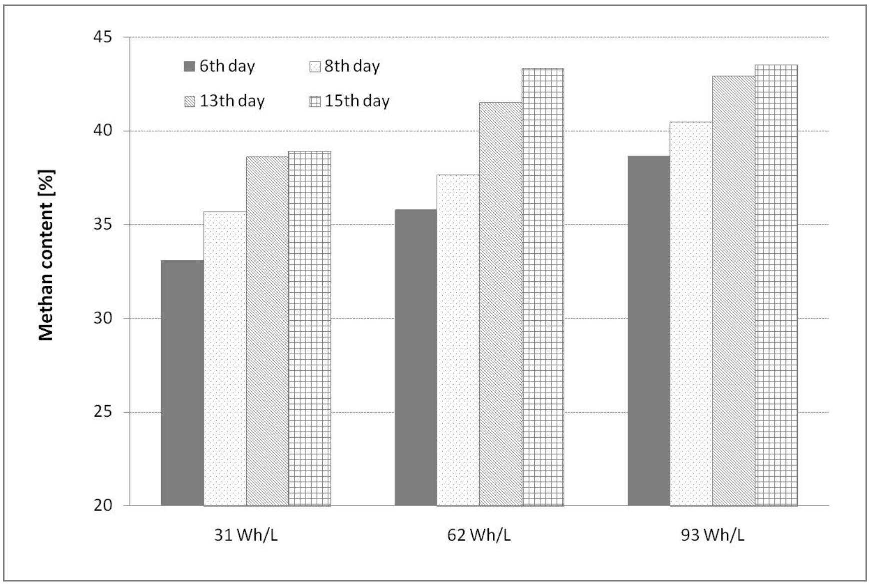
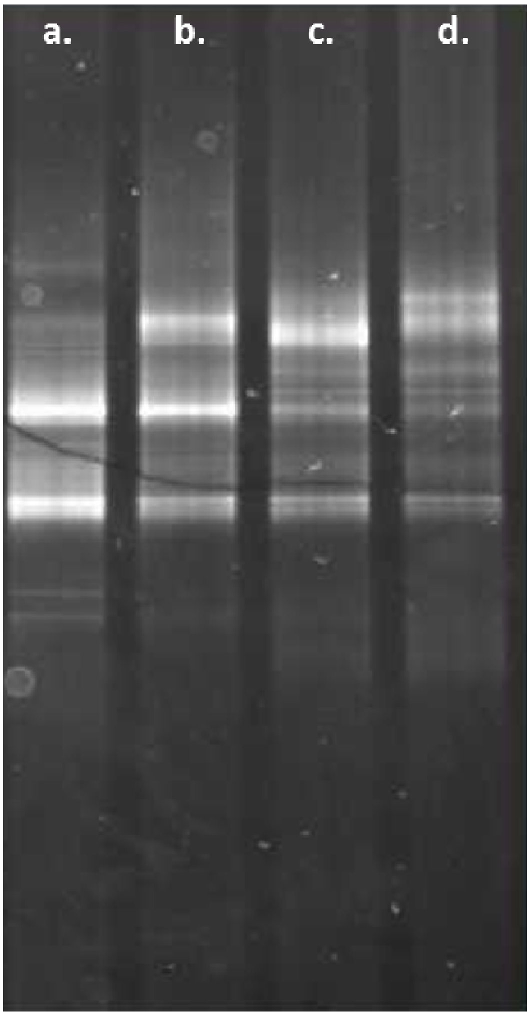


Figure 7.15 Methane content increase after ultrasonic treatment and during digestion time

This outcome, which is particularly interesting in terms of process application, is consistent with the results of the analytical determination of the soluble fraction of pretreated samples. The greater amount of soluble organic carbon related to sonolysis application determined the production of larger methane quantities, which can be conveniently recovered for energy generation.
According to these results, ultrasonic pretreatment can enhance anaerobic digestion yields of OFMSW digestate by increasing the solubilisation of carbonaceous organic matter, which is related to both substrate disintegration and biomass cell disruption.
In order to verify the contribution of microbial cell disruption to COD solubilisation, microbiological tests were performed.
Figure 7.16 shows the results of the DGGE analysis.



a. **0 Wh/L;** b. **31 Wh/L; c. 62 Wh/L; d. 93 Wh/L**

Figure 7.16 Microbial DNA changes in OFMSW digestate after sonolysis

It is evident that, for increasing energy inputs, any relevant changes in microbial population occurred in pretreated samples. This evidence suggested that biodiversity is not affected by ultrasonic treatment in this kind of substrates.
Such a result is particularly interesting if methanogenic bacteria are considered. It is well known that this kind of microorganism is

characterized by very low kinetics: their partial removal could, therefore, result in a serious disadvantage for sonolysis application as anaerobic digestion pretreatment. In continuous reactor, the gradual disappearance of methanogens could be not compatible with their growth kinetics.
It should also be highlighted that the fingerprint showed in Figure 7.16 differs from the typical ones, which are characterized by very thin bands.
The large bands appearing on the obtained fingerprint is representative of highly concentrated biomass samples.
The same consideration was strengthened by DAPI staining results, which gave a qualitative indication of the great living microorganism concentration still present in OFMSW samples after sonolysis.
Figure 7.17 reports two images of the same sample, pretreated with an ultrasonic energy input of 93 Wh/L (correspondent to almost 6300 kJ/kg_{TS}).
The analysis of both images highlights an immediate difference in the concentration of the clearer stains, which indicate the presence of alive microbial cells.
In both cases, high concentration of living microorganisms can be observed. However, their estimation in the sample under investigation is not possible, as their distribution looked not uniform in the sample itself.
This aspect was related not only to the high concentration of stained microorganisms, but also to the relevant presence of solid particles that represents a further obstacle in the living cell counting. These particles, which are identified through the darker spots, sheltered different amounts of living cells, altering their estimation.
The DAPI staining on OFMSW digestate samples proved to be not suitable, due to the great heterogeneity of the substrate, which constituted an obstacle also for the analysis of ultrasound disintegration effects by scanning electronic microscopy (SEM).

