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HIGH-FREQUENCY ULTRASOUNDS FOR THE DECONTAMINATION AND INTENSIVE STABILIZATION OF SEWAGE SLUDGE

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1. INTRODUCTION

1.1 SLUDGE MANAGEMENT: FROM SEWAGE TO AGRICULTURE

1.1.1 Critical issues of wastewater treatment

Up to date municipal wastewater treatment plants (WWTP) were seen as an end-of-pipe treatment just before discharge, having the aim to avoid eutrophication and hygienic health hazard in surface water. Due to the global demographic trends as well as new legislations (e.g. the Water Framework Directive, WFD) increased focus is put on quantity and quality of effluents: more and more seen as interface between sanitation and environment, WWTP are delivering resources to the environment and for human activities (recharge of drinking water reservoirs, recycling of nutrient, efficient energy use).

In fact, modern water management requires WWTP to cover a variety of goals. Initially centralized WWTP where introduced for reducing the organic load conveyed by urban sewer systems and emitted as point sources into the aquatic environment. Between late 1960s and the early 1980 the requirement of nutrient removal in WWTP became evident by the eutrophication of surface waters. Recent evidence is showing that WWTP are on the verge of a second upgrading round, since environment protection as required by new legislation (e.g. Water Framework Directive) as well as drinking water customer protection require a paradigm shift in urban wastewater treatment:

This situation has implications on the quality goals set for WWTP products:

Existing focus:	*New focus:*
● nutrient removal	● nutrient recycling
● pathogens removal	● micropollutants and ecotoxicity removal
● energy optimization	● energy production
● sludge disposal	● reuse of sludge and of its resources

These objectives will be achieved by optimal integration of various (proven and innovative) treatment steps. The most prominent change in sewage treatment in near future will be the focus on managing all relevant toxicity issues of wastewater. Several of these issues (e.g. removal of pesticides) are managed within safe drinking water production, even though the aquatic environment would require similar protective measures. According to efficiency considerations the tuned combination of waste design with source monitoring and control (e.g. product labeling, ban of hazardous compounds), source separation options (storm water, hospital and industrial wastewater treatment) and centralized treatment, will be required to improve the present situation of uncontrolled (and to a significant extent un-assessed) toxicity currently being discharged from centralized municipal wastewater treatment.

In developed countries the energy spent for municipal wastewater treatment is in the range of 3 to 10 $W\cdot cap^{-1}$ (mainly in the form of electricity used for aeration and pumping; energy consumption per amount of wastewater treated: 0.2 - 0.6 $kWh\cdot m^{-3}$; amount of wastewater: 0.4 $m^3\cdot cap^{-1}\cdot d^{-1}$). Ten times more energy is currently used for household warm water production. Considering the importance of water resources and aquatic ecosystems, this represents a small amount, compared to the total primary energy consumption of society ranging between 6,000 and 12,000 $W\cdot cap^{-1}$ in Europe and the US respectively (EEA -European Environment Outlook, European Environmental Agency, Copenhagen, 2005). This indicates that wastewater treatment is not a main focus for reducing the overall energy consumption. Nevertheless, efficiency is a major issue for achieving a sustainable society, and the water sector is no exception at this.

The chemical energy harnessed in wastewater in form of organic contaminants corresponds to 20 $W\cdot cap^{-1}$ (chemical oxygen demand of loads typically discharged into wastewater 120 $gCOD\cdot cap^{-1}\cdot d^{-1}$; specific oxidation enthalpy 14 $kJ\cdot g^{-1}$). With anaerobic sludge digestion up to 7 $W\cdot cap^{-1}$ can be recovered in the form of biogas (calorific value 6.5 $kWh\cdot m^{-3}$ biogas, production 27 $L\cdot cap^{-1}\cdot d^{-1}$). Due to the efficiency of power generation (30%), with state of the art wastewater treatment energy self-sufficiency is not achievable unless significant amounts of industrial wastes can be added to the anaerobic digester (i.e. improving the biogas production).

Besides the exploitation of chemical energy contained in wastewater, also improving the energy efficiency may substantially contribute in lowering energy consumption for a given quality target for the treated wastewater.

In Europe the Water Framework Directive (Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy, European Union, 2000) explicitly aims at preventing deterioration of surface water and at improvement of the aquatic environment considering human use. Against the background of increasing population and increasing urbanization, centralized municipal wastewater treatment is going to play a role of growing importance by contributing to the following water status aspects: a) nutrient conditions and b) pollution by priority substances and other specific substances.

Foy (Foy R.H., 2005) reviews that both nitrogen and phosphorus inputs critically influence the ecology (i.e. the trophic state) of surface waters. The implications of eutrophication on environment, human health and economy (e.g. tourism) are summarized by Volterra and Boualam (Volterra and Boualam, 2002). For both nutrients the main inputs are coming from agriculture and natural background as main diffuse sources, and municipal wastewater as major point source. The relative importance strongly depends on a) agricultural activity, b) local population density and c) technology standard implemented in WWTPs. Roughly, agriculture is contributing two third of the N and one third of the P emission into surface and marine waters (OECD ,2001). The rest is originating mainly from municipal discharges. Switzerland may serve here as an example of a country with a fairly high fraction of the population connected to WWTP (95%) but still significant potential for improving nutrient removal: the current 30% of the nitrogen load in municipal wastewater denitrified could be raised to the European standard of 60%, and the 80% of P removal to 90%. Since these point sources are currently responsible for 40% and 58% of total N and P loads into the aquatic environment the optimization of WWTP would have impact on the WFD goals. This illustrates, that municipal wastewater still bears a significant optimization potential to further improve the nutrient condition of the aquatic environment. Concerning organic micropollutants, a quantitative discussion about the relevance of the various compounds (e.g. comparing the impact of pesticides, industrial chemicals, pharmaceuticals and hormones) and about their sources (e.g. agriculture, manufacturing and household consumption) is currently not possible and not yet enough quantifiable (Sumpter, J. P., 2005). Nevertheless the following considerations are suggesting, that municipal wastewater represent a significant emission source as well as an opportunity of applying centralized removal processes. The consumption of pesticide in developed countries may be expected in the range of 0.2 - 1.6 $kg \cdot cap^{-1} \cdot a^{-1}$ (OECD, 2001), which is significantly higher than for pharmaceuticals. But in surface water compounds originating from agriculture and from human settlements may have similar relevance: for compound of both groups the concentration range observed in surface waters is up to low $\mu g \cdot L^{-1}$ levels (Starner, 2005; Ternes,1998). This is understandable, since only a low percent share of the applied pesticide fraction is being lost to surface water (Leu, 2004) while a significant number of pharmaceuticals is being discharged first into the sewer and later into the receiving surface waters, because many are not efficiently removed in current WWTP (Joss, 2005).Further WWTP may contribute significantly to the total pesticide load emitted into the aquatic environment. Finally also other micropollutant sources passing through WWTP may be of quantitative relevance.

The following groups of bioactive compounds are found in municipal wastewater in quantities that may justify focusing wastewater treatment on their targeted removal:

- hormones and other endocrine disruptors
- antibiotics, disinfectants, antiseptics and other biocides
- pharmaceuticals such as diclofenac and cytostatic agents
- personal care products: UV-protecting agents, fragrances
- brominated flame retardants
- polyfluorinated and anionic surfactants

1.1.2 Definition and types of sludge

Nowadays, management of the sludge produced in the wastewater treatment plants (WWTP) represents a global environmental and economic issue. The volume of the sludge extracted from primary and secondary settling tanks is about 2 % of the volume of treated wastewater (WW). In spite of this negligible volume, sludge treatment and disposal entails very high capital and operating costs, which can account for as high as 50 % of the total costs of the WW treatment plant. Sewage sludge disposal is a serious worldwide problem. Because of increased environmental awareness and stringent environmental standards governing the disposal of sewage sludge (set by different environmental protection agencies), its utilization in agricultural production has been gaining increasing interest and attention in recent years. It offers economic and nutrient recycling advantages over the traditional disposal options, such as incineration for dry sewage and sea disposal (Stone et al. ,1998). Nevertheless, potential risks derived from the accumulation of heavy metals and organic compounds, as well as pathogen contamination, must be taken into consideration.

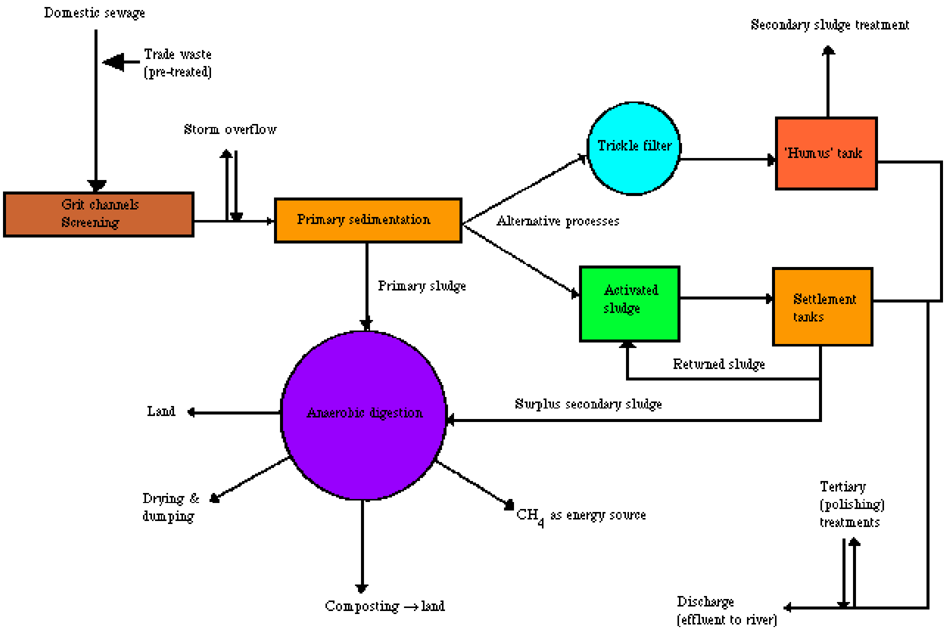


Fig. 1 Scheme of a wastewater treatment plant

Sewage sludge, also called biosolid, is a generic term for solids separated from suspension in a liquid. This material usually contains significant quantities of 'interstitial' water (between the solid particles). Commonly sludge refers to the residual, semi-solid material left from industrial wastewater or sewage treatment process. It can also refer to the settled suspension obtained from conventional drinking water treatment and numerous other industrial processes. Moreover, the definition given in the Working Document on Sludge, 3rd draft, (EU 2000) is the following one: sludge is the “mixture of water and solids separated from various types of water as a result of natural or artificial processes” and in particular sewage sludge is “sludge from urban waste water treatment plants”

Sludge from conventional treatment plants is derived from mechanical and chemical-physical processes (removal of solid material from suspension) and from biological processes aimed to degrade organic substance. A typical conventional WWTP generally contains primary, secondary and even tertiary treatment processes (Fig. 1).

Primary sludge is extracted from primary settling tanks upstream of biological reactor, while secondary sludge originates from secondary settling tanks downstream of reactor.

Generally, conventional WWTPs have also a pre-treatment stage, which consists of various physical and mechanical operations such as sieving, screening, blast cleaning, oil separation and fat extraction. This stage allows to remove voluminous materials, grease and sand, which cannot be considered as sludge and they are disposed in landfills.

Secondary sludge consists predominantly of excess biomass produced during the biological treatment (Ramalho, 1983) for breaking down the remaining organic materials in wastewaters. In the biological processes microorganisms, mostly bacteria, use the available organic matter as substrate to grow. The most common biological treatment are activated sludge, lagooning, bacterial beds and biofiltration.

Primary and secondary sludge present very different characterises: secondary sludge is richer in nutrients (nitrogen and phosphorus) than primary one and is also poorer of contaminants like heavy metals and organic micropollutants. On the basis of various international studies, the presence of nitrogen and phosphorus in secondary sludge was determined to be 1.8 – 2.0 times than that in primary sludge, precisely nitrogen and phosphorus concentrations are 7-8% and 7 % respectively in secondary sludge and 2.5% and 1.6% in the primary one (Mininni, 2004).   Moreover secondary sludge is more diluted and refractory to anaerobic digestion with respect to the primary one. In fact, digestion performance of secondary sludge is quite unsatisfactory (VS destruction in the range 35-40%), due to the difficult and rate-limiting cell lysis.

Tertiary sludge originates from an additional process aimed to remove the remaining nutrients from sludge, particularly nitrogen and phosphorus. Tertiary treatments are generally high performance biological and chemical processes.

1.1.3 Sludge production, reuse and disposal

The land area presently occupied by the EU is smaller than that of the U.S., but its population is larger (Table 1) so that the average population density is four times as high in the EU. Consequently, the table shows that estimated past and projected future overall sludge production is higher in the EU than in the U.S.

Table 1

Parameter	**Year**	**U.S.**	**EU**
Population	2000	283,230,000	376,722,000
Land area (km2)a		9,629,090	3,242,690
Population density (people/km2)	2000	29	116
Agricultural area (km^2)[a]	1999	4,182,500	1,420,840
Sludge production (dry metric ton/annum)	1992		7,387,000[d]
			5,511,000[e]
	1997	6,900, 000[b]	
	1998	6,900, 000[c]	6,588,000[e]
	2005	7,600,000[c]	8,331,000[e]
	2010	8,200,000[c]	
Sludge for beneficial use (% of total)	1998	60[c]	42d,52[e]
	2005	66[c]	54[e]
	2010	70[c]	
Sludge density (dry metric ton/annum.km2 of agricultural land)	1998	1.6	4.6

a (Food and Agriculture Organization. 2002.

c[U.S. Environmental Protection Agency. 1999. *Biosolids generation, use, and disposal in the United States.* EPA530-R-99-009
d[National Research Council. 2002. *Biosolids Applied to Land: Advancing Standards and Practices (Prepublication Copy),*Washington, D.C: National Academy Press.

E(Magoarou, P.,(1999)
excluding Italy and Sweden (approximately 11 and 3% of total).

On the other hand, the total area for agriculture in the EU is about one third of what is now occupied by U.S. agriculture and the ratio of sludge produced to available agriculture area is about 2.5 times as high in the EU as in the U.S. Hence, biosolids management is generally considered a more urgent issue in the EU, with more sludge being produced and less agricultural area available for beneficial use (Iranpour, 2004).

Tabel 2 (Greater Moncton Sewerage Commission, 2008) shows the sludge production per capita in different countries.

Table 2. Per capita sludge production (g/person×d) in different countries

Country	Sludge production (g/person × d)
Austria	**55**
Brazil	**33**
Canada	**76**
Italy	**38**
Finland	**94**
Hungary	**48**
Portugal	**60**
Slovenia	**20**
Turkey	**60**
Medium value	**54**

The sludge production range is quite large (20 – 94 g/person × d) thus indicating the different approach to WW treatment and sludge management in different countries. Sludge production more or less directly depends on the pollutants removal efficiency and chemicals added for phospahte precipitation and flocculation. Too high values, however, can indicate that the biological stabilization process is not completed. We generally can say that the appropriate values should be in the range of 45-65 g/(person × d), unless phosphorus removal process by chemical precipitation is performed.

Sustainable sludge management may be defined as methods that meet requirements of efficient recycling of resources without supply of harmful substances to humans and the environment (Fytili, 2008).

In fact, typical treatments for a large WW treatment plant include a first phase of concentration, generally carried out by gravity thickening, a biological aerobic or anaerobic stabilization, aimed to reduce biodegradable solids, odours and pathogens, and mechanical dewatering by centrifugation, belt-pressing or filter-pressing. In most cases sludge processing is designed according to the conventional systems, which might not be suitable for producing sludge with proper characteristics for its final disposal or utilization according to the legislative standards and avoiding any detrimental effects for the environment and any risk for the human health.

The current situation in most European countries as regards sludge disposal is quite diverse. The Table 3 shows the current sludge disposal options in some European countries (Greater Moncton Sewerage Commission, 2008).

Table 3. Current disposal options in European countries (% of sludge produced)

	Landfill sites	Thermal treatments (incineration)	Composting	Agricultural utilisation	Non agricultural utilisation	Other
European Union	18	23	7	45		7
Austria		35	50	15		
Bulgaria	100			Only few cases		
Czech Republic	13	<1	50	17		20
Finland			73	3		
Flanders		88			12	
Germany	3-6	20 (mono-incineration)+17 (other thermal treatments including co-incineration)		32	25 (landscaping)	
Hungary	60	0.8		39		
Netherland		58 (mono-incineration + 27 (thermal dried)	15			
Norway	7			65	12	16
Slovenia	30	47 (export to incineration)	15	7		1
U.K.	1.5	19.5		67	5.2	1.8

It may be seen that in many countries agricultural utilisation still plays an important role while in others it was completely banned. Composting seems to increase its importance while more stabilized and sanitized sludge could be obtained by using this process, due to the intense stabilization and the process temperatures as high as 70°C. The application of thermal treatments is increasing as well as a result of progressively closure of the landfill disposal and agricultural use.

In countries where technology is less developed, direct agricultural application or landfilling are the typical ways for secure sludge outlet from the WW treatment plants. In countries where the stakeholders and policy makers practically have forbidden land application and where the landfill directive implementation into the national legislation was stringent, only the high temperature destruction methods are available.