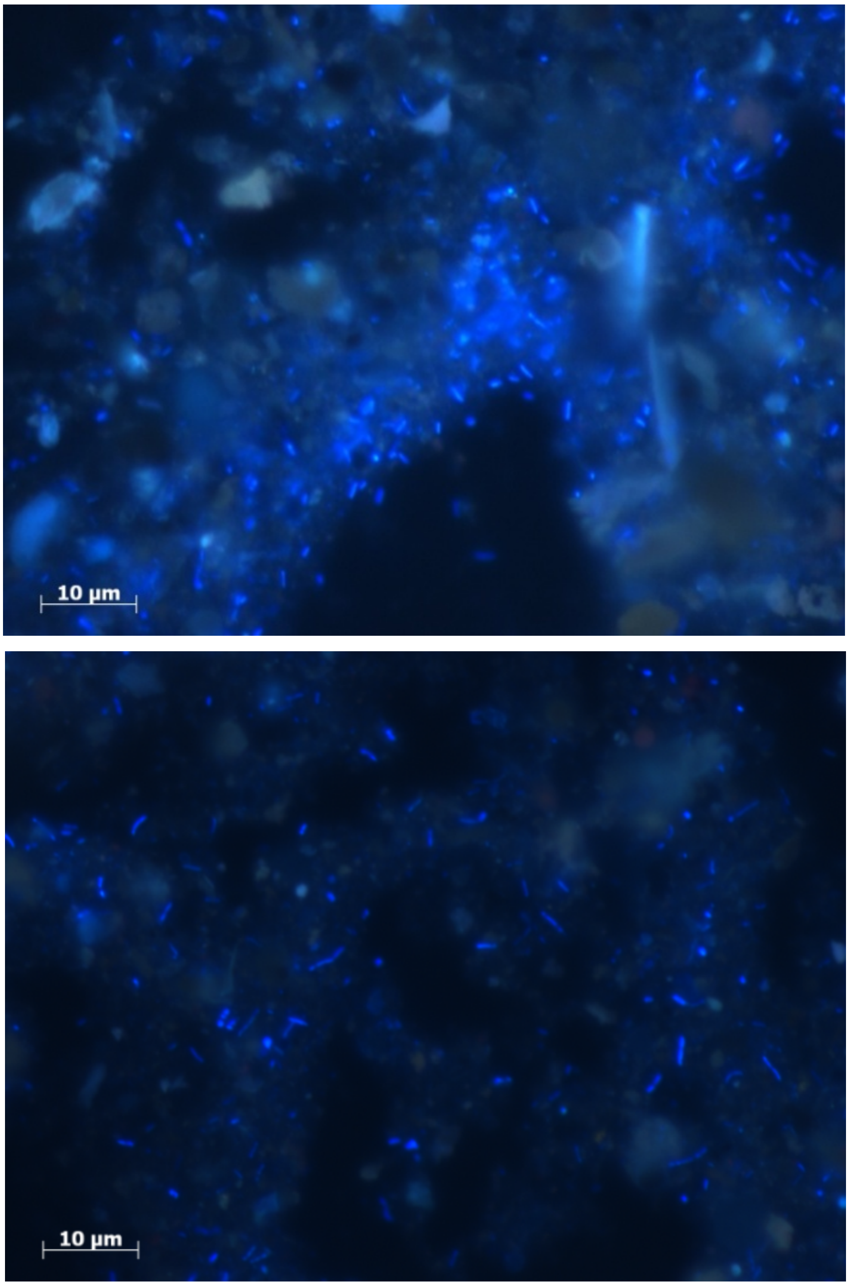


Figure 7.17 DAPI staining on sonicated OFMSW digestate sample (Ultrasonic energy input: 93 Wh/L)

The complexity of OFMSW digestate samples is better visualized in Figure 7.18. It is evident from the picture that the heterogeneity of the sample made almost impossible the observation of phenomena that can be visualized in other kinds of organic samples, under the same analysis operating conditions.

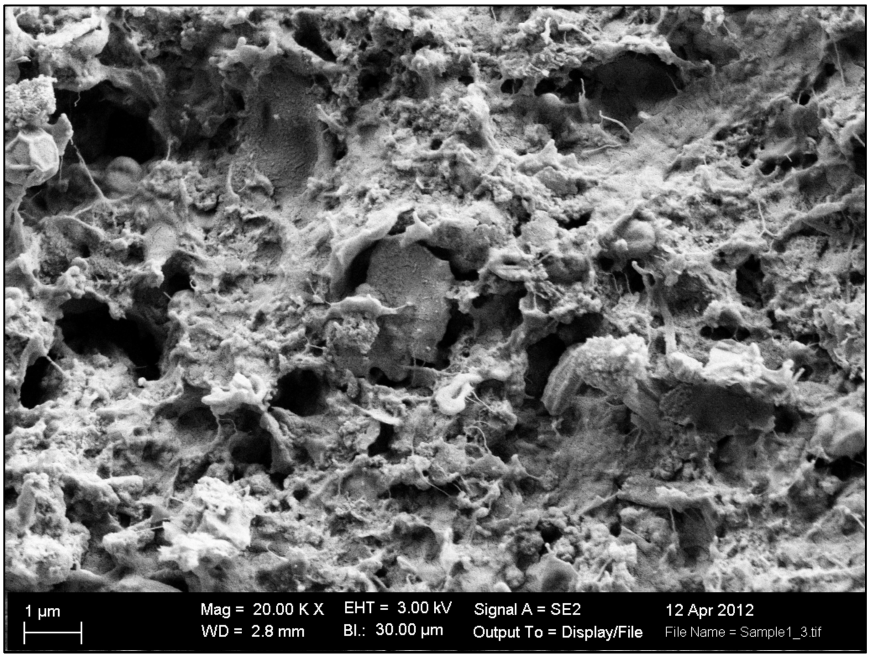


Figure 7.18 SEM observation of untreated OFMSW digestate samples (Mag=20.00 k x)

Even when focusing on similar organic matter structures, at the same magnification, any significant difference could be observed, as shown in Figure 7.19, where the comparison between untreated and sonicated (93 W h/L) OFMSW digestate samples is provided.

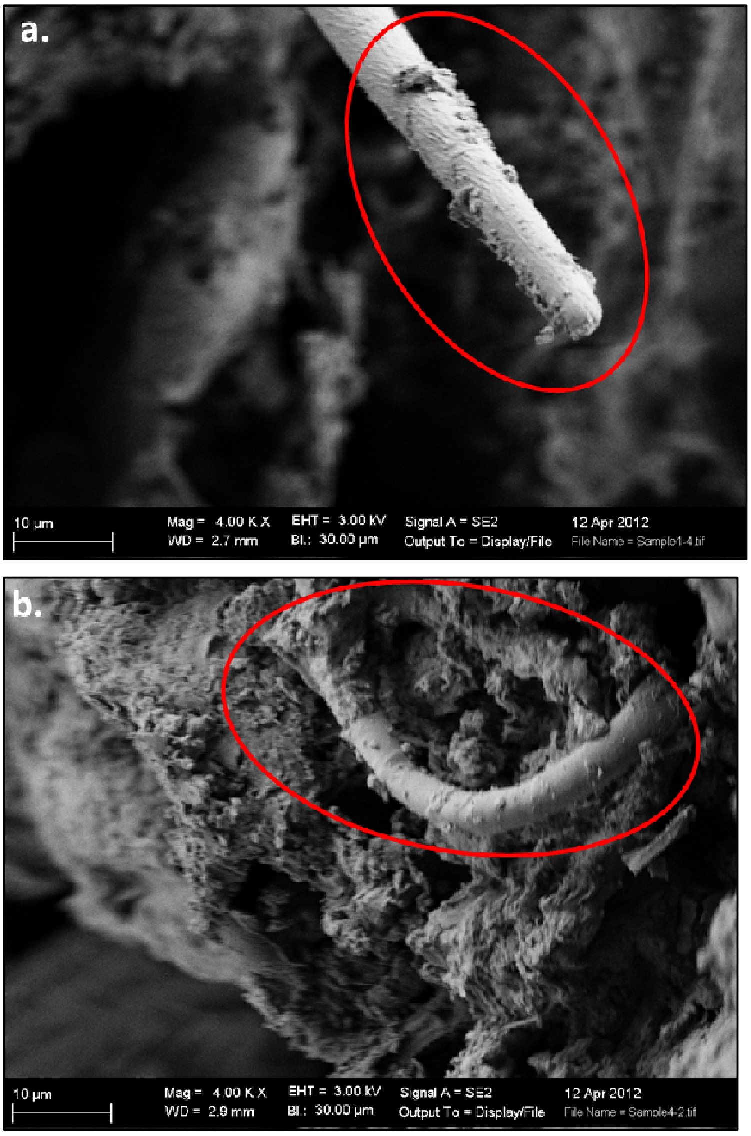


Figure 7.19 Comparison between SEM analysis of untreated (a.) and sonicated (b.) OFMSW digestate samples

Conversely, when only sludge is analysed, sonolysis effects are clearly visualized with SEM technique, as shown in Figure 7.20.

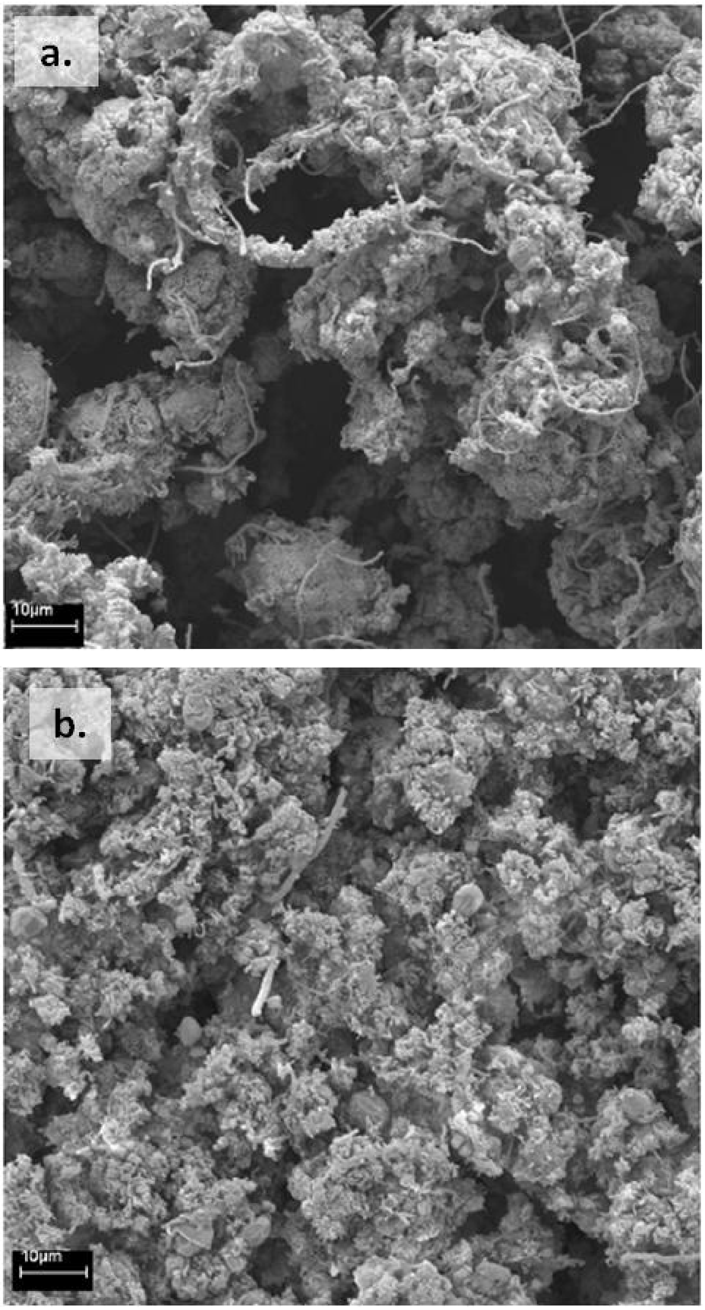


Figure 7.20 Comparison between SEM analysis of untreated (a.) and sonicated (b.) sludge samples (Neis et al., 2012)

Despite the substrate heterogeneity, according to experimental outcomes, sonolysis proved to be effective in increasing biogas yields of OFMSW digestate. Batch anaerobic digestion test results were consistent with the ones of chemical and physical determinations as well as with scientific literature reports dealing with ultrasonic pretreatment of sludge. The main difference between these substrates is related to heterogeneity level, which is much higher for OFMSW digestate than sludge, which is mainly composed of biomass. This item represents an obstacle in the estimation of ultrasonic disintegration effects on microorganisms by means of the conventional analytical techniques, which can only provide qualitative information.
However, the high biomass concentration in OFMSW digestate ensures the maintenance of high biodiversity level after the application of sonolysis, which proved not to determine any negative contribution to the microbial population.

7.2.2 Effects of sonolysis on protein-rich substrates

The effects of energy inputs applied to OFMSW digestate were also investigated on DDGS (Dried Distilled Grains with Solubles). This protein-rich substrate was analysed both in its rough and partially degraded form.
Figure 7.21 plots the comparison, in terms of soluble COD variation, between DDGS and DDGS digestate, along with OFMSW digestate.
The graph shows that, for increasing ultrasonic energy values, soluble COD improved only for the partially degraded materials. In particular, the increase in soluble COD obtained for DDGS digestate is definitely higher than the one obtained for the digested OFMSW.
This difference is, however, reduced if disintegration degree values are considered, as shown in Figure 7.22 and it can be related to the different composition of investigated substrates, in terms of soluble compounds. Before ultrasonic treatment, both the concentration of soluble COD (Figure 7.21) and the COD released by NaOH addition (COD_{NaOH}), which is assumed to result in complete disintegration of organic matter, were higher for DDGS digestate, as reported in Table 7.2.

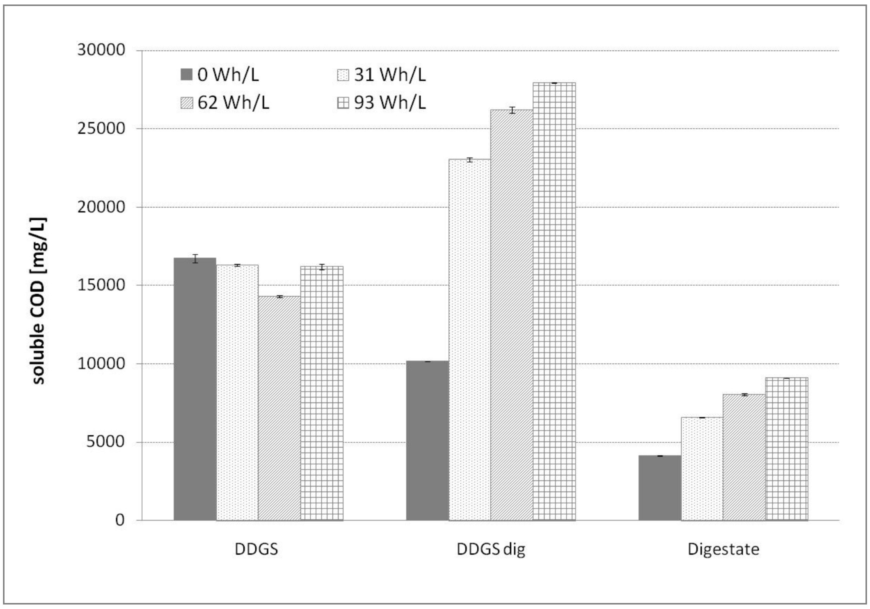


Figure 7.21 Soluble COD variation for investigated samples, after different pretreatment conditions

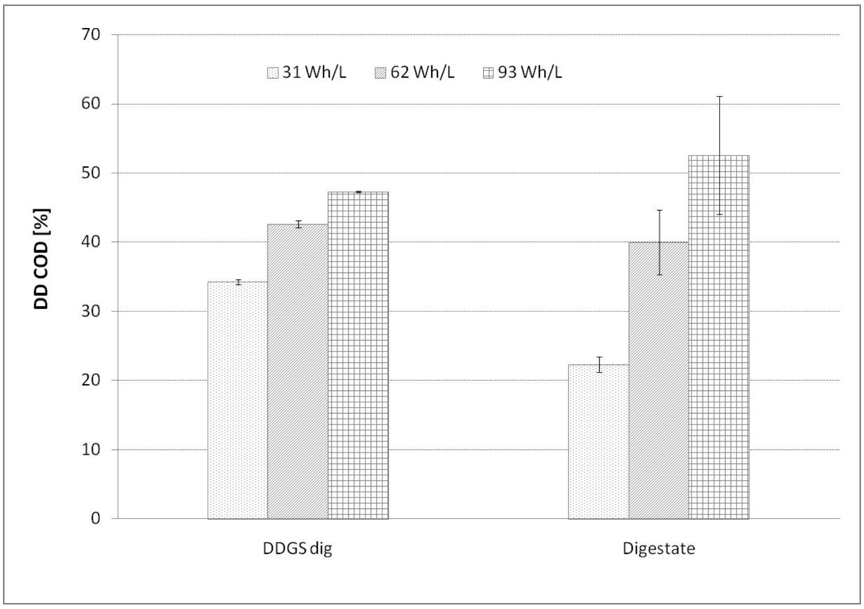


Figure 7.22 Disintegration degree for digested substrates after different ultrasonic treatments.

Table 7.2 Soluble compounds in untreated DDGS and OFMSW digestates

Substrate	sCOD [mg/L]	COD_{NaOH} [mg/L]
DDGS digestate	10 170 ± 3	47 766 ± 58
OFMSW digestate	4124 ± 20	14 788 ± 36

Conversely, improving sonolysis extent proved to determine slightly decreasing solubilisation effects on the rough protein-rich substrate. This result, however, was not related to the occurrence of mineralization phenomena. Figure 7.23, displays the volatile solid content of the investigated substrates that did not change due to sonolysis application.