At present the major ways of disposing of sewage sludges are deposition, landfill and incineration, only part of the sludges are used in agriculture. Application of sewage sludge to agricultural land may be beneficial because it can improve the physical, chemical and biological properties of soils which may enhance crop growth (Beck et al. ,1996). To achieve this, sludge application cannot just be a way of disposing of the sludges but a deliberate application in order to recycle nutrients and to restitute organic matter to soils in order to prevent over-exploitation of agricultural soils in the Community (Marmo, 2000). In addition the use of sludge as a fertilizer would decrease the amounts of chemical fertilizers needed in agriculture (Tideström ,1997) and supply micro-nutrients which are not commonly restored in routine agricultural practice. Thus sludge use in agriculture could help save non-renewable materials or energy, a prerequiste to achieve sustainable production (OCDE 1992 in Sequi et al. 2000).

Even if land application of sludge is an attractive option for beneficial use of biosolids, there is still a great concern about human and environmental health, because sludge can contain pathogens and various pollutants. Both in U.S. and in European Union, government agencies have promulgate regulations for biosolids land application in order to limit risks for humans and environment.

The most important European regulation regarding agricultural use of sludge is the Directive 86/278 (Sewage Sludge Directive), containing 18 articles. Each EU member state is allowed to adopt more stringent standards than those established at Community level.

The Directive 86/278 contains requirements and limit values for the application of sludge to soil:

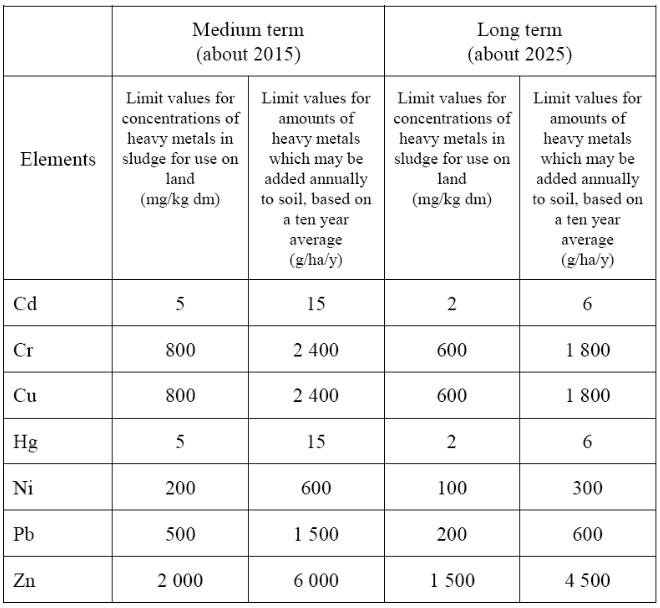
- (i) the concentrations of heavy metals in soil; (ii) those in sludge , and (iii) the maximum annual quantities of heavy metals which can be introduced into the soil must comply with the limits given in three Annexes;
- sludge must be treated before being used in agriculture, with biological, chemical or heat treatment, long-term storage or any other appropriate process, so as significantly to reduce its fermentability and any health hazard;
- sludge shall be used taking primarily into account the nutrient needs for plant growth, and that the quality of the soil and the surface- and ground-waters is not impaired.

In Italy the agricultural use of sewage sludge is disciplined by a specific guideline: the D. Lgs. 27 January 1992, Nr. 99 (D. Lgs. 99/92) derived from key Directive 86/278/EEC. This regulation influences the sludge management given that deals with some important topics:1. Concentration limits for heavy metals in both sludge and soils; 2. Agronomical and microbiological characteristics of sludge; 3. Maximum amount of sludge for land application. According to data reported more than ten years ago, agricultural use of sludge in Italy was scarce: only 18% of the total wastewater treatment sludge produced was utilized in agriculture and almost entirely in the North or Central area of the country. During the same period 80% of this sludge was disposed into landfills and, due to strong opposition of the public opinion against incineration, only 1% of the sludge was managed by this method. For the following years a great reduction of landfill disposal and an augment of agricultural recycling of sewage sludge were foreseen. In fact, data collected by the Italian Environmental Administration in the period 2001-2003 already showed a growing percentage of land application near to 32%.

Another step in the field of European waste management was constituted by the Directive 91/156 also designed as Waste Basin Directive, which contains general principles for the methods to utilize and dispose sludge; for the development of waste management plans and for the approval and monitoring procedures. In the same year, European Union promulgated the Urban Waste Water Directive (91/271/EEC) which sets more stringent quality standards for wastewaters. The most important article of this directive concerning sludge is Article 14, which declaims that “sludge arising from waste water treatment shall be re-used whenever appropriate”; in addition member States are forced to “ensure that by 31 December 1998 the disposal of sludge to surface waters by dumping from ships, by discharge from pipelines or by other means is phased out”.

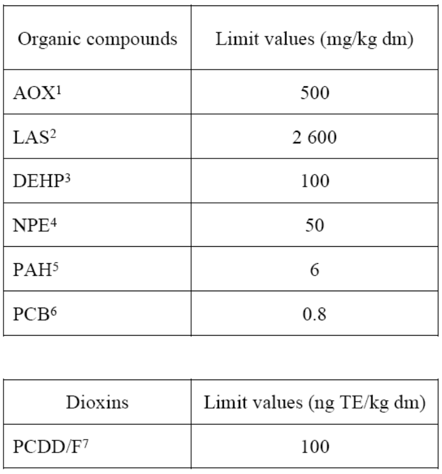
The Sewage Sludge Directive has now been revised to improve the current sludge management and new requirements and limits have been proposed in 2000 with the Working Document on Sludge, 3rd Draft. This document fixes more stringent limits for heavy metals in (Table 4), but also the concentrations for some organic pollutants (Table 5). Nevertheless, it must be note that metal contamination of sludge is worldwide considered as more important than organic contamination, as regards to human health, and often the concentrations of organic pollutants in sludge exceed the limits of the Working Document.

Table 4



	Medium term (about 2015)		Long term (about 2025)	
Elements	Limit values for concentrations of heavy metals in sludge for use on land (mg/kg dm)	Limit values for amounts of heavy metals which may be added annually to soil, based on a ten year average (g/ha/y)	Limit values for concentrations of heavy metals in sludge for use on land (mg/kg dm)	Limit values for amounts of heavy metals which may be added annually to soil, based on a ten year average (g/ha/y)
Cd	5	15	2	6
Cr	800	2 400	600	1 800
Cu	800	2 400	600	1 800
Hg	5	15	2	6
Ni	200	600	100	300
Pb	500	1 500	200	600
Zn	2 000	6 000	1 500	4 500

Table 5

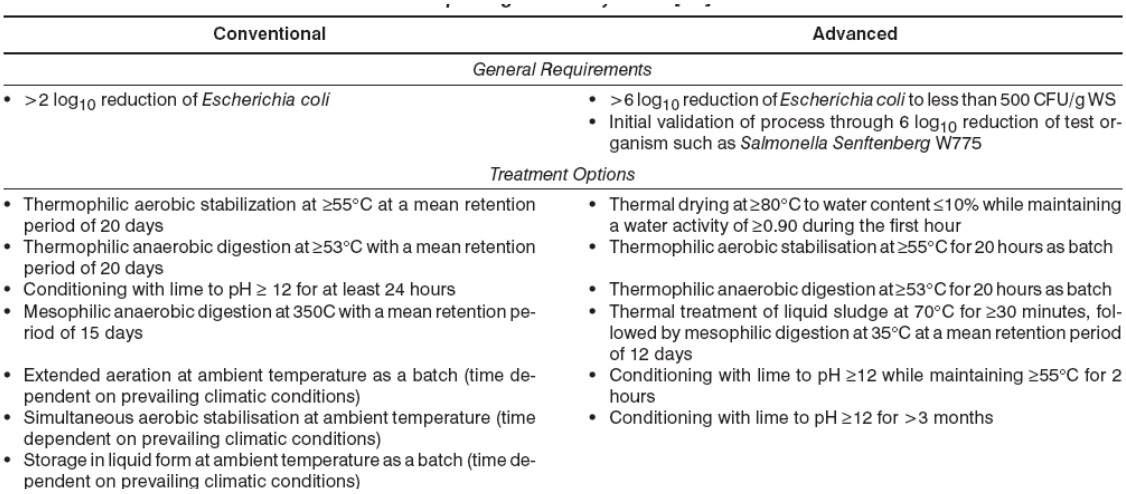


Organic compounds	Limit values (mg/kg dm)
AOX[1]	500
LAS[2]	2 600
DEHP[3]	100
NPE[4]	50
PAH[5]	6
PCB[6]	0.8

Dioxins	Limit values (ng TE/kg dm)
PCDD/F[7]	100

The most important change is pathogens regulation: the previous Directive 86/278 does not specify the limits for pathogens densities, but only requires sludge treatments before land application in order to reduce pathogens risk; the recommended or accepted treatments are different for each member state. The regulations of the 2000 working document are more specific towards pathogens reduction, treatment processes and site restrictions in land application (Table 6).

Table 6

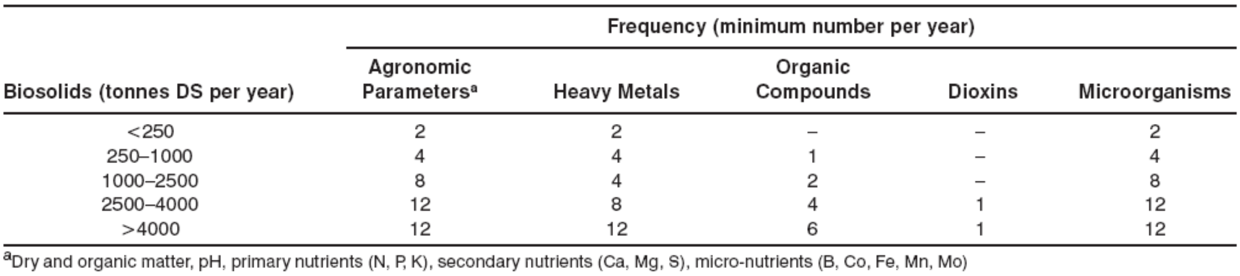


Conventional	Advanced
General Requirements	
• $>2 \log_{10}$ reduction of *Escherichia coli*	• $>6 \log_{10}$ reduction of *Escherichia coli* to less than 500 CFU/g WS • Initial validation of process through 6 $\log_{10}$ reduction of test organism such as *Salmonella Senftenberg* W775
Treatment Options	
• Thermophilic aerobic stabilization at ≥55°C at a mean retention period of 20 days • Thermophilic anaerobic digestion at ≥53°C with a mean retention period of 20 days • Conditioning with lime to pH ≥ 12 for at least 24 hours • Mesophilic anaerobic digestion at 350C with a mean retention period of 15 days • Extended aeration at ambient temperature as a batch (time dependent on prevailing climatic conditions) • Simultaneous aerobic stabilisation at ambient temperature (time dependent on prevailing climatic conditions) • Storage in liquid form at ambient temperature as a batch (time dependent on prevailing climatic conditions)	• Thermal drying at ≥80°C to water content ≤10% while maintaining a water activity of ≥0.90 during the first hour • Thermophilic aerobic stabilisation at ≥55°C for 20 hours as batch • Thermophilic anaerobic digestion at ≥53°C for 20 hours as batch • Thermal treatment of liquid sludge at 70°C for ≥30 minutes, followed by mesophilic digestion at 35°C at a mean retention period of 12 days • Conditioning with lime to pH ≥12 while maintaining ≥55°C for 2 hours • Conditioning with lime to pH ≥12 for >3 months

The document distinguishes between advanced treatments, i.e. those comparable to Processes to Further Reduce Pathogens, PFRPs, in the part 503 Biosolids Rule for Class A biosolids of the U.S. EPA regulation, and conventional ones, as the PSRPs of the Part 503 Biosolids Rule for Class B biosolids. Sludge treated with advanced processes have no restrictions in land application, while biosolids produced with conventional treatments have much more limited applicability.

The 2000 document also regulates the frequency of analysis sampling, based on the quantity of sludge produced (Table 7).

Table 7



Biosolids (tonnes DS per year)	Frequency (minimum number per year) Agronomic Parameters[a]	Heavy Metals	Organic Compounds	Dioxins	Microorganisms
<250	2	2	–	–	2
250–1000	4	4	1	–	4
1000–2500	8	4	2	–	8
2500–4000	12	8	4	1	12
>4000	12	12	6	1	12

[a] Dry and organic matter, pH, primary nutrients (N, P, K), secondary nutrients (Ca, Mg, S), micro-nutrients (B, Co, Fe, Mn, Mo)

The development of agricultural recycling of sludge depends mainly on the possibilities to improve the quality of the sludge itself and increase confidence in sludge quality.

This sets the problem of preventing pollution of the waste water at source by reducing the possibilities for heavy metals and organic compounds to enter the waste water sewage system, improving sludge treatment and ensuring the monitoring of sludge quality. These technical solutions will require major investment both from the water companies and local authorities, although these costs will remain low when compared to the overall costs of managing water and urban wastewater. The resistance against sludge agricultural recycling originates mainly from the uncertainties over the possible risks for human health and the environment, in particular about the transfer of pollutants (especially the organic ones) to the environmental media and the food chain, and the possible effects of organic pollutants and pathogens in sludge. (EU synthesis report, 2002).

Some of the processes described as ‘conventional treatment’ in the EU 2000 third draft document would be appropriate to improve the sludge quality: in particular, if sufficient organic material is present, mesophilic anaerobic digestion at 35°C with a mean retention period of 15 days is a possibility. Anaerobic digestion is an appropriate technique for the treatment of sludge before final disposal and it is employed worldwide as the oldest and most important process for sludge stabilization.

1.1.4 Anaerobic digestion and sludge stabilization

Generally, sewage sludge is very putrescibile because of its high content of biodegradable organic material and the presence of pathogen microorganisms. A treatment, named stabilization, is necessary to degrade organic substance by various bacteria groups. Stabilization can be chemically and biologically obtained. Chemical treatment is less used than the biological one because of its high operating costs. Generally, biological stabilization is carried out in digesters through a process which can be aerobic or anaerobic. The anaerobic stabilization or anaerobic digestion is described as a series of processes involving microorganisms to break down biodegradable material in the absence of oxygen. This process occurs naturally in the environment and consists in the breakdown of complex organic substrates, through a series of parallel and sequential steps, by several groups of microorganisms. As stated by McInerney and Bryant : “ While it is possible to do this in a scheme, it is emphasized that these bacterial groups cannot be separated in the discussion of their metabolism since the efficient metabolism of each group is dependent on the others”. The overall result of anaerobic digestion is a nearly complete conversion of the biodegradable organic material into methane (biogas), carbon dioxide, hydrogen sulfide, ammonia and new bacterial biomass. Buswell proposed a generic formula describing the overall chemical reaction of the anaerobic fermentation process of organic compounds which can be used for the prediction of biogas production:

$$C_aH_bO_cN_d+\frac{4a-b-2c+7d}{4}H_2O\rightarrow\left(\frac{a}{2}\frac{b-3d}{8}-\frac{c}{4}\right)CH_4+\left(\frac{a}{2}-\frac{b}{8}+\frac{c}{4}-\frac{5}{8}d\right)CO_2+dNH_4HCO_3$$

The anaerobic digestion process can be divided in four steps. The first one is the **hydrolysis** of the complex organic substances in order to break down the organic and inorganic polymers into their monomers. These monomers, in particular sugars, amino acids and long chain fatty acids are converted into CO_2, H_2, NH_4, organic acids and alcohols in the second step, named **acidogenesis** or fermentation. In the third phase, **acetogenesis,** the fermentation products are converted into acetic acid, H_2 and CO_2.CH_4 and CO_2 production occurs in the last step called **methanogenesis** by methanogenic bacteria. The following scheme (Fig. 2) shows the 4 steps involved in the anaerobic digestion.

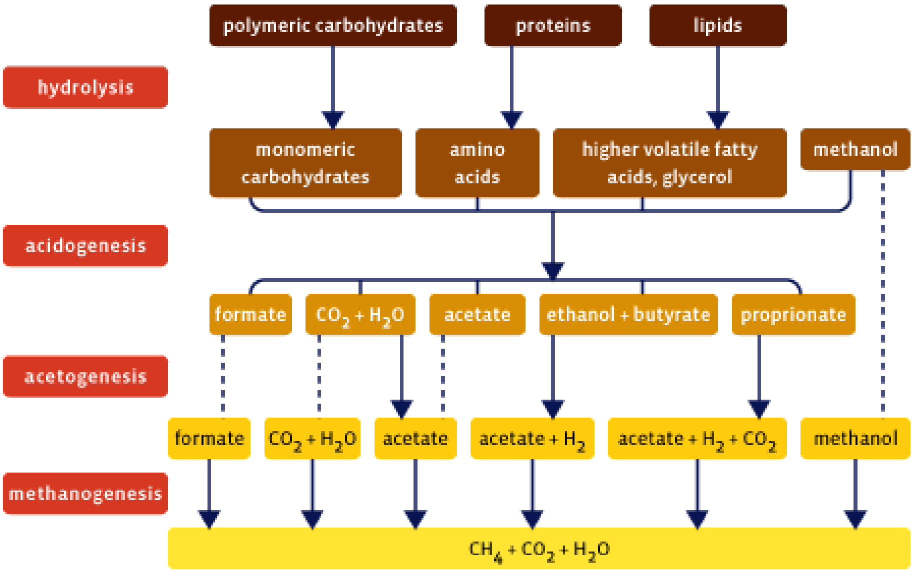


Fig. 2 Scheme of the anaerobic digestion process

Hydrolysis

Anaerobic microorganisms cannot use directly complex organic substrate; so proteins, carbohydrates and lipids need to be transformed into their monomers. The products of their enzymatic degradation are monosaccharides, amino acids and long chain fatty acids. This process occurs during the hydrolysis step, which is the rate limiting phase of the overall anaerobic digestion process.