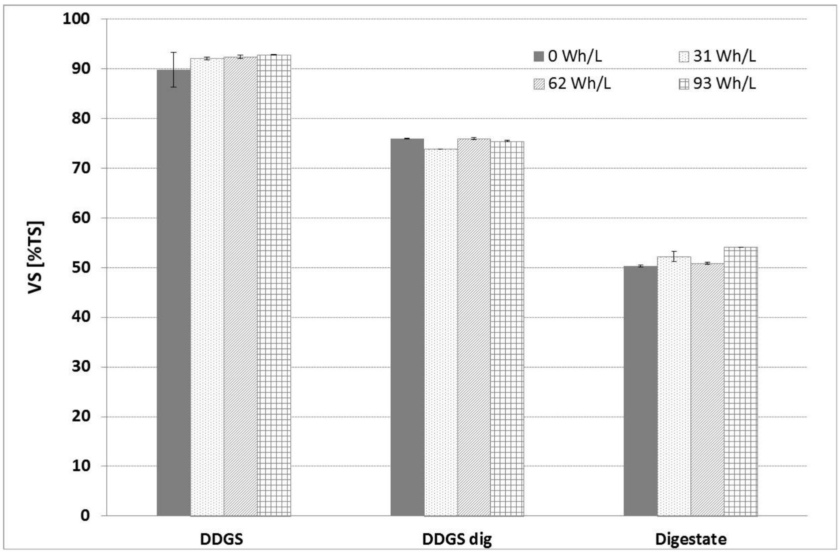


Figure 7.23 VS variation for investigated samples, after different pretreatment conditions

The analysis of the soluble fraction of investigated samples highlights, for DDGS digestate, a direct correlation between both dissolved organic carbon and nitrogen concentrations and soluble COD (Figure 7.24 and Figure 7.25). This evidence suggested that soluble COD increase was caused by the quantitative variation of soluble compounds, determined by release of both carbon and nitrogen in solution, as already discussed for OFMSW digestate.

Conversely, any meaningful relation could be established, considering the same parameters, for DDGS.

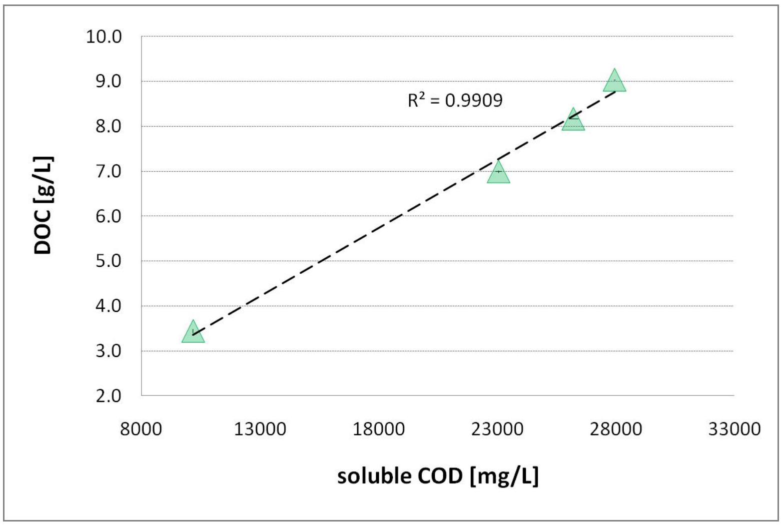


Figure 7.24 Correlation between soluble COD and DOC for DDGS digestate

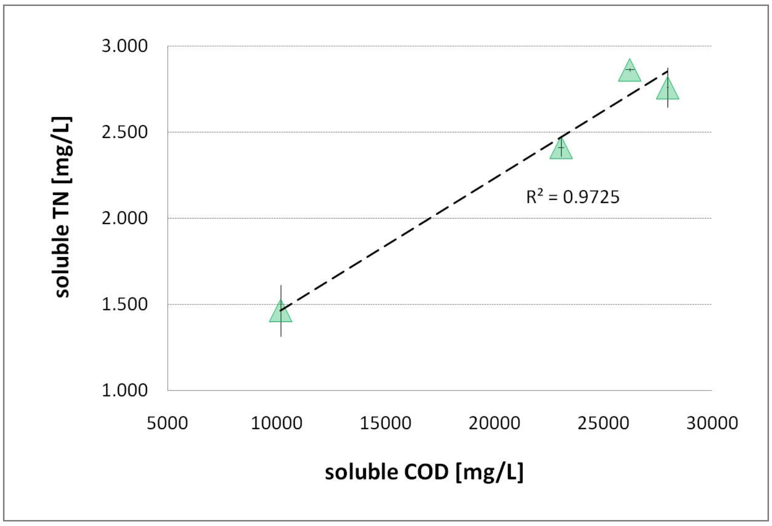


Figure 7.25 Correlation between soluble COD and soluble nitrogen for DDGS digestate

Table 7.3 reports the results of main chemical and physical parameters analysed in order to detect sonolysis effect on DDGS.

Table 7.3 Sonolysis effect on DDGS, in terms of main chemical parameters

Ultrasonic specific energy [kJ/kg$_{TS}$]	sCOD [mg/L]	DOC [g/L]	soluble TN [mg/L]
-	16 744 ± 269	5.88 ± 0.16	341 ± 6
2835 ± 63	16 317 ± 67	5.94 ± 0.06	361 ± 16
5630 ± 122	14 319 ± 67	5.98 ± 0.03	317 ± 1
8493± 252	16 198 ± 168	6.10 ± 0.10	351 ± 5

According to these results, sonolysis proved to be ineffective in increasing solubilisation of organic matter, both in terms of organic carbon and nitrogen. Similarly, any qualitative variation in terms of solubilisation was observed, as soluble COD values vary in a very narrow range for increasing ultrasonic specific energy provided.

Despite solubilisation results, anaerobic digestion tests proved that, specific biogas production from DDGS enhanced for increasing energy inputs, as shown in Figure 7.26.

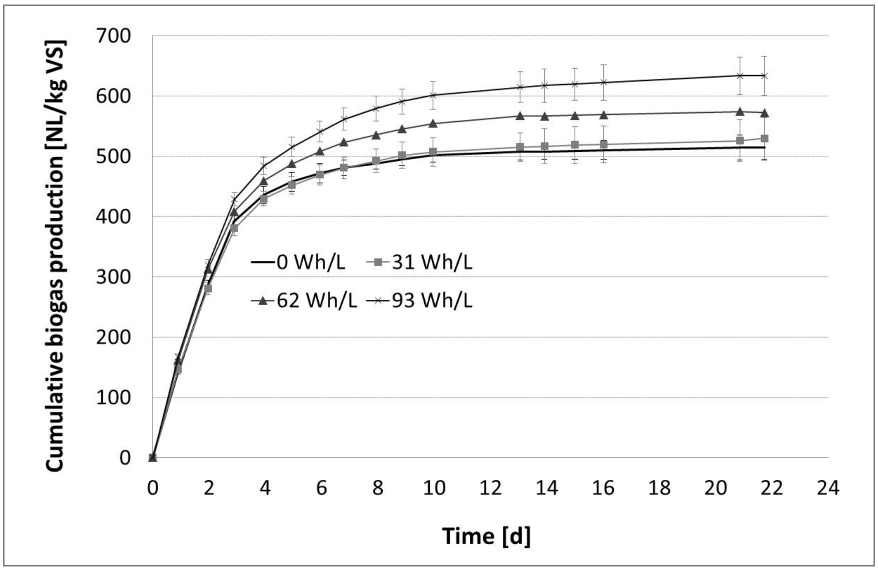


Figure 7.26 Specific biogas production of untreated and sonicated DDGS samples

Methane content remained constant, at an average value of 65.1% (with a standard deviation of 1.0). This evidence was consistent with the results of chemical and physical tests: the amount of carbon, which could be converted into biogas within the anaerobic process, did not change due to sonolysis.

Therefore, the main effect of ultrasonic energy input was reasonably the change of the substrate structure.

DDGS is mainly composed by proteins. The microscope observation of untreated samples allowed the identification of both globular and fibrous proteins, as shown in Figure 7.27 and Figure 7.28, respectively.

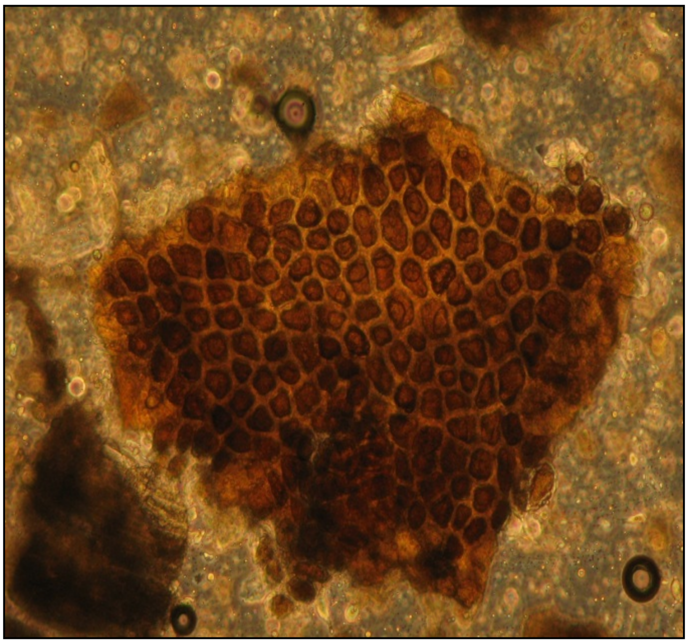


Figure 7.27 Globular protein in DDGS samples

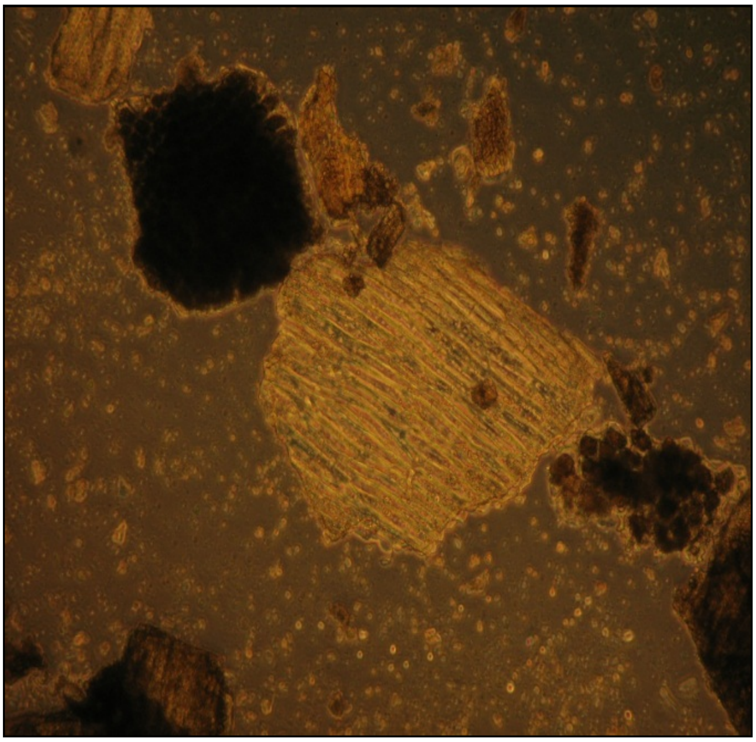


Figure 7.28 Fibrous protein in DDGS samples

A protein is a polypeptide chain made up of amino acid, linked together in a definite sequence, known as primary structure.

Due to their chemical composition and structure, the amino acids of a polypeptide chain try to minimize their free energy of contact by folding in locally identifiable, discrete structures, known as secondary structures. The final spatial configuration of a polypeptide chain can be defined as the tertiary structure of a protein. The tightly folded tertiary structure of a protein is characterized by the existence of amino acids with hydrophobic side chains in the core of the structure and amino acids with hydrophilic side chains on the protein surface. Multiple folded polypeptide molecules can, finally, be clustered together to form a quaternary structure (Sadowski and Taylor, 2010).

All these structures define the native configuration of a protein.

When ultrasound is applied to protein-rich substrates, it may determine a variation in protein structure, as reported in several studies dealing with protein rich food processing. The alteration of the native structure of a

protein from the loss of its quaternary, tertiary and secondary structure, resulting in its unfolding, is called denaturation.
Environmental conditions, such as changes in temperature, pH, exposure to shear forces, contact with organic solvents and chemical denaturants may induce destabilization and cause denaturation of the protein (Schiffter, 2011). Therefore, the collapse of gas bubbles, which produces high-intensity shock waves, microjets, shear forces and turbulence, can promote the same effects.
Denaturation usually results in the loss of biological function and/or specific properties of the protein, such as the reduction of its solubility (Chandrapala et al., 2012) and the increase in digestibility (Ljøkjel et al., 2000; Sagum and Arcot, 2000), as found in this investigation.
The simplification of protein structure, by its unfolding, makes the protein itself more easily available to microorganism attack. The same phenomenon can influence solubility by allowing the exposure of the hydrophobic side chains rather than the hydrophilic ones.
Therefore, the increase in biogas volumes can be related to the occurrence of denaturation phenomena, which could also explain the absence of solubilisation.
The extent of denaturation phenomena depends on both denaturating conditions and the original protein configuration. Under the same operating conditions, effects on the investigated rough protein-rich substrate proved to be different from the ones obtained on its partially degraded form.
Despite the enhancement in soluble COD provided by sonolysis on DDGS digestate, cumulative biogas production slightly decreased for increasing energy inputs (Figure 7.29) and methane content in biogas was constant at an average value of 60.8% (with a standard deviation of 1.6).

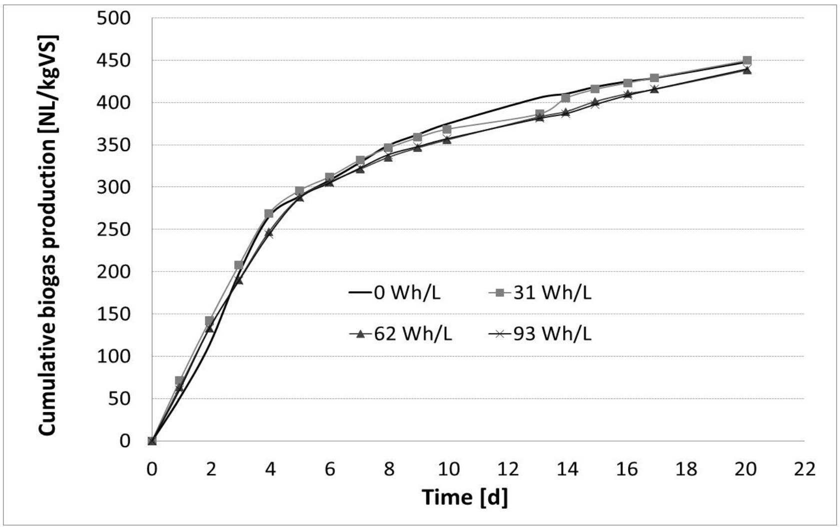


Figure 7.29 Specific biogas production of untreated and sonicated DDGS digestate samples

Results of the cumulative biogas production are also reported in Table 7.4, in order to highlight the slight variation of the average values plotted in the graph.

Table 7.4 Sonolysis effect on DDGS digestate, in terms of biogas production

Ultrasonic specific energy [kJ/kg_{TS}]	Biogas production in (21 days) [NL/kg_{VS}]
-	448.15 ± 29.57
2264 ± 4	449.63 ± 1.86
4581 ± 59	438.46 ± 6.03
6858 ± 46	439.80 ± 8.56

As the improved solubilisation did not result in increased biogas production from sonicated substrates, the increase in organic carbon and nitrogen solubilisation, reported in Table 7.5, could not be exclusively related to sonolysis effects on biomass. Under this hypothesis, soluble compounds would have been available to microorganism attack, resulting in higher biogas volumes.