For the hydrolysis of secondary sludge, also the cellular lyses of bacteria and microorganisms should be taken into account (Pavlostathis, 1986). The complex organic material of a secondary sludge is constituted mainly of biomass formed during wastewater treatment; the lyses of cell walls and the resulting release of intracellular substances are necessary in order to make that bind carbon is available to biodegradation. Moreover biomass occurs in aggregate and complex form (sludge flocs), constituted of a biological active area, inactive bacteria, colloids and inert particles. In fact bacteria have an external layer of extracellular polymeric substances (EPS), formed of carbohydrates, amino acids and uric acids, which play an important role in cell aggregation and adhesion, biofilm formation and cell protection from hostile environment. But it’s necessary that all these substances, EPS, inorganic particles and bacteria, are released in a medium (disintegration and cell lyses) in order that biodegradation finally occurs. Hydrolysis of organic molecules is performed by extra cellular enzymes, hydrolases, excreted by fermentative bacteria. In particular, carbohydrates, proteins and lipids are hydrolysed by cellulases, proteases and lipases respectively with different hydrolysis rate (Stryer, 1995). The cumulative effect of these parallel processes is described with a single first-order kinetic for the substrate biodegradation. Several studies demonstrate that the kinetic coefficient (*kh*) is strongly dependent on the experimental digestion conditions, as temperature, pH, particle size, stirring conditions and mainly inoculum/substrate ratio.

Acidogenesis

The acidogenesis step, or fermentation, is the degradation of organic soluble substances without an external electron acceptor. The main products of acidogenesis are: acetate, propionate, butyrate, CO_2, H_2 and other organic products such as lactate and alcohols. Acetate, CO_2, H_2 and single-carbon compounds can be directly used by methanogens in the final digestion step. During acidogenesis some mineral compounds like sulphates, nitrates and carbonates act as electron acceptors and, from a chemical point of view, this step practically is a dehydrogenation process (loss of 2 electrons with elimination of an hydrogen molecule, H_2). The removal and transfer of H_2 is an enzymatic process and occurs with transport co-enzymes NAD^+ and its phosphate form $NADP^+$, which firstly act as hydrogen acceptors and then they return to the oxidation state releasing

H_2 to other acceptors. Hydrogen transport through enzymatic chain provide energy, because high energy bonds are broken; this energy is used by microorganisms for their metabolic functions. The dehydrogenation of the hydrolysis products takes place in the bacterial cells. The fermentation pathway depends on the substrate and the microorganisms involved. Acidogenic bacteria grow faster and are less sensitive to pH variations than acetogens and methanogens .It has been shown that acidogens population generally represents about 90% of the total microbial population present in anerobic digesters (Zeikus, 1980).

Acetogenesis

In the acetogenic step, the fermentation intermediates are converted to acetate, CO_2 and H_2 by obligate H_2-producing acetogenic bacteria. In order to have acetogenesis, a syntrophic microbial relationship becomes necessary because the conversation of alcohols and fatty acids is not energetically feasible under standard conditions. These reactions are likely to occur when H_2 partial pressure is in the range of 10^{-4}-10^{-5} atm.

Methanogenesis

Waste stabilization occurs during methanogenesis, that is the CH_4 production by methanogenic archaea, which metabolize the fermentate products, mainly H_2, CO_2 and acetate. Also other single-carbon compounds (formate, methylamines and methanol) can be used by methanogenic archaea. Methanogens are strict anaerobes to which oxygen is inhibitory. Methan is essentially insoluble in water and readily separates from the sludge in the gas which leaves the system. Methanogenesis proceeds via two main pathways: acetoclastic and hydrogenotrophic pathways. Most of the CH_4 formed (approximately 72%) is produced from acetate cleavage (acetoclastic way):

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Even if this is the predominant reaction, only 2 methanogens genus are able to carry out this process: *Methanotrix*, obligate acetoclastic bacteria, and some genus of *Methanosarcina*, facultative acetoclastic.

The remaining 28% is generated in the hydrogenotrophic methanogenesis , which is the CO_2 reduction to CH_4 and H_2O using hydrogen as energy source:

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

Essentially this reaction can be considered as anaerobic respiration, performed by all methanogen bacteria, except *Methanotrix*. Despite of the limited CH_4 production, it has the important function of removing H_2 from the system and preventing organic acids build-up. Methanogenic archaea are extremely sensitive to temperature, pH variations and are inhibited by numerous compounds. Optimal conditions from methanogens growth are oxygen absence, temperature range of 30-40°C and 50-60°C and pH range of 6.5-7.5.

CH_4 production is directly related to a decrease of the chemical oxygen demand (COD) in wastewater during anaerobic treatment. Thus it’s possible to evaluate the biogas production considering that the theoretical CH_4 formed is equal to 0.35 Nm^3/kg of the degraded COD in standard conditions.

Important parameters in anaerobic digestion

The most important environmental parameters in anaerobic process are nutrients (micro and macro), pH, temperature and toxic substances. Mc Carty defined the optimal conditions for anaerobic digestion as 1) sufficient nutrients such as nitrogen, phosphorus and others; 2) pH of 6.5 – 7.6; 3) temperature range of 30-38°C (mesophilic process) or 50-60°C (thermophilic one); 4) complete absence of oxygen; 5) absence of toxic materials. Considering the overall process, the methane-forming bacteria are thought to be the most sensitive microorganisms to environmental changes.

Nutrients

The nutrients required in highest concentration are nitrogen and phosphorus. Nitrogen is used in the synthesis of proteins, enzymes, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA); while phosphorus is necessary for the synthesis of energy-storage compounds (ATP), of RNA and DNA too. Nitrogen requirement can be calculated on the basis of the empirical formula for bacteria $C_5H_7O_2N$ in which nitrogen comprises approximately 12% of bacterial cell mass. The amount of phosphorus necessary for bacterial growth is 1/7-1/5 of the nitrogen concentration.

Other nutrients are necessary in anaerobic process, although in lower concentrations than nitrogen and phosphorus: iron, nickel, cobalt, sulphur, calcium and some trace organics. Trace metals must be in solution to be available for bacterial growth.

pH

pH have a wide influence on the permeability of the cell membrane because it modifies membrane proteins and also favours the dissociation of electrically neutral compounds, making them permeate difficultly. The optimal pH range for efficient anaerobic digestion is 6.5-7.6. For lower pH level ($pH \leq 4$) methanogens activity is blocked; on the other hand, when pH increases up to 9, even though the same effects occur, the system recovery is slower with respect to the acid conditions. Methanogens are the most sensitive bacteria to pH changes. When a system imbalance occurs (caused by various factors like hydraulic characteristics, changes in organics or in temperature, or introduction of toxic materials), generally volatile acids produced by acetogenic bacteria increase at a faster rate than they are decomposed by methanogenic bacteria. So, if the system don’t have sufficient buffer capacity, pH will drop to unacceptable level and methane production will decrease and even stop. The most important buffering system in anaerobic digestion is the bicarbonate system (see equation), where buffering capacity is generally measured as alkalinity:

$$\left[H^+\right] = K_1 \frac{\left[H_2CO_3\right]}{\left[HCO_3^-\right]}$$

Maintenance of sufficient alkalinity prevents system failure due to pH drops, but, if an upset occurs, in most cases process performances can be restored by adjusting the pH to the neutral range as rapidly as possible.

Temperature

Anaerobic digesters are generally operated in two different temperature ranges: mesophilic (30-38°C) and thermophilic (50-60°C). Many studies (Buhr, 1977) demonstrated that thermophilic process results in higher digestion rate, improved sludge dewaterability and increased pathogen destruction. On the other hand, thermophilic digestion implies some disadvantages like higher energy required for heating, less stable biological process and poor supernatant quality. Both for mesophilic process and for thermophilic one, a general rule is that maintenance of a constant and uniform temperature range is necessary for efficient digestion operation.

Inhibiting substances

The anaerobic processes are negatively affected by various substances which, at certain concentrations, can even inhibit or halt the process. Many substances stimulate the reactions in low concentrations; however, as concentration increases, the effect becomes inhibitory . Anyway, the degradation of these inhibiting substances may also occur by acclimated bacteria, if the concentrations of these compounds are lower than the toxicity limits.

Ammonia

Ammonia is a substance which can become inhibiting for anaerobic digestion process. Mc Carty reported that concentrations between 50 and 200 mg/l are beneficial because ammonia-nitrogen is an essential nutrient, but, depending on the pH, it can be also toxic (Mc Carty, 1964). Ammonia comes from the decomposition of urea or other proteins and it presents as indissociate form (dissolved gas) or as ammonium cation by the following equilibrium equation:

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O$$

Lowering the pH ($pH \leq 7.2$) causes the equilibrium shift towards the formation of ammonium ion; on the other hand, increasing the OH^- concentration ($pH \geq 7.2$) produces an increase in the concentration of indissociate ammonia, which is considerably more toxic than the ammonium ion. So, in the presence of NH_4^+ ion, toxicity increases with increasing pH.

Sulphates

Also some sulphate compounds can influence the performances of anaerobic digestion: high concentrations of sulphates (in excess of 200 mg/L) facilitate the growth of sulphate-reduction bacteria (SBR) which are competitors of methanogens bacteria (MPB) for utilizing H_2 and acetate.

Heavy metals

It's also well known that anaerobic processes are particularly sensitive to high loadings of heavy metals. The toxicity of heavy metals depends mainly on the various chemical forms in which metals may present under anaerobic conditions and at near-neutral pH. Mosey (Mosey,1975) showed that heavy metals cause anaerobic digestion failure only when the concentrations of metals in soluble forms (free ions) exceed a certain threshold concentration, which is directly related to the concentration of divalent sulphide ions. Another study by Ghosh (Ghosh, 1972) demonstrated that although low concentrations of some metals are toxic, even high concentrations could be tolerated in anaerobic processes if there is sufficient concentration of sulphide ions available for precipitation.

Cations

Many researchers studied the effect of the cations Na^+, K^+, Ca^{2+}, Mg^{2+} and NH_4^+ on the performances of anaerobic digestion: the anaerobic processes failure is greatly dependent on the cations concentration. The optimal environment contains 0.005M of divalent cations and 0.01 of monovalent ones. However, the addition of antagonistic cations can reduce or eliminate the toxicity caused by the alkali and earth-alkali cations.

In many central and northern European countries, mesophilic anaerobic fermentation is the most commonly

applied process for stabilization of sewage sludge (around 70% of sewage sludge is treated by anaerobic digestion); although due to increased demands on sewage sludge treatment, it is being supplemented or complemented with a thermophilic treatment (Odegaard, 2004).

Recently, new developments of sewage sludge treatment by using a combination of aerobic and anaerobic processes and different kinds of pretreatments (mechanical, thermal, chemical, oxidative) have been discussed to reduce harmful sludge constituents before they can enter the environment (CEC, 2004, Carballa, 2007).

Most of these studies are focused either on enhancing sludge biodegradability, and consequently, biogas production. However, to our knowledge, little information is available on the effect of these advanced treatment schemes on digested sludge quality.

An alternative sustainable sewage sludge management, addressed to improve agricultural utilisation of sewage sludge, could be attained through a separation of primary and secondary sludge before their treatment and disposal. It would thus be possible to maintain agricultural utilisation for the biological sludge (secondary) and to convert to inert material by different inertization processes only the primary sludge (Mininni et al. 2004).

1.1.5 The innovative sludge management: the separation of primary and secondary sludge treatment[1]

Sewage treatment plants generate quite a large amount of sludge. Although rich in nutrients, organic substance and microelements, in some countries sewage sludge is not accepted as agricultural fertilizer due to the awareness regarding risks for contamination of typical products like milk, because of the possible presence of toxic compounds. A good stabilization is a preliminary condition both for sewage sludge agricultural use or landfill disposal. Anaerobic digestion was always considered the principal process to stabilize sewage sludge in the past years, at least for the big plants.

Separation of primary and secondary sludge could lead to more sustainable sludge management. Such an innovative concept is based on the following considerations: secondary sludge, poorer in pollutants than primary sludge, should be segregated and treated separately from primary sludge to increase chances of secondary sludge utilisation in agriculture. For example, linear alkylbenzene sulfonates (LAS) concentration was reported to be 4,000-7,000 mg/kg dry solids for primary sludge and of 300 mg/kg for activated sludge due to sorption on particulate material of the raw sewage and aerobic degradation in biological treatment (De Henau et. al., 1989; Siegrist et al., 1989).

Due to the low water solubility and lipophylic properties of polycyclic aromatic hydrocarbons (PAHs), these compounds are typically removed from sewage by adsorption on the particulate material in the primary settling tank (Angelidaki and Ahring, 1999). Khan and Ongerth (Khan and Ongerth, 2002) have reported estimation of pharmaceutical residues in primary and secondary sewage sludge and they predicted concentrations up to 0,8 mg/L in fresh primary sludge, which really appears a very high value. Therefore, if secondary sludge is blended with primary sludge it may become no longer acceptable for agricultural use, due to excessive content of heavy metals and organic contaminants.

Secondary sludge, on the other hand, is much richer in nitrogen and phosphorus than primary sludge (5-8% instead of 1.5-5% of dry solids for nitrogen, and 1.6-6.0% instead of 0.3-1.5% for

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[1] Mininni G, Braguglia C.M., Gianico A., Gallipoli A., (2010), “Energy balance in a novel approach to sludge processing”, Full paper at RESIDUAL AND BIOSOLIDS 2010, Water Environment Federation, p. 543-559, Savannah

phosphorus), with consequent better profit for crops, which need smaller amounts of sludge to balance nutrient requirements.

Separation of primary and secondary sludge treatment could also be advantageous from a technological point of view. Primary sludge, in fact, can be thickened, digested and mechanically dewatered much easier alone than in a mixture with secondary sludge (Kopp and Dichtl, 2001). Moreover, primary sludge treatment can be more easily optimised for energy recovery and for the final incineration.

On the other hand, separate treatment of secondary sludge can be impaired by its poor characteristics in terms of biodegradability and thickenability thus requiring pre-treatments aiming of increasing digestibility (biogas production) and, consequently, quality of digested sludge, regarding organic contaminants and pathogens.

Scope of this Ph.D work is, in fact, the assessment of the ultrasound pretreatment efficiency to improve secondary sludge biodegradability and remove contamination by sonolysis.

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2. OBJECTIVES

The development of wastewater treatment technology together with the implementation of stringent environmental legislation has successfully protected the aquatic system from pollution in many of the countries of the world. However, sewage sludge, semisolid, nutrient-rich by-product of this treatment is becoming worldwide an environmental problem because of its increasing production and its high contents of organic waste and pathogens, as well as xenobiotics and heavy metals. The sharp increase in sewage sludge production expected in the next few years should change the disposal routes also in Italy. The agricultural recycling of sewage sludge therefore appears to be an interesting alternative for the national authorities, although an increase in the importance of this disposal route is expected to be limited in the coming years. One of the main limiting factors appears to be the size of farms in Italy which, with an average area of 5.9 ha are rather small, limiting the development of land spreading for technical reasons.

To date, the primary disposal methods for the excess sludge produced are landfilling, incineration and application in agricultural.

Application of sewage sludge to agricultural land may be beneficial because can improve the physical, chemical and biological properties of soils enhancing crop growth. In addition the use of sludge as a fertilizer would decrease the amounts of chemical fertilizers needed in agriculture and supply micro-nutrients which are not commonly restored in routine agricultural practice. Thus sludge use in agriculture could help save non-renewable materials or energy, a prerequiste to achieve sustainable production.

The European Union has developed on 2000 the draft of a “Working document on sludge” to promote the use of sewage sludge in agriculture while improving the safety and harmonize quality standards. It proposes limit values for concentrations of heavy metals and organic compounds that should restrict the use of sewage sludge in agriculture if the limits are exceeded and provides suggestions for good practice in treatment and agricultural use.

One of the objectives of this work was to give an overview on the occurrence of organic, and inorganic compounds, and nutrients in different types of sewage sludges deriving from italian WWTPs, in order to assess new strategies in sludge management.

In this context, as reported in detail in the EU document “Organic contaminants in sewage sludge for agriculture use” (2001) it is also necessary that to prevent elevated levels in digested sewage sludge, organic substances must be aerobically and anaerobically degradable. Such properties must be postulated in particular for chemicals like the components of laundry- and dish-washing detergents and surface cleaners which are used in high amounts directly in water. If this condition is not fulfilled, problems of residual levels in sludge-treated soils will be encountered.

Main purpose of this study was to investigate the potential of an innovative decontamination treatment using high frequency ultrasounds to remove anionic surfactants (like linear alkylbenzensulfonates) directly in sewage sludge.

Sonolysis of pollutants has been studied in the last years uniquely in water media, the idea to apply this treatment also in the case of complex media like sewage sludge before digestion is absolutely on of the strong points of this work.