Table 7.5 Increase in organic carbon and nitrogen solubilisation after ultrasound application to DDGS digestate samples

Ultrasonic specific energy [kJ/kg$_{TS}$]	sCOD [mg/L]	DOC [g/L]	soluble TN [mg/L]
-	10 170 ± 3	3.44 ± 0.04	1463 ± 151
2264 ± 4	23 055 ± 134	7.00 ± 0.02	2413 ± 52
4581 ± 59	26 210 ± 198	8.17 ± 0.00	2864 ± 8
6858 ± 46	27 950 ± 35	9.04 ± 0.00	2760 ± 115

This evidence is reasonably related to the prevailing occurrence of sonolysis effects on the protein residues of DDGS digestate.
In DDGS digestate, proteins have undergone a biological degradation process, which necessarily results in the alteration of the native configuration of protein itself. Therefore, the presence of both folded and unfolded proteins could be expected in DDGS digestate.
The interaction between the native and unfolded states of proteins, which is schematically represented in Figure 7.30, has been traditionally recognized as the explanation of larger-molecular-weight aggregates (Schiffter, 2011), reasonably difficult to biodegrade.

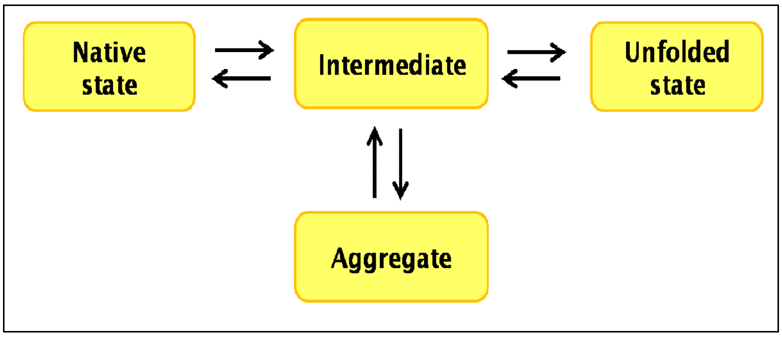


Figure 7.30 Schematic representation of relationship between possible protein states (adapted from Shifter et al., 2011)

Although analytical results suggested this hypothesis, further analysis are required to monitor the actual occurrence of the proposed mechanisms due to sonolysis application. To this end, electrophoresis techniques can be applied.
However, these analytical techniques involve the alteration of the protein structure. Their effective implementation must be, therefore, carried out on specific single proteins rather than on their mixture, so that the

resulting alteration can be exclusively ascribed to sonolysis and not to the many variables affecting the analysis of complex protein-rich substrates, as the one considered in the present work.

7.3 THE FEASIBILITY OF SONOLYSIS AS PRETREATMENT OF ORGANIC WASTE FOR ANAEROBIC DIGESTION

The results of experimental activity showed that sonolysis can positively affect anaerobic digestion yields of both OFMSW and DDGS. However, as the composition of the organic substrate proved to play a fundamental role, different consideration raised for both OFMSW and DDGS, if the feasibility of sonolysis as treatment prior to anaerobic digestion is assessed for its possible scale up.

Sonolysis focused studies were performed with the basic unit of the ultrasonic device designed by Ultrawaves GmbH (Hamburg, Germany): therefore, this device was considered to discuss the process scale up with reference to both investigated substrates.

7.3.1 Sonolysis pretreatment of OFMSW

The effectiveness of sonolysis on the organic fraction of municipal solid waste addressed to anaerobic digestion process was successfully investigated.

The disintegration provided by ultrasonic cavitation phenomena was studied both on the rough and partially degraded form of this substrate.

In the latter case, as already pointed out, a reduction in energy inputs can also be achieved. Moreover, sonolysis of rough substrate requires the addition of water, thus limiting its installation within plants operating with wet (TS < 10% in the reactor) anaerobic digestion technologies. Conversely, the treatment of OFMSW digestate widens sonolysis application, as this material is characterized by dry matter content compatible with the direct application of the ultrasonic technology.

Results of the experimental activity highlighted that the ultrasonic treatment of OFMSW digestate, in the range 31-93 Wh/L, determined:

- absence of mineralization phenomena;
- disintegration degree improvement varying between 22 and 53%;

- increase in specific biogas production ranging between 46 and 71%.

Moreover, methane content enhanced for increasing ultrasonic energy supply as well as during digestion time.

According to these considerations, within anaerobic digestion facilities treating OFMSW, the implementation of ultrasonic equipment can be considered as represented in the process scheme of Figure 7.31.

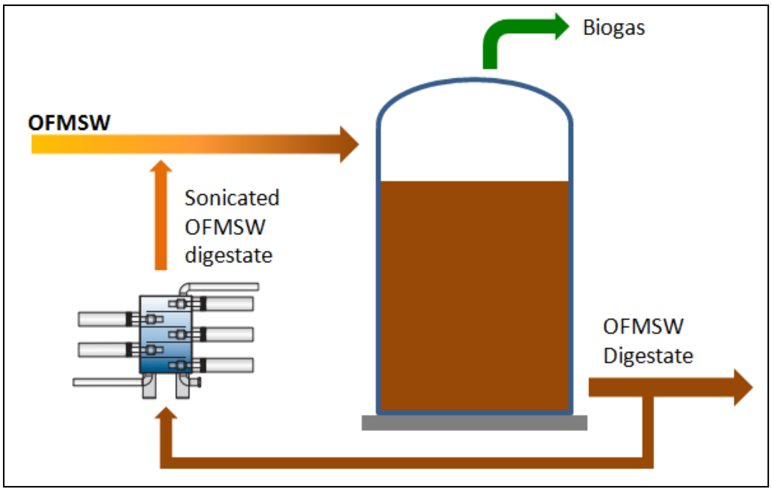


Figure 7.31 Process scheme for sonolysis application within OFMSW anaerobic digestion facilities

As the recirculation of digestate is often used to provide addition mixing system, sonolysis can also be considered for the upgrade of existing plants.

The economic feasibility of the combined sonolysis/anaerobic digestion process depends on the additional methane recovery that can be achieved by means of the ultrasonic treatment. The operation of an ultrasonic device is affordable if the incomes originated by the energy production can balance the costs of the energy consumption required to run the device itself.

This kind of analysis is highly influenced by several factors, including main anaerobic digestion operating parameters, such as the organic loading rate and the hydraulic retention time. These factors typically influence both the produced amount of biogas and the characteristics of

the digestate that is further processed by means of the combined sonolysis/anaerobic digestion process.
Similarly, the design of the ultrasonic device can properly amplify cavitation phenomena, thus influencing disintegration effects.
The equipment under investigation was designed following a pilot scale experimentation carried out, in continuously operating systems, to verify the performances of ultrasonic treatment prior to sludge anaerobic digestion processes running with different retention time of the organic substrate. It was proved that the portion of non-degradable matter that exists in each type of biosolids was reduced 15% (Nickel and Neis, 2007).
Therefore, it can be reasonably assumed that approximately 15% increase in biogas production can be achieved by means of the investigated ultrasonic device, in continuously operated systems.
The reliability of this assumption has already been proved by monitoring the results of anaerobic digestion yields in full-scale facilities where the considered ultrasonic device was installed.
Samples withdrawn in these plants, treating both wastewater and maize, and investigated under the operating conditions used in this research, showed that the disintegration degree was in direct correlation with ultrasonic energy input. Figure 7.32 shows the trend for both sludge and farm biogas plant samples (Vural et al., 2011) and highlights that results of the present research are comparable with them.

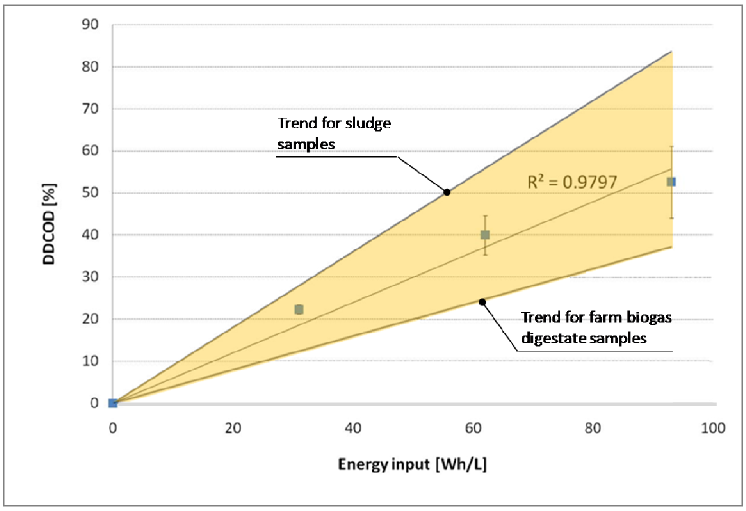


Figure 7.32 Operating condition diagram for sonolysis application

The evaluation of the economic feasibility of an ultrasonic installation was carried out considering the operating parameters of Luebeck plant, where the OFMSW digestate under investigation was sampled.
Results can, however, be extended to other facilities working under the operating conditions reported in Table 7.6.