Among the available treatment technologies, anaerobic digestion is commonly used at medium and large STPs as sludge stabilization technique resulting in the reduction of sludge volatile solids and the production of biogas. However, anaerobic stabilization is a slow process; because the first step in digestion, biological hydrolysis, has been identified as the rate-limiting step. Therefore, long residence times in the fermenters and large fermenter volumes are required for digestion.

In order to improve the hydrolysis rate, various types of sludge pretreatment methods have been widely studied in the last decade. These methods lead to rupture of the cell wall and membrane of bacteria in waste activated sludge resulting in release of organic substances to the outside of the cell. Therefore, sludge disintegration was introduced to solubilise and convert slowly biodegradable, particulate organic materials to low molecular weight, readily biodegradable compounds. Disintegration may be performed biologically, chemically, thermally, mechanically or by a combination of these methods.

Ultrasonication at 20 kHz is one effective mechanical method, used commonly as digestion pretreatment. There are several parameters that affect the efficiency of sonication as pretreatment on waste activated sludge. Therefore, before the full scale installation of ultrasound unit, lab scale and pilot scale experiments should be carried out to analyze these parameters such as sonication energy and sludge characteristics.

Moreover, as mentioned before, considerable interest in the last years was addressed to the application of ultrasound at high frequency as a destructive process for the treatment of hazardous contaminants in water whereas no application has been investigated for sludge.

In this thesis the effect of 200 kHz ultrasound treatment on floc disintegration was studied and compared with the results obtained with the "conventional" 20 kHz treatment.

In fact it is worth to note that all the literature published on ultrasonic disintegration report the application of 20 kHz ultrasound up to full scale plants, but surprisingly the effect of other frequencies on sludge disintegration was never investigated.

Different parameters have been employed to evaluate sludge disintegration efficiency. They can be collectively classified into three categories namely, physical (such as change in particle size distribution and microscopic examination), chemical (such as increase in soluble COD concentration and release of protein and carbohydrate), and biological (anaerobic degradability).

Food to inoculum microorganism (F/I) ratio is the ratio of substrate amount as waste activated sludge to the inoculum amount as anaerobic digested sludge added to the reactor. It is an important digester parameter since volatile solids loading to the digester affects the performance of the process. There is lack of information in literature on the effect of F/I ratio on digester performance for sonicated sludge.

For this reasons, within the scope of this study, the effect of 200 kHz sonication on waste activated sludge was examined first during the disintegration studies and then, batch anaerobic reactors were set for anaerobic biodegradability tests in order to examine the effect of this innovative pretreatment and of the food/inoculum ratio on the anaerobic digestion process.

The main scope of this part of activity was to understand the potentialities of the new 200 kHz pretreatment in terms of anaerobic digestion improvement. Both understanding the mechanisms of the anaerobic digestion process of untreated and sonicated sludge also in terms of fate on micropollutants, and the evaluation of the advantages of the pretreatment incorporation for quality enhancement are important goals to optimize in the future this type of sludge treatments.

3. COLLECTION AND EVALUATION OF DATA OF PRIMARY AND SECONDARY SLUDGE QUALITY: MONITORING OF ITALIAN FULL SCALE PLANTS[2]

Three WW treatment plants in the Rome area were selected for a monitoring campaign, with the aim to assess the pollution load and the nutrient contents of primary and secondary sludge. This monitoring campaign was specifically designed to verify whether the idea of sludge separation, reported in the previous chapter, may be a solution to sustain sludge utilization in agriculture of the biological sludge that should be basically a clean soil improver where the presence of the non polar contaminants is expected to be negligible.

The 3 plants are Co.Bis. Cesano, 90,000 Population Equivalent (PE), Fregene 33,000 - 76,000 (PE) and Roma Nord 780,000 (PE). The quality of sewage sludge is subjected to some variation due to the mode of operation (sludge age) and maintenance of some units (especially the secondary settling tanks of Roma Nord WW plant).

3.1 Waste water treatment plant description

The municipal “Roma-Nord” WW treatment plant is one of the four wastewater treatment plants serving the city of Rome. The plant is conventional including screening, primary clarification and secondary treatment by activated sludge. This treatment plant serves about 780,000 p.e. and is operated with very high sludge age (20 d). The plant is designed to treat an average WW flow rate of 4.1 m^3/s. Both primary and secondary sludges are mixed together and treated in two anaerobic digesters with a total capacity of 18,000 m^3.

The Co.bis. Cesano WW treatment plant, which serves the cities of Cesano, Anguillara Sabazia, Bracciano, Manziana, Oriolo Romano e Trevignano, is designed to treat an average WW flow rate of 250 L/s. It is a conventional plant with primary clarification and secondary treatment by activated sludge; the sludge is aerobically treated and finally dewatered.

The Fregene WW treatment plant is located on the sea coast and therefore it is subjected to a quite large fluctuation of quantity and quality of the WW treated, due to the seasonal resident changes. This plant is designed to operate with a winter population of 33,000 p.e., treating 140 L/s of wastewater and with a summer population of 76,000 p.e., treating 336 L/s. The plant includes a primary settlement and a secondary treatment by activated sludge; the sludge treatment includes a pre-thickening step followed by a mechanical dewatering step (without any biological stabilization).

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[2] Mininni G., Braguglia C.M., Gallipoli A., Gianico A., Mascolo G., “ L’attivita’ dell’IRSA sui fanghi di depurazione nel progetto europeo NEPTUNE”. Full paper for ECOMONDO 2010, 3/11/2010-6/11/2010, RIMINI (ITALY), p. 831-836.

3.2 Sampling strategy

The sampling campaigns were performed on Roma Nord, Co.Bis. and Fregene WW treatment plants, taking both primary and secondary sludge 2 times a day for 5 consecutive days to achieve a representative sample. In the three WW treatment plants the sampling campaigns were carried out as reported in Table 8.

Table 8. Sampling campaing in the three WW treatment plants

Roma Nord	Co.bis.	Fregene
June 2007	January 2008	
May 2008	August 2008	March 2009
December 2008	January 2009	May 2009

In each campaign 10 L of primary sludge and 25 L of the secondary sludge were collected. Moreover, 10 L of stabilized sludge were also collected in the WW treatment plants of Roma Nord and Co.bis., equipped with an anaerobic digester and an aerobic stabilization basin, respectively. As in the Fregene plant the sludge is not stabilized, the mixed sludge (about 0.5 kg) was taken after dewatering by centrifugation.

In the WW treatment plants of Roma Nord and Co.bis. the primary sludge was sampled directly on the sludge extraction line from the primary settling tank. For Roma Nord plant this line takes the sludge to the pre-thickener while for the Co.bis. plant directly to the aerobic stabilization tank. The secondary sludges were sampled on the recycle line to the aeration basin.

In the Fregene plant primary sludge was taken directly from the primary settling tank and secondary sludge in the excess sludge line.

Table 9 shows the main characteristics of the treatment units of the three WW treatment plants.

Table 9. Main characteristics of the WW treatment plants

	Primary sedimentation	**Biological section**	**Secondary sedimentation**
Roma Nord	**4 settling tanks** **Φ=60 m** **H=2 m** **Residence time=2 h** **S_{tot}=11,310 m^2**	**2 aeration tanks** **1 diffused air** **1 rotors** **V_{tot} oxidation=56,000 m^3**	**8 settling tanks** **Φ=60 m** **H=3,5 m** **Residence time=5 h** **S_{tot}=22,619 m^2**
Co.bis.	**4 settling tanks** **Φ=18 m** **V_{tot}=2,000 m^3** **S_{tot}=1,018 m^2**	**4 aeration tanks + 2 for denitrification** **V_{tot} oxidation=2,800 m^3** **V_{tot} denitrific.=2,400 m^3** **Residence time=2.8 h**	**4 settling tanks** **Φ=22 m** **H=2.2 m** **S_{tot}=1,520 m^2**
Fregene	**2 settling tanks** **Φ=13 m** **V_{tot}=1,600 m^3** **S_{tot}=265 m^2**	**1 aeration tank** **V_{tot} oxidation=720 m^3** **Residence time=1.3 h**	**2 settling tanks** **V_{tot}=2,000 m^3** **S_{tot}=402 m^2**

A third part of each sample was used to carry out the VS, TS, CST, MBAS, TKN and Total Phosphorus analyses; the other two third parts were freeze-dried to carry out the Heavy Metals, Organic Micropollutants and Thermal analyses, as shown in Fig. 3.

3.3 Chemical characterization

Total and volatile solids (TS and VS) were determined according to the standard methods (APHA, AWWA and WPCF, 1995). The analysis of volatile solids, determined in triplicates, was carried out by a preliminary drying of samples at 105°C for 24 h and determining the concentration of dry solids. In a second step, the dry solids were brought to 550°C for 2 h. The residue typically represents the inorganic part of the dry solids. The difference between the total dry solids and the inorganic ones gives the volatile solids.

The CST was measured with a patented standard CST device. The Model 319 Multi-CST Triton Electronics was designed to comply with European and U.S. electromagnetic compatibility standards. Two 10- and 18-mm diameter funnels were applied for *fast* and *slow* filtering of light and heavy sludges respectively. Sludge was poured into a small open funnel (circular tube) placed on a piece of special chromatographic paper. Filtrate is extracted by capillary suction and a cake is formed at the bottom of the funnel. The CST is obtained from two electrodes placed at a standard interval from the funnel. The time taken for the waterfront to pass between these two electrodes constitutes the CST. CST typically represents a measure of filterability.

Nutrients (TKN and Total Phosphorus) were determined in triplicates by photometric determination using Nitrogen and Phosphate cell test by Spectroquant Merck.

MBAS were measured in duplicates by the standard method (APAT-IRSA 5170), after an ultrasonic extraction from dried sludge to a methanol phase.

C10-C40 – (EPA 5021 A 2003 + EPA 3540 C1996 + EPA 8015D 2003 Total hydrocarbons).

The heavy metals were determined using an Agilent 7500c ICP-MS equipped with a collision/reaction cell in the form of the Octopole Reaction System (ORS). The ORS-ICP-MS technique uses a collision/reaction cell to remove polyatomic interferences. The ions are extracted from the ICP-MS interface into an octapole cell which is positioned before the analyser quadrupole. Helium gas is bled into the cell operated in the Radio Frequency (RF) mode. The RF field focuses the ions, which then collide and react with molecules of the gas leading to the removal of polyatomic interfering ions. Analytic ions are then directed toward the quadrupole analyser. The instrumental operating parameters are reported in Table 10.

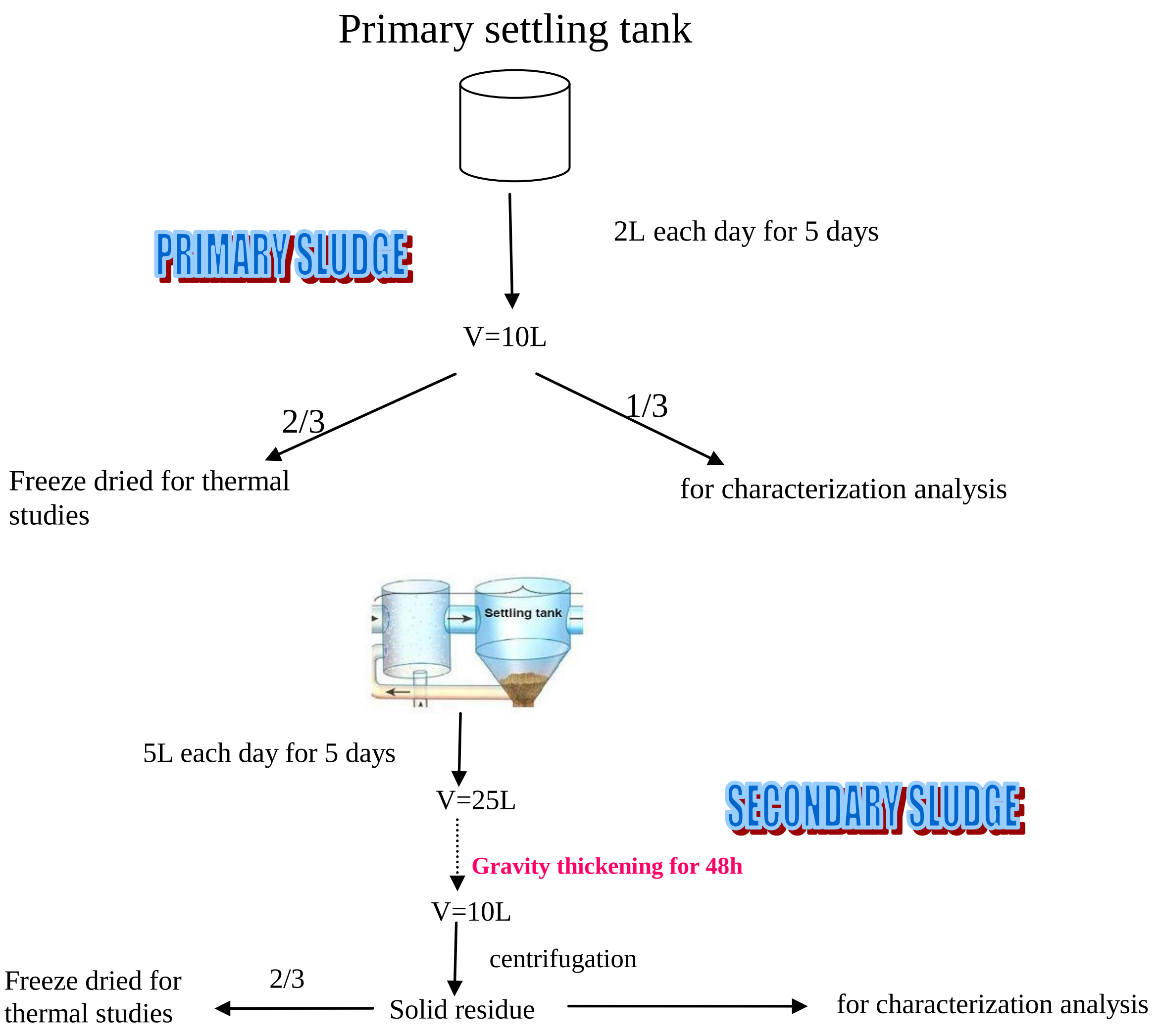

Fig. 3 Sampling strategy

Throughout this study the octapole cell was pressurized with ultra high purity He gas. The Instrumental Detection Limit (IDL) values were calculated according to the section 3.5 of method 200.8 by USEPA (1994).

The Extractable Organic Halogens (EOX) were determined according to EPA 9023 method using the ECS 1000 analyzer by Euroglas Analytical Instruments (Delft, Netherlands). This method does not allow detecting specific organic halogenated compounds, but only the total amount of Chlorine, Bromine and Iodine due to the organic halogens of the sample.

Table 10. Operating conditions adopted for ORS-ICP-MS analyses

RF power	1500 W
Sample depth	7 mm
Nebuliser	Babington
Nebuliser gas flow	1.10 L/min Argon
Spray chamber temperature	2° C
Sampler and skimmer cones	Nickel
QP bias	- 13.0 V
Octapole bias	- 7.0 V
Cell gas flow	2.5 mL/min He
Measurements	Integration time 0.3 s; 10 replicates
Instrumental tuning	Solution containing ^{7}Li, ^{59}Co, ^{89}Y, ^{205}Tl, ^{140}Ce at 5 □g/L each; $^{156}CeO^{+}/^{140}Ce^{+}$; $^{70}Ce^{2+}/^{140}Ce^{+}$

3.3.1 Nutrients

Error! Reference source not found. Tables 11, 12 and 13 **Error! Reference source not found.**show the characterization, in terms of total and volatile solids and nutrient contents, of analyzed sludges sampled at Roma Nord, Co.bis. and Fregene WWTPs.

Table 11. Solid and nutrient content of Roma Nord sludges

	Roma Nord (1°campaign)		Roma Nord (2°campaign)		Roma Nord (3°campaign)	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
Total Solids TS (g/L)	33.5	11.4	45.7	6.9	67.2	12.7
Volatile Solids VS (g/L)	15.6	6.2	19.9	3.2	22.7	4.5
VS/TS (%)	46.6	54.5	43.6	47.1	33.8	35.0
Total P (%TS)	1.0	1.4	0.8	1.4	0.7	1.2
Total N (%TS)	3.5	5.5	2.3	4.7	2.1	4.3

Table. 12 Solid and nutrient content of Co.bis. sludges

	Co.bis. (1°campaign)		Co.bis. (2°campaign)		Co.bis. (3°campaign)	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
Total Solids (g/L)	11.8	5.3	9.1	5.3	9.3	5.0
Volatile Solids (g/L)	8.7	3.8	6.8	3.9	6.2	3.4
VS/TS (%)	73.7	71.7	74.6	72.4	66.5	68.1
Total P (%TS)	1.4	3.5	0.5	0.8	0.2	0.5
Total N (%TS)	4.6	8.6	3.2	4.9	4.2	6.4

The average nutrient concentrations confirm that in the three WW treatment plants secondary sludge is richer in nitrogen (+60%, +130% and +170%) and phosphorus (+85%, +65% and +190%) than primary sludge. Volatile concentration of the Roma Nord sludges are quite low depending on the very prolonged sludge age and probably the inefficiency of the degritting unit.

Table 13. Solid and nutrient content of Fregene sludges

	Fregene (1°campaign)		Fregene (2°campaign)	
	Primary	Secondary	Primary	Secondary
Total Solids (g/L)	9.4	5.7	27.8	5.8
Volatile Solids (g/L)	5.4	2.2	19.1	3.7
VS/TS (%)	57.2	39.4	68.8	62.9
Total P (%TS)	0.9	1.2	1.4	5.0
Total N (%TS)	3.3	3.9	0.4	6.8

Some values of nutrients reported in Table 11, 12 and 13 are outside the typical range for secondary sludge (N 5-7 % of TS and P 1.5-3.0% of TS). Regarding the Rome Nord plant the values are a bit lower than the typical concentrations probably due to the quite diluted wastewater of Rome where we have a typical hydraulic loading of 500-700 L/(per × d). In the case of Cobis plant, it must be pointed out that a sewerage system collects all the discharges around the lake of Bracciano, which might be loaded with nutrients due to soil-leaching during the raining periods. Finally, as regards Fregene the low values of 1st campaign are certainly due to the high values of inert solids.

3.3.2 Heavy metals

Heavy metals are one of the main chemical classes of substances of water and soil pollution and can be found in various concentrations in wastewaters coming from both industries and municipal sewage (Chipasa, 2003). These elements, with a density above 5 g/cm^3 (Nies, 1999), have a well-known toxicity to microorganisms and their effects on a wide range of physiological processes are continuously studied (Ehrlich, 1997; Jezierska and Witeska, 2001). Generally, metal ions dissolved in any aquatic environment are in a range between $3 \cdot 10^{-11}$ and $3 \cdot 10^{-3}$ mol/L. Higher concentrations are often due to anthropogenic activities.

Adsorption of these ions on suspended particles and their deposit on sediments can cause damage to the equilibrium of aquatic systems. In many countries, water authorities are concerned with the problems related to heavy metal sludge disposal, because sludge with significant concentrations of toxic heavy metals is unsuitable for agricultural recovery and presents a potential environmental risk (Page et al., 1987).

Furthermore, it has been reported that significant increases of contamination potentially due to the presence of heavy metals in sludge-amended soils are produced by the formation of metal forms which are more easily extractable. The speciation of heavy metals significantly influences their environmental mobility and bioavailability determining a potentially high danger of environmental contamination. It is accepted that the primary environmental concern associated with the recycling of sewage sludge is the potential migration of heavy metals to the biosphere by the absorption in plants and consequently into the aquatic environment. The mobility of some of the most common toxic metals (Zn, Cd, Cu, Pb) usually contained in sewage sludge is very high and sludge application to soil increases the accumulation of metals in plants (Roy and Couillard, 1998).

Tables 14 and 15 show the metal content of primary and secondary sludges of Roma Nord and Co.bis.

Table 14. Heavy metals content (mg/kg) of “Roma nord” sludges

	1st campaign		2nd campaign		3rd campaign		Limits of the Directive 86/278 for agricultural use
	Primary	Secondary	Primary	Secondary	Primary	Secondary	
Cd	0.04	0.05	0.7	0.8	0.3	0.2	20-40
Hg	0.89	0.64	0.7	0.6	0.2	0.1	16-25
As	16	15	20	17	9.5	12.7	
Ni	25	22	25	24	14	16.3	300-400
Cr	47	45	96	97	25.1	36.8	
Pb	140	124	142	134	115.8	119.3	750-1,200
Cu	184	162	174	179	80.2	110.4	1,000-1,750
Mn	293	283	271	274	187.5	226.1	
Zn	544	465	617	612	258.1	360.1	2,500-4,000

Table 15. Heavy metals content (mg/kg) of Co.bis. sludges

	1st campaign		2nd campaign		3rd campaign	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
Cd	0.05	0.06	0.6	0.4	0.3	0.4
Hg	0.33	0.8	0.9	0.9	0.4	0.3
As	20	12	29	13	9.5	7.9
Ni	13	18	29	21	9.9	10.7
Cr	33	42	102	40	36.1	23.9
Pb	71	67	97	78	73.8	78.2
Cu	282	369	357	354	91.8	184
Mn	142	681	172	686	124	395.9
Zn	595	460	9.741	586	300.8	381.9

In spite of the negligible difference between primary and secondary sludge in heavy metals content, it must be pointed out that primary sludge gets strongly reduced in digestion compared to secondary sludge. The heavy metal concentration of primary digested sludge should therefore be higher than the one of digested secondary sludge. All values are well below (1-3 order of magnitude) the present European limits.

The metal concentrations measured are found to be far from the maximum concentrations permitted according to Goi D. et al. (Goi, 2006), demonstrating that these sludges are well suited for agricultural use.

3.3.3 Micropollutants

3.3.3.1 Organic halogen compounds

Contributors to the total amount of organic halogen compounds in municipal wastewaters and in sludge are washing detergents, disinfection byproducts, pesticides, varnishes, stain removers, flame retardants, cosmetics, pharmaceuticals, deodorants, etc. used typically in domestic activities, together with many other halogenated solvents and other synthetic materials consumed in handicraft activities and small industries connected to municipal sewers. The most common halorganics which can be found in municipal wastewaters are chlorinated hydrocarbons, chlorophenols (trichlorophenols, 2-chlorophenol, dichlorobenzene), trichloroethane, trichloroethylene, aldrin, dieldrin, polichlorinated biphenyls (PCBs).

The concentrations of these substances in municipal wastewaters usually are low so their toxicity phenomena on biomass are not a concern in traditional biological treatment plants. Biological processes are not very effective for their biodegradation, but rather these chemicals can be adsorbed and concentrated on biomass (WPCF, 1986; Bornhardt et al., 1997). Presence of organic halogen compounds in effluents of municipal WW treatment plants can preclude water reuse (Jekel et al., 1991).

It is important to underline that treatments which are traditionally carried out on sludge usually have no effect on the chemical properties of the halorganic compounds and therefore their removal from sludge can be carried out only through specific and generally expensive processes (Wild and Jones, 1989). If sludge is used to amend soils, these persistent halorganic polar compounds, which are less absorbed by soil, may reach the ground aquifer and pollute groundwaters (Klopffer, 1996).

In Fig. 4 the extractable organic halogens contents of both primary and secondary sludge of Roma Nord and Co.bis. are reported.

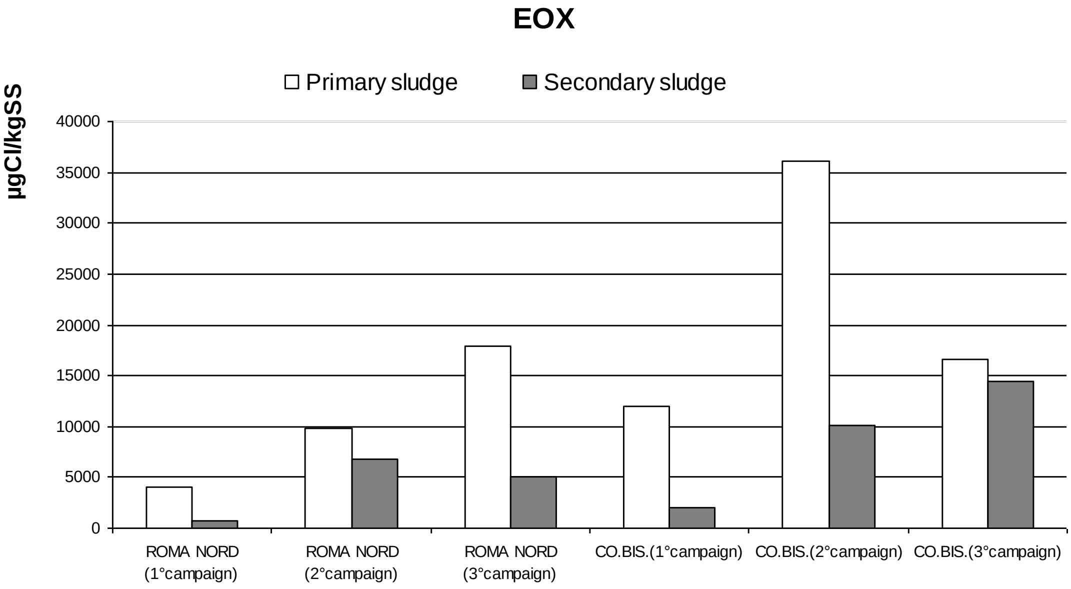

Fig. 4 Extractable organic halogens (EOX)

Primary sludge is always richer in EOX content than the activated one.

3.3.3.2 Total Hydrocarbons

Total hydrocarbons are apolar compounds consisting of carbon and hydrogen, which are classified as aromatic or aliphatic, according to their content or absence of benzene rings. Hydrocarbons originally derive from various petroleum fractions and their toxicity increases according to the scale: paraffins, naphthalenes, olephines, and aromatic compounds. Generally, the smallest and most volatile molecules are the most toxic ones. Hydrocarbons are used as fuel, lubricants, solvents and raw materials for the chemical industry.

WWTPs located in urban areas were much likely to receive wastewaters contaminated by hydrocarbons and PAHs from road traffic and phtalates from industrial activities. These compounds could then be concentrated in sludge during primary settling. Some microorganisms are able to degrade organic compounds; for instance, in biological processes such as sludge composting or anaerobic digestion some PAHs and PCBs were reported to be eliminated by biodegradation (Lazzari et al., 2000; Bernal-Martinez et al., 2005). In settling tank, where the primary sludge is produced, oxygen concentration is low causing a slow fermentative biodegradation. On the contrary in aerated basin, where biological sludge is produced, oxygen concentration is higher and a high oxidative biodegradation is expected.

In Fig. 5 the total hydrocarbons contents of both primary and secondary sludge of Roma Nord and Co.bis. are reported.

3.3.3.3 Methyl Blue Active Substances (MBAS)

Household detergents are the principal source of inorganic and organic chemical substances of domestic origin which are discharged into the environment, normally through wastewater drainage systems. The active matter of detergents is composed of natural and/or synthetic surfactants of different types. Linear alkylbenzene sulphonates (LAS) are the most frequently employed synthetic anionic surfactants. (Prats et al., 1997) and typically they represent 70-80% of MBAS.

Fig. 6 shows the MBAS content in primary and secondary sludge of Roma Nord, Co.bis. and Fregene.

These results show that anionic surfactants, which are one of the focus pollutant affecting sludge utilization in agriculture, are mostly adsorbed in primary sludge.

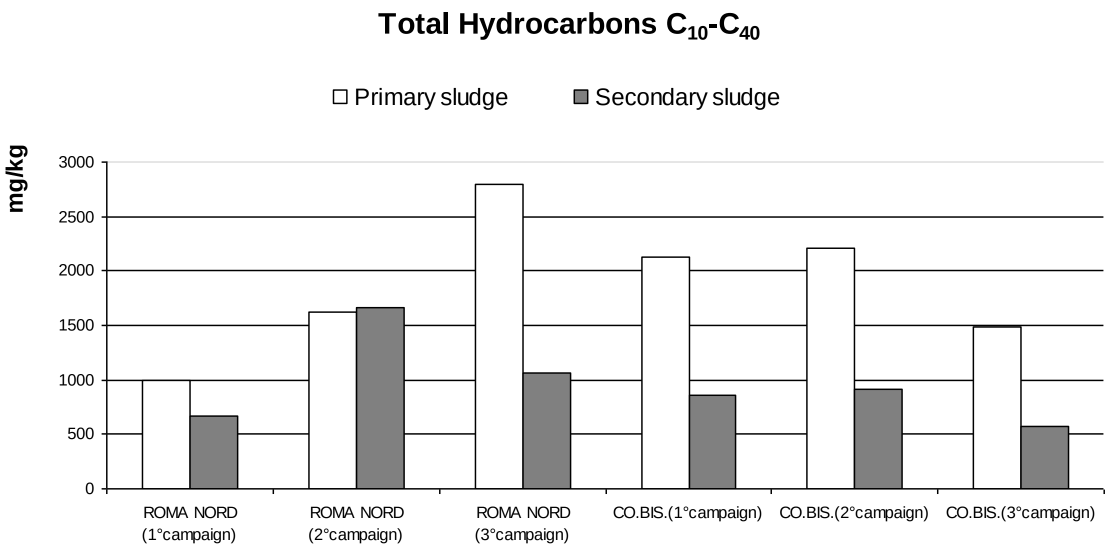

Fig. 5 Hydrocarbons

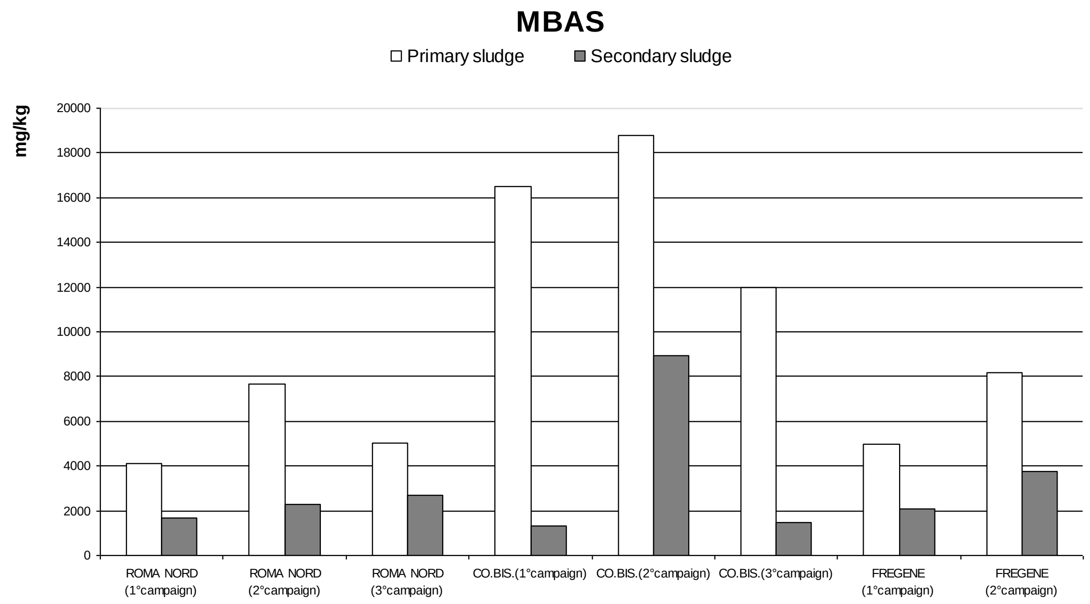

Fig. 6 Anionic surfactants (one of the focus pollutant because inserted in the third draft of the proposed new directive on sludge utilization in agriculture) are mostly adsorbed in primary sludge (MBAS = methylene blue active substances)

As said before, MBAS determination was performed adapting the standard method APAT-IRSA 5170 (which was developed for surface waters, groundwater and urban and industrial wastewater) to sludge analyses. This method permits the determination of the total amount of anionic sulphate and sulphonates surfactants, but it is subjected to various interferences, both positive and negative ones. Positive interferences are generated by the organic substances which can react with methylene blue to give chloroform soluble salts and by the inorganic substances which can form ionic couples with methylene blue. Instead, negative interferences are due to those substances which can react with the anionic surfactants or reduce the methylene blue. So, MBAS quantification in a complex matrix like sludge leads to an overestimation of the anionic surfactants concentrations.

3.4 Dewaterability of sludge

Capillary suction time is widely used to asses sludge dewaterability. The test is very rapid (some seconds) and as such is therefore a good alternative for determining filterability that normally should be assessed by specific resistance to filtration that is quite time consuming test. Only when sludge solid concentration is high and the predominant presence of particulate as colloidal fraction renders filtration very difficult, CST may be as high as hundreds of seconds.

Table 16 shows the CST values of the Roma Nord, Co.bis. and Fregene, respectively. It may be seen that practically there is no difference among different sludge types which show very good filterability.

Table 16. Sludge dewaterability

	Roma Nord					
	Primary			**Secondary**		
Sampling campaign	**I**	**II**	**III**	**I**	**II**	**III**
Capillary suction time (s L/g dry solid)	**2.3±0.3**	**3.67±0.16**	**1.78±0.12**	**0.61±0.01**	**1.12±0.06**	**0.85±0.07**
	Co.bis.					
	Primary			**Secondary**		
Sampling campaign	**I**	**II**	**III**	**I**	**II**	**III**
Capillary suction time (s L/g dry solid)	**5.1±0.2**	**3.0±0.12**	**1.94±0.12**	**0.78±0.05**	**0.94±0.06**	**1.82±0.02**
	Fregene					
	Primary			**Secondary**		
Sampling campaign	**I**	**II**	**III**	**I**	**II**	**III**
Capillary suction time (s L/g dry solid)	1.58 ± 0.08	1.65 ± 0.15		1.60 ± 0.03	1.94 ± 0.12	

3.4.1 References

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4. RESULTS

4.1 Effects of ultrasound pretreatment on sludge

4.1.1 Application of ultrasound on sludge: state of the art and theory principles

Ultrasound assisted biological sludge degradation has been extensively studied in laboratory, pilot and full-scale (Lafitte-Trouque and Forster, 2002; Müller et al., 2003; Bougrier et al., 2005; Tiehm et al., 2001; Odegaard, 2004; Brown et al., 2003; Braguglia et al., 2006; Khanal et al., 2007; Eder and Guenthert, 2002; Groenroos et al. 2005; Nickel and Neis, 2007; Pilli et al., 2010). Many of research studies presented the SCOD (soluble COD) increase with respect to sonication duration, which makes it harder to compare one study to the other. This is because ultrasonic disintegration depends on several factors such as TS content, frequency, sludge type, ultrasonic density, temperature, duration of sonication, etc., and such information is not well described in the literature. For better comparison, the SCOD release needs to be correlated with the specific energy input, by means of the following equation:

$$E_{spec} = \frac{P \times t_t}{V \times TS}$$

Fig. 7 shows a typical SCOD increase pattern at different specific energy inputs . As evidence from the figure, better disintegration of biological and non-biological solids was achieved (as evident from continuous increase in SCOD) at a longer sonication time, and thus at a higher specific energy input. This is because at longer sonication time or at higher specific energy input, there was ample opportunity for cells and debris to come under perpetual attack of large numbers of collapsing cavitation bubbles. However, the release in SCOD slowed down at an energy input of over 35 kJ /g TS. This is most likely due to exhaustion of readily disintegrable biological and non-biological organic particles near the collapsing cavitation bubbles or exhaustion of dissolved gases that aid cavitation bubble formation. Based on this finding, an energy input of 35 kJ/gTS was found to be optimal for maximal sludge disintegration at 3% TS content (Khanal et al., 2007). It is important to point out here that optimization of energy requirements for efficient sludge disintegration is extremely important for cost effective digestion of sonicated sludge.