Table 7.6 Luebeck plant fermenters: operating conditions (information provided by Luebeck plant operators)

Parameter	Value
Input flow [m^3/d]	300
Retention time [d]	15
Operating volume [m^3]	4500
Organic load [kg_{VS}/m^3 d]	4,77
Biogas production [m^3/d]	4000-5000
Methane content [%]	63
Energy production [kWh/year]	3 000 000

A 15% increase in biogas production, with respect to the lower declared yields (4000 m^3/d) results in additional 600 m^3/d of biogas: total biogas production after ultrasonic pretreatment of a 1-2 m^3/d of OFMSW digestate is 4600 m^3/d.
Assuming 60% methane in biogas, so that its lower calorific value is 5.9 kWh/m^3, and 38% electrical efficiency (as estimated by Ultrawaves GmbH), it is possible to evaluate the economic benefits, summarized in Table 7.7.

Table 7.7 Economic benefits from ultrasonic installation

Parameter	Value
Energy surplus [kWh_{ele}/d]	1345
Energy surplus [kWh_{ele}/y]	490 998
Electricity price [€/kWh]	0.12
Energy saving [€/d]	159
Energy savings [€/y]	57 938

The price for electricity was deducted by Eurostat data, updated to 2011 and it was assumed constant in time. It refers to the average price estimated in the European Union area, which is 40% lower of the one which was estimated to be applied in Italy in the same year.

Almost 60 000 €/year can be saved by the additional electrical energy that can be produced by means of the ultrasonic installation. The device, however, requires energy consumption related to:

- the operation of the reactor itself as well as the one of the pumping system;
- the routine maintenance;
- the replacement of sonotrodes, which are damaged by the cavitation itself.

The evaluation of the annual costs associated to the operation of the single ultrasonic device is reported in Table 7.8.

Table 7.8 Operating costs associated to ultrasonic equipment operation

Item	Description	Value	Total cost [€/y]
Reactor operation	Power requirement [kW]	5	5168
	Working hours [h/a]	8760	
	Electricity price [€/kWh]	0.12	
Pumping system operation	Number	1	1551
	Power requirement [kW]	1.5	
	Working hours [h/a]	8760	
	Electricity price [€/kWh]	0.12	
Ordinary maintenance	Work hours [h/week]	1	1560
	Specific price [€/y]	30	
Replacement of sonotrodes	Number of sonotrodes	5	2000
	Price of sonotrode [€/unit]	800	
	Replacement frequency [y^{-1}]	2	
Total annual cost for ultrasonic system operation			**10 279**

On the basis of these results, a net electrical saving of approximately 47000 €/year was estimated.

Additional advantages, which were not considered in the evaluation of the process economic feasibility, are related to:

- the reduction of digestate to handle at the end of the process;
- the improved characteristics in terms of digestate viscosity provided by the ultrasonic treatment, which allows the reduction of energy requirement for mixing system within the digester.

It is also possible to state that the capital costs for the whole ultrasonic system, which account to approximately 110 000 €, including interest charge (3% interest rate), can be recovered approximately in three years, as reported in Table 7.9.

Table 7.9 Evaluation of capital cost amortization

Time [year]	Initial capital [€]	Interests [€]	Final capital [€]
1	- 110 000	- 3300	- 65 641
2	- 65 000	- 1969	- 19 952
3	- 19 952	- 599	27 109
4	27 109	813	75 581

Both the technical and economic feasibility assessments show the benefits of ultrasound as pretreatment of OFMSW anaerobic digestion, thus widening the application field of this technology.

7.3.1 Sonolysis pretreatment of DDGS

According to the experimental results, different considerations should guide the effectiveness of sonolysis as DDGS pretreatment for its anaerobic digestion.
As already pointed out, the improvement of anaerobic biodegradability of the protein rich substrate was proved only for its rough form. Sonolysis of DDGS digestate, despite an improvement in solubilisation, did not result in any variation in biogas production.
On the basis of the results of anaerobic digestion tests, a possible process scheme is showed in Figure 7.33.

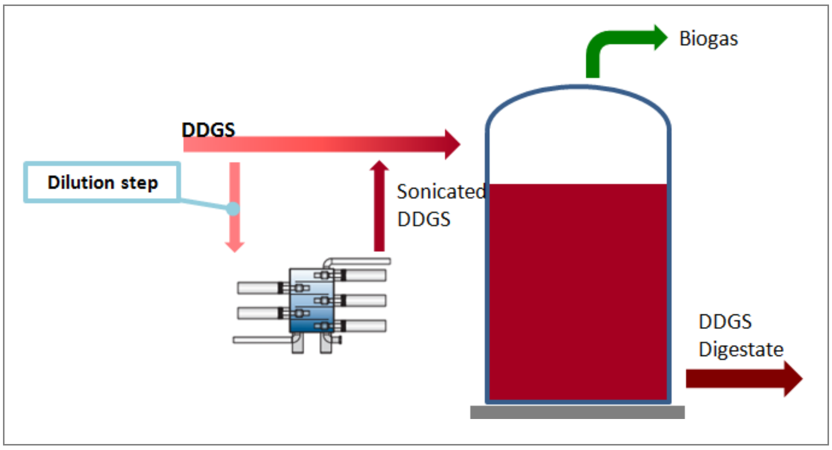


Figure 7.33 Process scheme for sonolysis application as DDGS pretreatment for anaerobic digestion

Dilution step is fundamental to provide the occurrence of cavitation phenomena. Its presence, however, limits sonolysis application to wet anaerobic digestion technologies and involves additional capital as well as operating costs.
However, water addition could be avoided if the whole DDGS production process as well as the different mass flows involved are considered.
As already mentioned, DDGS originates from the drying process of the stillage originated during ethanol fermentation. According to the kind of feedstock used within the process, stillage can be characterized by a dry solid content up to 10% (Davis et al., 2005), which is favourable for direct ultrasound application.
In this context, benefits would be achieved by:

- the elimination of the energy consuming drying phase;
- additional biogas production, which can be provided by ultrasound application.

It should be, however, pointed out that, under investigated operating conditions, DDGS sonolysis provided biogas improvement in the range 3-23%. The same energy inputs allowed a higher increase in biogas production of OFMSW digestate, in the range 46-71%.
Therefore, different results are expected from the study of the combined sonolysis/anaerobic digestion process operating continuously.
The absence of a direct correlation between disintegration degree and energy inputs as well as between solubilisation and biogas production suggested the occurrence of reaction mechanisms different from the ones monitored for ligno-cellulosic materials.
Further research is required to provide an in-depth analysis of those mechanisms.
To this end, sonolysis should be applied to singular proteins, in their rough as well as partially degraded form, in order to verify the denaturating effects, both in terms of solubilisation and anaerobic digestibility.
Moreover, as the substrate composition influences mechanisms effects, additional research should be directed towards the definition of any relation between sonolysis effects and different mixtures of proteins.
It seems, therefore, untimely to assess the technical feasibility of the combined ultrasound/anaerobic digestion process for DDGS or stillage treatment.

Further studies are required to clarify sonolysis effectiveness as function of the different factors influencing the yields of its application on protein-rich substrates.

If adequate research demonstrates the effectiveness of sonolysis as pretreatment of theses substrates for its anaerobic digestion, the economic feasibility can be assessed, with particular reference to the possible up-grade of combined bioethanol and biogas production processes.

The ethanol stillage can be utilised for biogas production, in order to recover energy for the process requirements. Depending on the form of biogas utilisation and plant size it is possible to cover more than 76% of the whole energy demand of the bioethanol process (without DDGS production) by the use of the biogas originated from the residues of the bioethanol production (Pfeffer et al 2007).

In this context, sonolysis can promote the reduction of digestion time, thus providing a higher capacity within biogas plants integrated with biorefinery facilities.