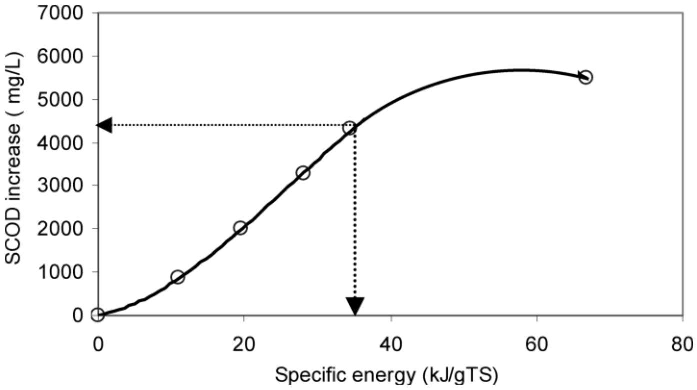

Fig. 7. Typical SCOD increase pattern at different specific energy inputs

Muller proposed an equation to calculate DD_{COD} as given by Schmitz et al. (Schmitz, 2000):

$$DD_{KW} = \left[\frac{COD_{ultrasound} - COD_{original}}{COD_{NaOH_{22hr}} - COD_{NaOH_0}}\right]\left[\frac{COD_{NaOH^*}}{COD_{hom\,ogenisation}}\right] \times 100(\%)$$

In the above equation, $COD_{ultrasound}$ is the COD in the supernatant of the sonicated sample (mg/L); $COD_{original}$ is the COD of supernatant original (untreated sample) (mg/L) and COD_{NaOH} is the maximum COD release in the supernatant after NaOH digestion at 22 hours. Tiehm et al. (Tiehm, 2001), Rai et al (Ray, 2004), Bougrier et al. (Bougrier, 2005) and Nickel and Neis (Nickel, 2007) used the degree of disintegration (DD_{COD}) modified by Muller (modified version from Kunz and Wagner, (Kunz, 1994) for evaluating the disintegration efficiency.

The effect of total solids (TS) concentration on degree of disintegration was evaluated by Neis et al. (Neis, 2000). Increase in TS, the solubilisation of COD increased leading to an optimum, beyond which solubilisation of COD decreased. Higher solids concentration generates more cavitation sites and more hydro-mechanical shear forces due to implosion of more bubbles formed, and beyond the optimum concentration the homogeneous distribution of the acoustic waves are disrupted by absorption effects (Neis et al., 2000). However, according to Show et al. (Show, 2007) the optimal range of solids content for sonication lies between 2.3% and 3.2% TS; if the solids concentration is too high, increased viscosity hinders cavitation bubble formation. The degree of disintegration is lower at higher specific input, therefore the effect of ultrasonic density is more significant than specific energy input (a contradictory statement when compared with previous studies, saying that specific energy is more important than ultrasonication density for higher sludge disintegration).

The effect of input power of the bulk solution were evaluated by Chu et al. (Chu, 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 power level of 0.33 W/mL, the ratio of BOD/TCOD increased from 66% to 80%.

The effect of ultrasound on different types of sludges was also performed. The solubilisation effect during in the sonication of primary and secondary sludge was evaluated (Mao, 2004). The solubilisation of COD was higher in the secondary sludge (SCOD increased 4 times in the primary sludge and 7.7 times in the secondary sludge) and linear correlation was observed between SCOD and sonication time for primary and secondary sonicated sludge . Bougrier et al. (Bougrier, 2005) evaluated the effect of specific energy (kJ/kg TS) on COD solubilisation. With increase in specific energy input the soluble COD increased. The rate of solubilisation was higher initially between specific energy of 0–8000 kJ/kg TS, beyond which increase in specific energy reduces the rate of total solid solubilisation. The soluble COD ratio (SCOD/TCOD) increased from 4% to 32% with increase in specific energy from 0 kJ/kg to 10,000 kJ/kg TS, and the optimum solubilisation occurred at a specific energy of 10,000 kJ/kg TS.

Braguglia et al. (Braguglia, 2009) observed a significant increase in terms of soluble COD and particle charge density after sonication WAS pretreatment in the range 1250-5000 kJ/kg TS. This increase might be attributed principally to the release of extracellular polymeric substances during the floc disintegration. In fact release of intracellular material is negligible by this range of sonication explored (up to 5,000 kJ/kg TS). Only by increasing the energy input (8,000 kJ/kg TS) break-up of cell walls may occur (Khanal et al. 2006).

Khanal et al. (Khanal, 2006) investigated the impact of sonication on the structure of flocs in WAS by microscopic observation. They reported that sonication resulted in a considerable structural changes in flocs and disappearance of filaments in WAS. Khanal et al. (Khanal, 2006) conducted scanning electron micrograph (SEM) to assess the impact of sonication on biological properties of sludge. The results revealed that even short time sonication (2 minutes) disrupt structural integrity of flocs as well as filaments. They showed that longer sonication (10 to 40 minutes) could lead to disruption of majority of bacteria. The results obtained by Wang et al. (Wang, 2005) indicated that protein was the predominant part of organic material which was released by sonication of WAS.

In activated sludge, almost all bacteria are able to produce EPS (extracellular polymeric substances) that participate in microbial aggregation of flocs. Flocs consist of microbial cells enveloped in a matrix of polymers that are located outside of the cells and have a high molecular weight. In addition to aggregation of bacterial cells, the EPS matrix can play several roles in sludge and wastewater such as formation of a protective barrier providing resistance to harmful shocks, improving adhesion of cell surfaces, accumulation of some enzymatic activities such as digestion of exogenous macromolecules for nutrients and accumulation of nutrients from the environment by sorption of exogenous organic compounds and providing a stable composition of microbial community in a highly dense condition in order to allow microorganisms to live continuously (Wingender, 1999) . Various polymeric materials exist in EPS in activated sludge including neutral and acidic polysaccharides, lipopolysaccharides, proteins, nucleic acids and humic acids (Urbain et al., 1993; Frolund et al., 1996). Bura et al. (Bura,1998) demonstrated that polysaccharides play the most important role in flocculation and settlement as compared to proteins although proteins were found to be the dominant component of EPS. The dominance of protein in activated sludge could be due to the presence of a large quantity of exoenzymes in the flocs (Frolund et al., 1995). The substrate compounds, cell lysis and the destruction of flocs can also affect the complexity of extracellular proteins (Dignac, 1998).

There has not been a considerable study of pretreatment impact on EPS of WAS in the literature. Pretreatment can inactivate or disrupt cells and impact on EPS loss. In addition, a pretreatment process can destroy EPS or release bonds with cells. Due to the short period of pretreatment, a comparison between the EPS content of WAS before and after pretreatment can be employed to assess the short term impact of pretreatment on EPS including disruption and disappearance. Pretreatment may facilitate EPS production in the anaerobic digestion process. Poxon and Darby (Poxon and Darby, 1997) indicated that an increase in concentration of glucose caused an increase of EPS content of anaerobic sludge in full and laboratory scale digesters. The provision of glucose as an easily assimilated substrate that is readily available for bacteria in initial stages of digestion stimulated the production of EPS. The experiments showed that acidogens were responsible for EPS production and the addition of the other materials such as propionic acid did not accelerate the rate of EPS production. Since, pretreatment can enhance the hydrolysis stage in anaerobic digestion of WAS, dissolved concentrations of carbohydrates as a suitable substrate for acidogenic bacteria may increase. Accordingly a higher formation of EPS due to pretreatment of WAS may occur in a digester.

The knowledge of EPS content of WAS and the impact of pretreatment technologies on EPS is beneficial to interpret the other pretreatment impacts such solublization or rates of biodegradability. Since a considerable fraction of organic materials in WAS are in EPS form (Liu and Fang, 2002), the composition and level of solublization might depend on the composition of EPS and the strength of bonds between EPS and microorganisms. For instance an increase in the level of colloidal COD due to pretreatment can be interpreted as the solublization of EPS and the biodegradation rate of colloidal material might depend on the chemical composition of EPS in WAS.

Moreover, if sludge disintegration degree is too high, many fine colloidal particles will be produced, which is not beneficial for sludge conditioning. Chu et al. (Chu, 2001) reported that the CST for 0.33W/ml sonicated sludge had been increased 2.5 times after 1 hour of sonication. In this case, the dewaterability of sludge was therefore seriously deteriorated after ultrasonic treatment. Such an observation was attributed to the fact that a great amount of water could be attached onto the large surfaces provided by the small particles after ultrasonication (Wang, 2006).

4.1.2 Materials and methods

4.1.2.1 Sludge characterization and ultrasound pretreatments

Sludge

The anaerobic digestion experiments described in this section were performed on two different types of sludge: secondary sludge, untreated and sonicated at 20 kHz. Excess sludge was obtained from the municipal "Roma Nord" wastewater treatment plant, one of the four wastewater plants of the city of Rome (Fig. 8). Secondary sludge was sampled from recycle stream before thickener to avoid the addition of polyelectrolyte, which can alter the charge density determinations. It was gravity thickened for 24 h at 4°C before carrying out the sonication experiments.

Fig. 8 .View of Roma Nord treatment plant

Determination of soluble COD (Chemical Oxygen demand)

COD or chemical oxygen demand is used to indirectly measure the amount of organic substances. It is expressed in mg/L which indicates the amount of oxygen consumed per liter of solution.

Sludge aliquots were centrifuged at 4000 rpm for 10 min and then 10 ml of supernatant were drawn and passed on acetate cellulose filter paper with a 0.45 micron pore size.

Soluble COD (chemical oxygen demand) in sludge supernatant was measured by photometric determination of chromate consumption by the organic compounds, after digestion in concentrated sulphuric acid solution at 148°C for 2 h by means of COD Cell Test by Merck Spectroquant (EPA method 410.4). COD determinations were done in duplicate.

Determination of total and volatile solids

Total solids content represents the amount of solids remaining after heating the sample at 105°C to constant weight. The total solids are composed of two components, volatile and fixed solids. The volatile solids are organic compounds of animal or plant origin.

Volatile solids (VS) were determined drying the samples at 105°C for 24 h to obtain the concentration of dry solids. In the next step, the dry solids were incinerated at 550°C for 2 h. The residues after incineration represent the inorganic part of the dry solids. The difference between the total solids and the inorganic ones gives the volatile solids (IRSA, 1984).

EPS content

Sludge aliquots are filtered through glass filters with 1.2 µm pores (GF/C Whatman); the supernatant is used for protein and carbohydrates determination.

Protein content is calculated by means of the modified Lowry Kit for Protein Determination (Sigma-Aldrich P 5656).

Carbohydrates determination is based on a modified DuBois method (DuBois, 1956).

Particle charge detector

The charge density determinations were performed by a Particle Charge Detector PCD02 (Mϋtek GmbH,Herrsching). The PCD operates on the principle of the so-called “streaming current detector”. Inside a measuring Teflon vessel, a piston moves back and forth at constant frequency in a sample. Colloids (and other adsorbable material) adhere to the piston and vessel surfaces, such that the apparent characteristics of these surfaces become those of the attached colloidal materials. Due to the oscillating piston movement, the sample liquid phase flows in the annular space between pistons and vessel wall. The resulting streaming current, referred to as SC, is detected by electrodes at opposite ends of the flow path and amplified to give a digital output. The SC is proportional to the electric charge of the colloids and therefore provides a measure of particle charge similarly to zeta potential (Abu-Orf and Dentel, 1997). The overall charge can be quantitatively measured by a titration using a standard polyelectrolyte solution (0.001 N polydiallyldimethylammoniumchloride solution, called Poly Dadmac). A titrant (organic polyelectrolyte) of opposite charge to the sample is added until the latter reaches the point of zero charge. The original charge amount is therefore calculated from the titrant consumption. Sludge samples were centrifuged at 5,000 rpm for 10 min and the centrate was titrated in the PCD. This procedure was used to determine the charge related to the colloidal particles. Alternatively, the charge total quantity of sludge particles was evaluated using diluted sludge samples with deionised water (1:20), thus allowing free piston movement without interference due to solid phase.

Determination of capillary suction time

Capillary suction time (CST) typically represents a measure of filterability.

The CST was measured with a patented standard CST device. The Model 319 Multi-CST Triton Electronics was designed to comply with European and U.S. electromagnetic compatibility standards. Two 10- and 18-mm diameter funnels were applied for *fast* and *slow* filtering of light and heavy sludges respectively. Sludge was poured into a small open funnel (circular tube) placed on a piece of special chromatographic paper. Filtrate is extracted by capillary suction and a cake is formed at the bottom of the funnel. The CST is obtained from two electrodes placed at a standard interval from the funnel. The time taken for the waterfront to pass between these two electrodes constitutes the CST.

Microscope analyses

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

Ultrasound pretreatment at 20 kHz

The disintegration by ultrasound was performed with an ultrasonic processor UP400S (dr. Hielscher, Germany) operating at 255 W and 24 kHz. The power control had an amplitude of 20-100%. The sonotrode has a diameter of 22 mm making the device suitable for samples volumes from 100 to 2000 mL. Sonication was performed for 4 min on 500 mL of waste-activated sludge (2,5-3% TS) placed in 1 L beaker with the probe allocated at 3 cm above the beaker bottom. The specific energy was in the range 4300-5000 kJ/kg dry solids depending on the TS of the treated samples. The ultrasound treatment was carried out immediately before starting the anaerobic digestion experiment.

Ultrasound pretreatment at 200 kHz

Sonication was performed for 10 and 40 min on 300 ml of waste activated sludge. The ultrasound apparatus is constituted of an amplifier T&C Power Conversion and a sonoreactor Elac Nautik USW 51-051, operating at 200 kHz and at an average power of 90-100 W. The transducer is made up of a piezo-ceramic element with an active area of approx. 25 cm^2. The glass sonication cell is covered and water jacketed for cooling sonicated sludge mixture. The specific energy supplied was in the range 19500-44670 kJ/kgTS.

Sludge disintegration by ultrasound

To assess the degree of sludge disintegration, the chemical oxygen demand (COD) in the sludge supernatant was determined. The degree of disintegration (DD_{COD}) is calculated as the ratio between COD increase due to sonication and the total COD:

$$DD_{COD} = \left[\frac{\left(COD_{sol,son} - COD_{sol,tq} \right)}{COD_{\max}} \right] \times 100$$

where $COD_{sol,son}$, $COD_{sol,tq}$ and COD_{max} are the supernatant COD (mg/L) respectively of the sonicated sample, of the untreated sample and of reference sample after complete chemical mineralization with H_2SO_4. The degree of disintegration is dependent on the specific energy supplied (E_{spec}) which can be calculated by the following equation:

$$E_{spec} = \frac{P \times t_t}{V \times TS}$$

where P is the power of the ultrasonic processor, t_t the sonication time, V the sludge treated volume and TS the sludge total solids content.

4.1.3 Ultrasound irradiation at 20 kHz

The ultrasonication is an emerging and promising mechanical disruption technique for sludge disintegration due to several inherent merits. The ultrasound pretreatment on sludge induces considerable changes on the physical, chemical and biological sludge properties. In fact, ultrasound de-agglomerate the biological sludge flocs and disrupt the large organic particles into smaller size ones. The shear forces generated by high-pressure waves break down bacterial cell wall and release the intracellular substances into aqueous phase.

In the first stage of sonication the structure of the sludge flocs is effectively disintegrated (Tiehm, 1997) but cell lysis doesn’t occur yet. As the energy supplied to the sludge increases, microrganisms cell walls are broken and intracellular organic material and EPS are released into the aqueous phase. Bougrier et al. (Bougrier, 2005) found out that the minimum energy required to destruct the cells is about 1000kJ/kg TS, that is about 20 kJ/L sludge which agrees with the Gonze et al. (Gonze, 2003) who have found a minimum energy of 30 kJ/L sludge and Lehne et al. (Lehne, 2001) found a higher value which is 3000 kJ/kg TS. Protein is an important building block of all microbial cells. The microbial extracellular polymeric substances (EPS), which provide the structural matrix for all microbial aggregates such as flocs in activated sludge, also contain protein. Wang et al. (Wang, 2006) reported protein contents of 698 and 11,338 mg/L, respectively in EPS and inside the microbial cells with 3% TS content. Therefore, quantification of sludge disintegration particularly WAS by protein measurement could be used reliably. However, for field application, protein measurement is still not common as none of the published studies employed protein measurement to assess the efficacy of ultrasonic sludge disintegration. The COD measurement will continue to be the method of choice for daily operation due to its simplicity (Khanal, 2007).