8 CONCLUSIONS

Anaerobic digestion has become widespread throughout Europe for the treatment of the organic fraction of municipal solid waste, as it allows the biological stabilization of organic matter along with the production of energy from biogas.

Although anaerobic digestion is a well-established technology, every attempt to improve its yields in terms of biogas production is of great interest, due to its technical and economic implications.

The pretreatment of anaerobic digestion feedstock aims to promote the hydrolysis of complex organic matter into soluble compounds, which has been recognized as the rate limiting step within anaerobic digestion processes. The improved solubilisation results in higher amounts of organic matter available within the biological process and, consequently, in enhanced biogas production.

This aspect is much more important if very heterogeneous materials, such as the organic fraction of municipal solid waste (OFMSW), are considered.

Scientific literature reports the application of several kinds of pretreatments to OFMSW addressed to anaerobic digestion.

Ozonation and sonolysis, which have been traditionally used as wastewater advanced oxidation processes and have been widely investigated as pretreatment of liquid substrates addressed to anaerobic digestion, are innovative technologies for OFMSW pretreatment.

Therefore, the research discussed in this work dealt with the application of both sonolysis and ozonation as pretreatment of solid organic waste to be processed by means of anaerobic digestion.

A comparative analysis was performed, in order to find out the process providing the better performances as pretreatment of OFMSW before anaerobic digestion.

Results proved that both ozonation and sonolysis can promote the solubilisation of organic matter.

However, ozonation did not show a significant improvement in biogas yields. In spite of a solubilisation increase ranging between 45 and 56%,

the production of biogas was lower than the one observed for untreated substrates.
This is likely due to the formation of by-products less biodegradable than rough material. The BOD_5/COD ratio of the sample soluble fraction decreases from 0.80 to 0.53 for ozone doses increasing between 0.16 and 1.2 g_{O3}/g_{TS}.
On the other hand, ultrasound technology proved to be effective in enhancing both solubilisation and biodegradability of OFMSW samples, so that biogas production increases from pretreated substrates was also observed.
A specific energy input of 15 000 kJ/kg_{TS} resulted in 9% soluble COD increase. Solubilised compounds were more biodegradable than rough OFMSW, so that biogas production from sonicated substrates proved to be approximately 15% higher than the one obtained from untreated ones.
As sonolysis enhanced both the amount and the biodegradability of soluble compounds, it proved to be more competitive than ozonation.
For the latter process, strict operating conditions in terms of ozone doses should be kept in order to avoid the formation of low-biodegradable compounds and this drawback would be a relevant obstacle to the process scale up.
According to these outcomes, further studies were focused on sonolysis, in order to assess the technical and economic feasibility of the process as OFMSW pretreatment for anaerobic digestion. Moreover the relation between ultrasonic pretreatment effects and organic matter composition was assessed.
To this end, the basic unit of a full-scale device was used.
Previous studies carried out with this device showed that comparable solubilisation and biogas production can be achieved by energy inputs up to eight-fold lower than the ones required to treat rough substrate. Therefore, the optimization of the combined sonolysis/anaerobic process, in terms of energy input reduction, required to apply ultrasound to OFMSW digestate rather than rough OFMSW.
Experimental results showed that specific energy inputs varying between 2102 and 6291 kJ/kg_{TS} (correspondent to 31-93 W h/L) allowed a disintegration degree improvement ranging between 22 and 53% and did not determine any mineralization phenomena. Moreover, an increase in specific biogas volumes between 46 and 71% was observed and methane content enhanced for increasing energy inputs as well.

Despite lower energy inputs, sonolysis effects on both solubilisation and anaerobic digestion of OFMSW digestate were higher than the ones obtained on rough substrate.
This evidence was related to the composition of the organic matter as well as to the design of the sonotrode, which allowed a more homogeneous distribution of ultrasonic effects into the whole sample.
The role of substrate composition was better highlighted from the comparison between sonolysis effects on OFMSW digestate, which is mainly a ligno-cellulosic material, and DDGS, which is a protein-rich substrate. DDGS was investigated both in its rough and digested form.
Experimental results proved that ultrasonic energy inputs in the range 31-93 W h/L can improve rough DDGS anaerobic degradability up to 23%, despite the solubilisation achieved.
This evidence can be explained by the occurrence of denaturation phenomenon, which is the alteration of the native configuration of a protein, resulting in its unfolding. The consequent simplification of the protein structure makes it more easily available to microorganism attack. The same process can differently influence solubility by allowing the exposure of the hydrophobic side chains rather than the hydrophilic ones.
The extent of denaturation phenomena depends on both denaturating conditions and the original protein configuration. Under the same operating conditions, effects on the investigated rough protein-rich substrate proved to be different from the ones obtained on its partially degraded form.
For protein-rich substrates, experimental results showed the absence of a direct correlation between disintegration degree and energy inputs as well as between solubilisation and biogas production, suggesting the occurrence of reaction mechanisms that need to be further investigated.
To this end, sonolysis should be applied to singular proteins, in their rough as well as partially degraded form, in order to verify the denaturating effects, both in terms of solubilisation and anaerobic digestibility. Moreover, additional research should be directed towards the definition of any relation between sonolysis effects and different mixtures of proteins.
Different consideration arises for OFMSW sonolysis.
Experimental results proved that OFMSW anaerobic digestion yields can be enhanced by feeding the reactor with sonicated digestate. To this end,

a full-scale installation should involve the recirculation of part of the digestate pretreated by ultrasound.

OFMSW digestate is usually characterized by dry matter content lower than rough OFMWS and compatible with the direct application of the ultrasonic technology. Conversely, OFMSW requires a dilution step to allow ultrasound application, thus limiting the installation of this technology within plants operating with wet (TS < 10% in the reactor) anaerobic digestion systems.

Therefore, the treatment of OFMSW digestate widens sonolysis application field and, as the recirculation of digestate is often used to provide addition mixing system into anaerobic digesters, sonolysis can also be considered for the upgrade of existing plants.

The combined sonolysis/anaerobic digestion process is economically feasible if the additional energy recovery provided by sonolysis ensures higher incomes than the operating costs of the ultrasonic device.

Following the analysis of the direct correlation between ultrasonic energy input and disintegration degree of OFMSW digestate samples, a 15% increase in biogas production can be expected by the installation of an ultrasound device in a full-scale continuously operated facility. Under this assumption, it was proved that:

- the energy savings coming from the additional biogas production cover the costs of the energy consumption required to run the ultrasonic device;
- the capital costs of the ultrasonic equipment can be recovered in approximately three years.

These results proved that sonolysis is a reliable and affordable technology, which can be successfully applied to promote the hydrolysis of ligno-cellulosic materials thus enhancing anaerobic digestion yields of complex and heterogeneous organic solid wastes.

As the results proved to be promising for sonolysis to be widespread within OFMSW anaerobic digestion facilities, further studies should be addressed towards the evaluation of the qualitative characterization of the digestate to be handled after the combined ultrasonic/anaerobic digestion process.

It is well-known that OFMSW digestate can be used, directly or after further treatments, for soil applications.

Therefore, its characterization in terms of toxic substances is fundamental in order to reduce the risk associated to the use of this substrate on soil, especially for agricultural purposes.

The removal of compounds such as pesticides, which can enter a digester along with organic waste, by means of digestate sonolysis would make this technology particularly attractive, as not only methane increase but also a qualitative improvement of the digestate could be provided.
This kind of research should be carried out along with the analysis of the main digestate properties, to be identified according to its specific use.
An additional interesting aspect to be further investigated is related to the rationalisation of both ultrasonic device design and installation feasibility assessment.
The creation and distribution of cavitational bubble fields is not adequately described by theoretical models, with few exceptions that are valid for simple single phase systems like water.
The existing models cannot be properly extended to sludge nor to solid organic substrates. In this context, the development of mathematical models could support the design of ultrasonic devices, which is currently based on empirical know-how.
Similarly, the technical and economic feasibility of an ultrasonic installation can vary according to the factors affecting the optimization of the combined ultrasonic/anaerobic digestion process, so that specific tests are usually required. The development of adequate theoretical models could, therefore, represent an alternative option to the undertaking of dedicated experimental activity.

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