The hydro-mechanical shear forces generated by the ultrasound disintegrate sludge flocs and the release of biopolymers into the liquid phase occurs. It has been demonstrated that the released biopolymers greatly affect the bio-flocculation process (Houghton and Quarmby, 1999): they are thought to be the glue that holds bioflocs together and they form functional groups such as hydroxyl and negatively charged carboxy groups (Zang, 2007). Thus, conditioning is required to neutralise the negative charge and usually a deterioration of the sludge dewaterability was observed (Novak, 2003.)

High-energy ultrasonic treatment can disrupt flocs and increase the number of fine particles and bound water. Therefore, only low energy ultrasound treatment is generally used to improve sludge dewaterability (Huan et al. 2009). Feng et al. (Feng, 2009) showed that application of low specific energy dosages (<4,400 kJ/kg TS) slightly enhanced sludge dewaterability, but larger specific energy dosages (>4,400 kJ/kg TS) significantly deteriorated sludge dewaterability. Chu et al. (Chu, 2001) report that the CST for 0.33W/ml sonicated sludge had been increased 2.5 times after 1 hour of sonication. In this case, the dewaterability of sludge was therefore seriously deteriorated after ultrasonic treatment. This may be due to the great amount of water attached onto the large surfaces provided by the small particles after ultrasonication (Wang et al. 2006).

But sonication mechanism is also influenced by ultrasonic frequency and characteristics of sludge (Show et al., 2006; Khanal et al., 2007).

Furthermore, it was figured out that the disintegration of sludge is better at low ultrasonic frequencies (20-40 kHz) (Atchley and Crum, 1988; Tiehm et al., 2001). The reason is the formation of powerful hydrodynamic shear at low frequency. The bubble radius is inversely proportional to the ultrasound frequency. Therefore, the application of low frequencies may create larger cavitation bubbles.

The evaluation of the degree of sludge disintegration is based on the changes in physical (as particles size distribution, microscopic examination, turbidity), chemical (increase of soluble COD, protein concentration, polysaccharides content of the supernatant, nitrate nitrogen and release of NH_3) and biological (heterotrophic count and specific oxygen uptake rate) properties.

4.1.3.1 Results and discussion

This section presents an overview of the results obtained with sludge ultrasound pretreatment at 20 kHz as regards the floc disintegration in terms of COD solubilization, charge and particle distribution and filterability (with CST).

The primary effect of ultrasound is the mechanical disintegration of sludge floc, and consequently the release of soluble and colloidal material. Fig. 9 shows the microphotographs of the untreated and sonicated sludge, at 10 and 40 min of ultrasonic treatment. The destruction of the sludge floc could be already observed after 10 min of sonication, whereas at 40 min the floc structure was completely broken and a high number of cells were dispersed in the surrounding liquid phase.

Quantitative chemical evaluation of sludge disintegration was assessed through the degree of disintegration modified by Muller (DD_{COD}). The disintegration degree is a biochemical parameter which represents the ratio between the soluble COD release after ultrasound treatment and the soluble COD obtained by chemical disintegration with NaOH. Chemical disintegration is assumed to completely disintegrate sludge flocs and therefore it is used as reference COD (Tiehm, 1997; Pilli, 2010). So, DD_{COD} describes the organic matter release caused by a mechanical disintegration of sludge flocs.

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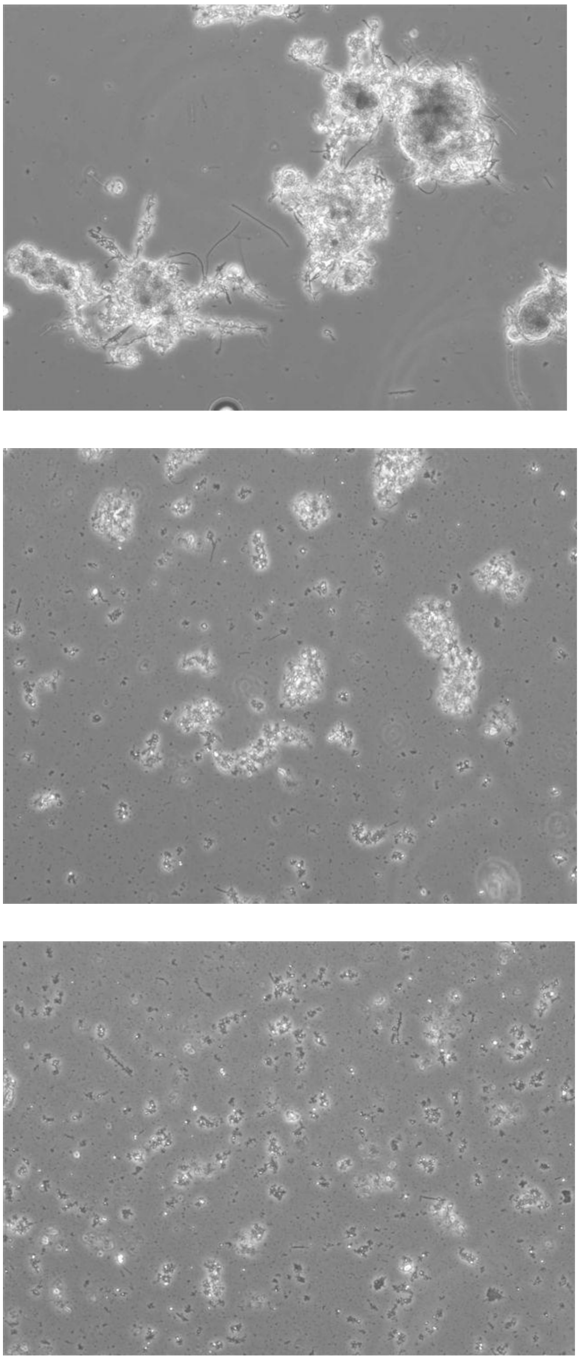

Untreated sludge

Sonicated sludge (10’)
Specific energy:
3049 kL/kgTS

Sonicated sludge (40’)
Specific energy:
6890 kJ/kgTS

Fig. 9. Microscopic photographs of untretaed and sonicated sludge, at 10 and 40 min.

Fig. 10 shows the DD_{COD} trend as function of the specific energy supplied to the sludge in the performed sonication experiments. The DD_{COD} steadily increased with increasing specific energy input up to 20000 kJ/kgTS; at high energy, the DD_{COD} increment was less evident and reached a plateau value. The DD_{COD} trend can be described with a logarithmic curve.

With energy input of 3000-4000 kJ/kgTS, a low disintegration degree (around 5%) can be obtained (as reported also by Lehne et al, 2001). This low DDcod indicated therefore the disruption of sludge flocs into smaller aggregates, without significant organic material release. With increasing the specific energy input, the COD release into the aqueous phase also increased; the maximum achievable DD_{COD} (50%) was reached for specific energy of 75000 kJ/kgTS.

To confirm that the disintegration degree is strictly dependent on the specific energy delivered to the sludge, different series of sonication experiments were performed at fixed specific energy and varying the sludge sample volume, the sonication time and the power of the ultrasonic device (parameter related to Espec). In all these experiments, the DD_{COD} calculated after sonication resulted almost constant.

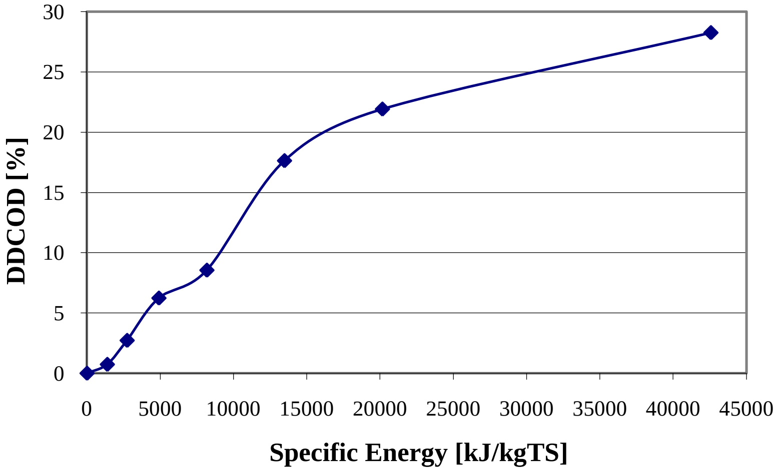

Fig. 10. DD_{COD} pattern as function of the specific energy

On the other hand, some authors pointed out that the degree of disintegration does not depend only on the specific energy. By applying the same energy input to different sludge samples the disintegration degree is not constant, because is strictly affected by the sludge characteristics (for example initial soluble and particulate COD) and by the volume of treated sample. For example, DD_{COD} of about 40% was obtained by Tiehm et al. at a specific energy of 60'000 kJ/kg TS (Tiehm et al., 2001), while Rai (Rai, 2004) reported a disintegration degree of 25% at an energy input of 64'000 kJ/kg TS and Khanal (Khanal, 2007) obtained DDCOD of 16.2 % with specific energy of 66'800 kJ/kg TS. Such differences can be attributed to different energy transfer efficiencies of the ultrasonic units used for the experiments.

The influence of the disintegration degree on the release of organic substances with different dimensions was also studied. Different sonicated sludge samples (i.e. at different energy inputs) were filtered by 0.45 and 1.2 µm pore size filters and the corresponding COD of the supernatants were measured.

As shown in Fig. 11 when DD_{COD} was lower than 10%, an increase in the concentration of the particles with dimensions between 0.45 and 1.2 µm was observed in the aqueous phase; with increasing the disintegration degree, the solubilisation of the smaller particles occurred. This may be explained because for DD_{COD} lower than 10%, the release of the colloidal extracellular polymeric substances and the fine intracellular colloidal and supracolloidal (>1.2 μm) material occurred. At higher disintegration degrees, the dimensions of these particles are significantly reduced.

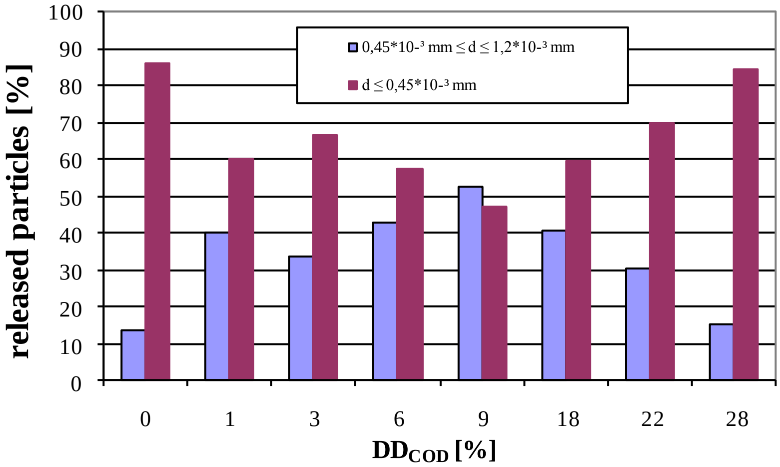

Fig. 11. Dimensional distribution of the particles in the sludge supernatatant

The disintegration efficiency can also be evaluated based on the release of the COD components such as carbohydrates, DNA and mainly proteins. In fact, the dry matter of the bacteria contains about 50-60% of proteins (Gonze, 2003). For different sludge samples (i.e. sonicated with different specific energies), the proteic COD was determined using the theoretical relationship: 1 g of proteins = 1.5 g of COD.

It was evident (Fig. 12) that the ultrasound treatment increased the protein release into the liquid phase and the solubilisation was dependent on the supplied specific energy. In the untreated sludge, the proteic COD was 29% of the soluble COD, and after the ultrasound treatment with a specific energy of 11516 kJ/kgTS, the proteic COD increased to 64%.

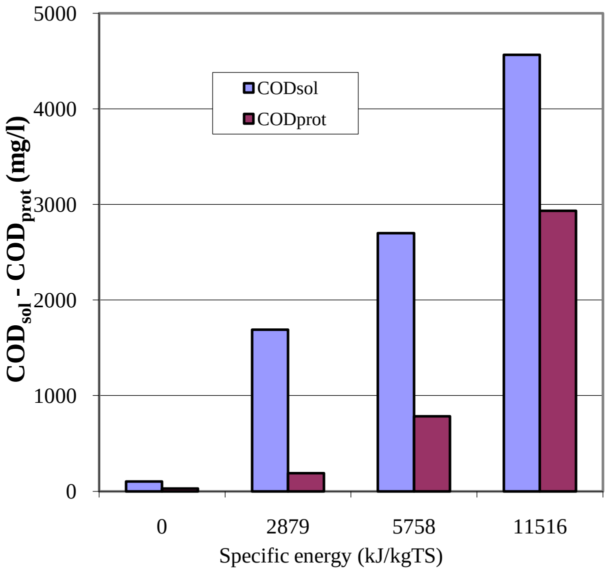

Fig. 12. Soluble and proteic COD

Activated sludge flocs are heterogeneous aggregates of microrganisms and organic and inorganic materials bound together by cations like Ca^{2+} and Mg^{2+} and biopolymers (Higgins, 1997). The biopolymers are constituted mainly by polysaccharides, proteins and also small amount of lipids, humic acids and DNA. Flocs in activated sludge usually carry negative charge at neutral pH. It has been found that the extracellular biopolymers contribute to the negative surface charge of the sludge flocs (Jia et al., 1996; Liao et al., 2001) and thus to the flocculation properties.

For this reason, surface charge density measures were performed on sludge samples, either untreated or sonicated, to relate this parameter to the ultrasound efficiency. The sludge particles presented in fact a negative charge. The specific surface charge measured for the untreated sludge was 2300±50 mC/gTS; for the sonicated sludge with a DD_{COD} of 5%, the surface charge was found to be 7520±60 mC/gTS and increasing the disintegration degree to 22%, also the surface charge increased to 22200±130 mC/gTS.

This phenomenon confirms the disintegration of sludge caused by the ultrasonic treatment leading to a significant decrease in particles dimensions. As a consequence, the specific surface and thus the surface charge density increased.

Fig. 13 shows the contribution (%) of the surface charge of the sludge supernatants filtered both with 1.2 and 0.45 µm filters to the sludge surface charge.

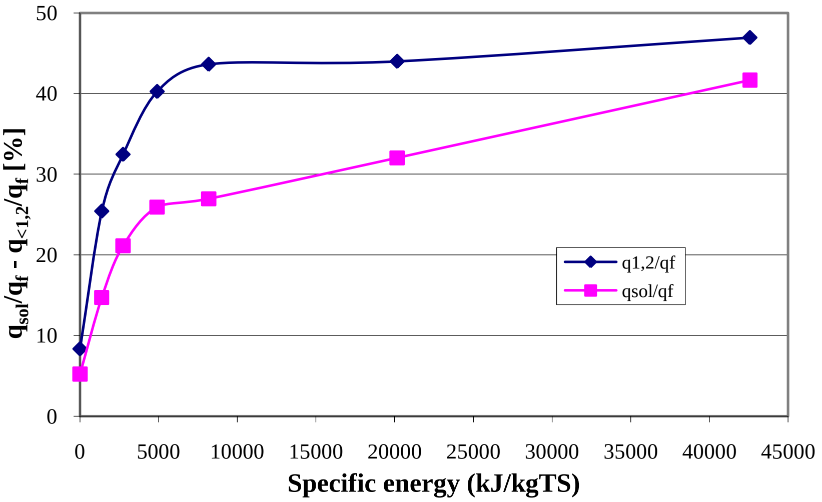

Fig. 13.

For specific energy inputs up to 10000 kJ/kg TS (corresponding to a DD_{COD} of about 10%), the percentage of the surface charge due to particles having dimensions <1.2μm increased steadily to 45% of the total sludge charge: this represented a plateau value because remained constant by increasing the specific energy input.

The trend of the surface charge contribution due to the particles smaller than 0.45 μm was different: for energies up to 10000 kJ/kgTS increased up to 25% of the total sludge charge and a continuous slight increment was observed by increasing the specific energy input. So, for low specific energy (i.e. <10000 kJ/kgTS) the difference between the surface charge contributions of the two types of particles may be due to the release of colloidal and supracolloidal material having dimensions in the range between 0.45 and 1.2 μm, confirming what reported before.

In order to understand the influence of the sludge total solids content on the disintegration efficiency of the ultrasound pretreatment, different sonication experiments were performed with sludge having TS contents in the range 5.88-27.70 g/L (Fig. 14).

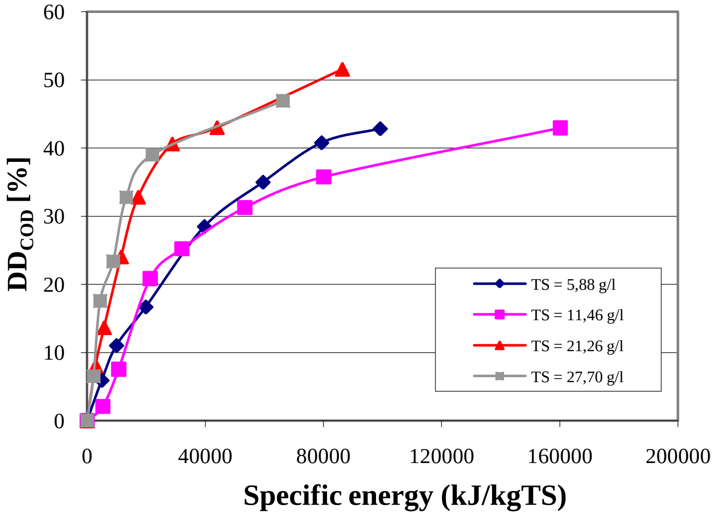

Fig. 14. DD_{COD} pattern at different TS content

For the sludge with low total solids contents (5.88-11.5 g/L), the obtained disintegration degrees were found to be almost constant. On the contrary, a significant effect of the solids on DD_{COD} was observed for higher TS contents (in the range 21-27.7 g/L): delivering the same specific energy input, the disintegration degrees were much higher with respect to those obtained pretreating sludge with low solids content. This is in agreement with the findings of a previous study by Neis (Neis, 2000) who reported that by increasing the total solids content the number of cavitation sites increased, thus leading to more efficient sludge disintegration. As a consequence, the use of thickened sludge resulted more convenient for the ultrasound pretreatment, and in the case of full scale applications (in Italy, as in Germany and Switzerland) activated sludge is generally prethickened and then sonicated.

Capillary suction time (CST) analyses were performed on the untreated and sonicated sludge to assess the influence of the ultrasound pretreatment on the sludge filterability. Fig. 15 shows the strict correlation between sonication and CST: by increasing the specific energy input supplied to the sludge, the CST increased, indicating a worsening of the dewaterability. This result confirms that by increasing the ultrasound energy floc disintegration increased in terms of fine particles released into the liquid phase. This could render the separation between the solid and the liquid sludge phases very difficult, because these fine particles blind the sludge cake and filter medium during filtration, resulting in increase in the capillary suction time. This may be due to the high amount of water bound to the reduced floc particles (Kopp&Dichtl, 2001).

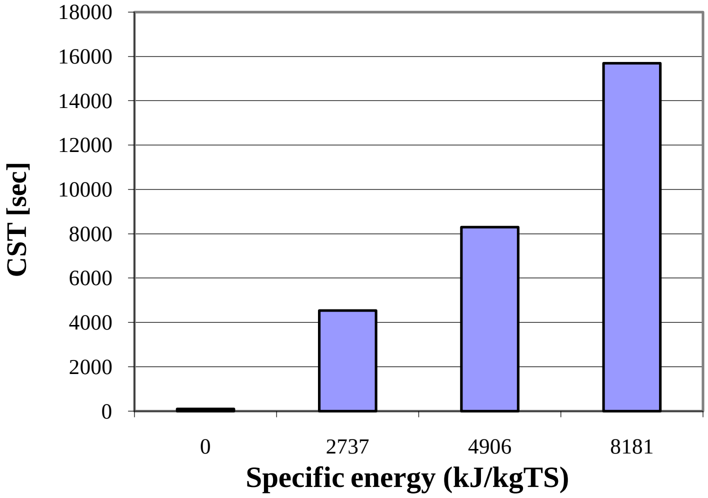

Fig. 15. CST as function of the specific energy

4.1.4 Ultrasound irradiation at 200 kHz

Operating frequency is an important parameter that controls the ultrasonic treatment efficacy. This is because operating frequency governs the critical size of the cavitation bubbles (Hua and Hoffmann, 1997). The resonant cavitation bubble is a function of the ultrasound frequency. Young (Young, 1989) approximated the ultrasonic cavitation radius as

$$R_r \approx 3.28 f_r^{-1}$$

where the resonant bubble radius is expressed in millimeters and f_r is the resonance frequency in kilohertz.

The application of low frequencies creates larger cavitation bubbles, and the hydrodynamic shear forces produced by ultrasonic cavitation are primarily responsible for particles disruption (Tiehm et al., 2001). Thiem evaluated the effect of ultrasound frequency, specific energy input and theoretical cavitation bubble size on degree of disintegration. With increasing in frequency, the degree of disintegration decreased gradually (DD_{COD} = 80% at 41 kHz and DD_{COD} = 7% at 3217 kHz). Mark (Mark, 1998) reported that sonochemical process, i.e. the production of hydroxyl radicals, were most significant at frequencies between 200 to 1000 kHz but not at 41 kHz. Thus, nearly all sludge disintegration researches have been performed at the lower frequency range of 20 kHz (Wang, 2005; Bougrier, 2005; Khanal, 2006).

In this Thesis sonication experiments at 200 kHz were performed to assess the disintegration efficacy available with higher frequency treatment and to make a comparison with the conventional treatment at 20 kHz.

4.1.4.1 Results

The evaluation of the efficacy of the ultrasound sludge pretreatment at 200 kHz is based on the variations of the dimensional, physical and chemical properties of sludge.

Ultrasound treatment experiments at 200 kHz were performed on the secondary sludge from Roma Nord wastewater treatment plant; sonication was carried out for 10 and 40 minutes.

In this section the results of these tests are reported and discussed.

Sonication time : 10 min

The specific energy supplied to the sludge sample during this sonication experiment was 7460 kJ/kgTS.

In Table 17 the flocs number and the percentage of the flocs belonging to five dimensional classes are reported for the untreated and sonicated sludge. For the untreated sludge, more than the half of the flocs had dimensions between 150 and 500 µm; after the ultrasound treatment applying 7460 kJ/kg TS the dimensional distribution of the flocs remained almost constant. In fact, the class between 150-500 µm still accounted for the 58.5% of the total sludge flocs. The results are reported in Fig. 16. The small particles, namely these with dimensions <50 µm, after sonication are halved.

Table 17 Dimensional distribution of the untreated and sonicated sludge (10 min) (a):flocs number; (b): percentage of flocs number

	Flocs number	
Dimensions (µm)	***untreated sludge***	***sonicated sludge (10')***
0-50	43	15
50-150	64	65
150-500	160	152
500-1000	30	26
1000-1500	3	1
Total flocs	300	260

(a)

	flocs number (%)	
Dimensions (µm)	***untreated sludge***	***sonicated sludge (10')***
0-50	14.33%	5.77%
50-150	21.33%	25.00%
150-500	53.33%	58.46%
500-1000	10.00%	10.00%
1000-1500	1.00%	0.38%

(b)

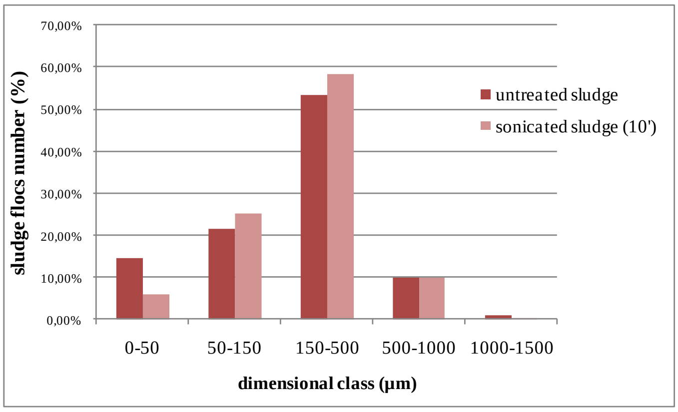

Fig. 16. Dimensional distribution of the untreated and sonicated sludge (10 min)

The slight sludge disintegration obtained with 10 minutes of ultrasonic treatment at 200 kHz was confirmed by the low increment of the soluble COD of the sonicated sludge with respect to the untreated sample (Table 18). Based on these results the disintegration degree was 2.9%.

Table 18. Soluble COD of the untreated and sonicated sludge

	SCOD (mg/L)
Untreated	490
sonicated (10')	917

Sonication time: 40 min

Due to the scarce disintegration efficiency obtained with the low energy ultrasonic treatment described before, the sonication experiment was carried out by increasing fourfold the treatment time. The specific energy delivered to the sludge sample was 44444 kJ/kgTS.

Table 19 reports the distribution of the untreated and sonicated sludge flocs on the basis of their dimensions in the five classes mentioned above.

Table 19. Dimensional distribution of the untreated and sonicated sludge (40 min) (a):flocs number; (b): percentage of flocs number

	Flocs number	
Dimensions (μm)	***Untreated sludge***	***Sonicated sludge (40 min)***
0-50	35	175
50-150	131	326
150-500	232	153
500-1000	24	3
1000-1500	2	0
Fiocchi totali	424	657

(a)

	flocs number (%)	
Dimensions (μm)	***Untreated sludge***	***Sonicated sludge (40 min)***
0-50	8.25%	26.64%
50-150	30.90%	49.62%
150-500	54.72%	23.29%
500-1000	5.66%	0.46%
1000-1500	0.47%	0.00%

(b)

When the sonication time was increased to 40 min, the disrupting effect of the ultrasound treatment at 200 kHz is more evident. In the untreated sample a high percentage of sludge flocs had dimensions between 150 and 500 µm; while, after the ultrasonic treatment, the number of flocs belonging to that class was considerably reduced and the percentage of flocs having smaller dimensions (50-150 µm) steadily increased (Fig. 17).

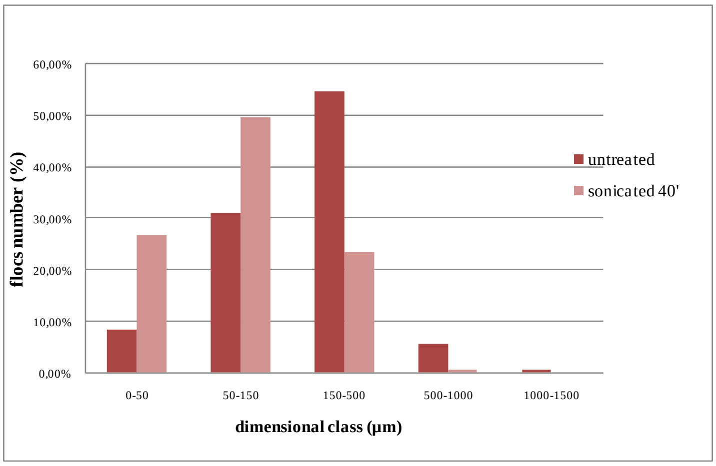

Fig. 17 Dimensional distribution of the untreated and sonicated sludge (40 min)

To assess the chemical changes due to the high-frequency ultrasound treatment at high energy, different sonication experiments were performed with increasing specific energy inputs. For all the experiments, a considerable increase of the COD in the soluble phase was observed. Fig. 18 shows the soluble COD increment observed after the ultrasonic treatment at 200 kHz in the 5 different experiments carried out during different periods of the work, on different sludge samples.

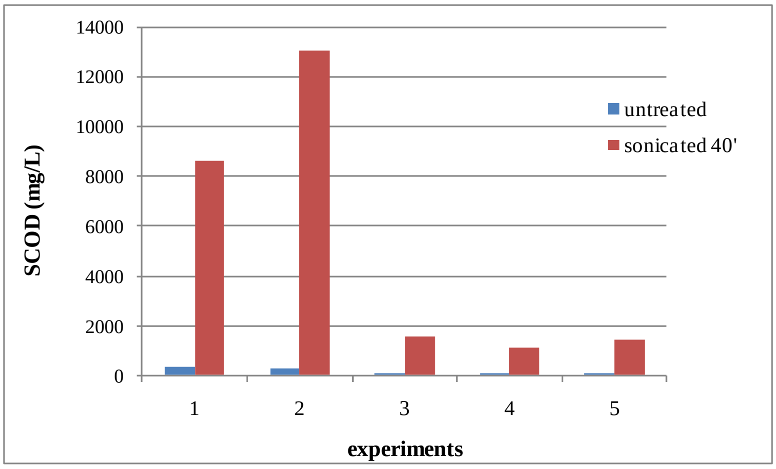

Fig. 18. Soluble COD of the untreated and sonicated sludge (40 min)

From SCOD values, the disintegration degrees were determined. In these experiments different specific energy inputs were supplied to the sludge samples; as a result, various levels of DD_{COD}

were achieved, ranging from 6 to 65%. As for the ultrasonic treatment at 20 kHz, DD_{COD} was greatly dependent on the specific energy supplied to the sludge sample.

As EPS are a considerable part of the organic matter released into the liquid phase after the ultrasonic treatment, protein and carbohydrates content of the untreated and sonicated sludge was also monitored. Table 20 reports the proteins and carbohydrates concentrations in the soluble phase of sludge, before and after the ultrasonic treatment.

Table 20. EPS (proteins and carbohydratres) content of the untreated and sonicated sludge (40 min)

	Proteins (mg/L)	**Carbohydrates (mg/L)**
untreated sludge	4,9	31,8
sonicated sludge (40')	252,9	423,6

Because EPS, and the proteins in particular, are the main responsible for the negative charge of the activated sludge, surface charge determination was also performed on the untreated sludge samples and the sonicated ones. Fig. 19 shows an example of the increment of the surface charge density which can was observed for the sonicated sludge of two different experiments with respect to the untreated samples.

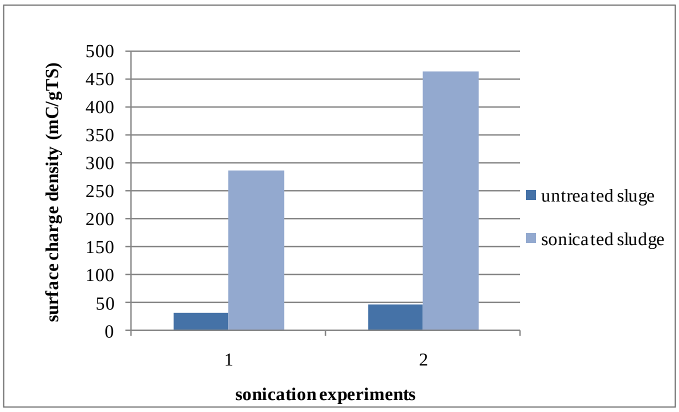

Fig. 19. Surface charge density of untreated and sonicted sludge (40 min)

4.1.4.2 Discussion

Ultrasound treatment is a very effective technique in reducing the particles size of sludge; it has been reported that the efficacy of size reduction is dependent by a high number of factors like sonication duration (Tiehm, 1997; Jorand,1995), ultrasonic density (Mao, 2004), sonication power (Show, 2007; Hua, 1997), sludge volume and characteristics (Feng, 2009; Mao, 2004).

Fig. 20 shows the percentage number of sludge flocs belonging to five dimensional classes in the untreated sludge and sludge samples sonicated for 10 and 40 min (sonication density: 0,23 W/ml). Most flocs in the untreated sludge had dimensions ranging from 150 to 500 μm; almost the same size distribution was found in the sample sonicated for 10 min. Increasing sonication time to 40 min resulted in an increase of the sludge flocs having smaller dimensions (50 to 150 and 0 to 50 µm).

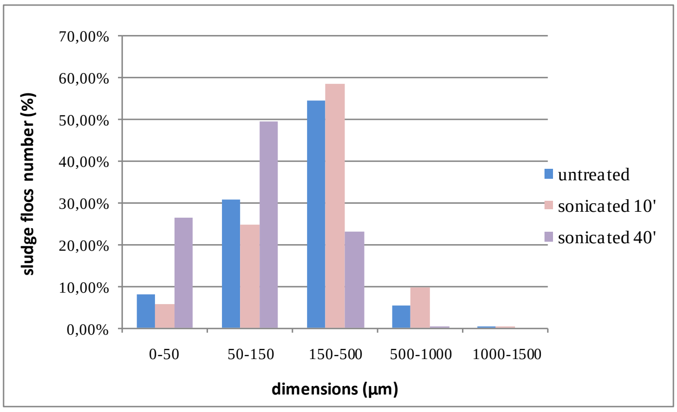

Fig. 20. Dimensional flocs distribution of the untreated and sonicated sludge

This is in agreement with Chu (Chu, 2001) who reported that only when power level has exceeded 0.22 W/ml the particles size would decrease after 40 min of ultrasonic treatment, suggesting the existence of an optimum power level and sonication time for sludge disintegration. As specific energy is proportional to sonication time, a correlation between the particles size distribution and specific energy can also be assessed.

Specific energy of 7460 kJ/kg TS is the calculated in the case of 10 min ultrasound treatment, whereas 44444 kJ/kg TS is the energy calculated for 40 min of sonication.

Fig. 21 shows microphotographs of the untreated and sonicated sludge, for 10 and 40 min of treatment. The untreated sample had a compact floc structure and no cell dispersion was observed in the surrounding liquid phase; after 10 min of ultrasonic treatment, the floc structure was somewhat looser, but the architecture of floc was basically the same as the original sample. In addition, protozoa (red arrows) resulted still alive. After 40 min of sonication, the structural integrity of flocs was completely broken, high cell dispersion was observed and only dead microorganisms were present. These observations are confirmed by the findings of Zhang (Zhang, 2007) who reported that sludge disintegration and cell lysis occurred continuously during sonication but sludge (biomass) inactivation occurred mainly in the second stage (10–30 min).

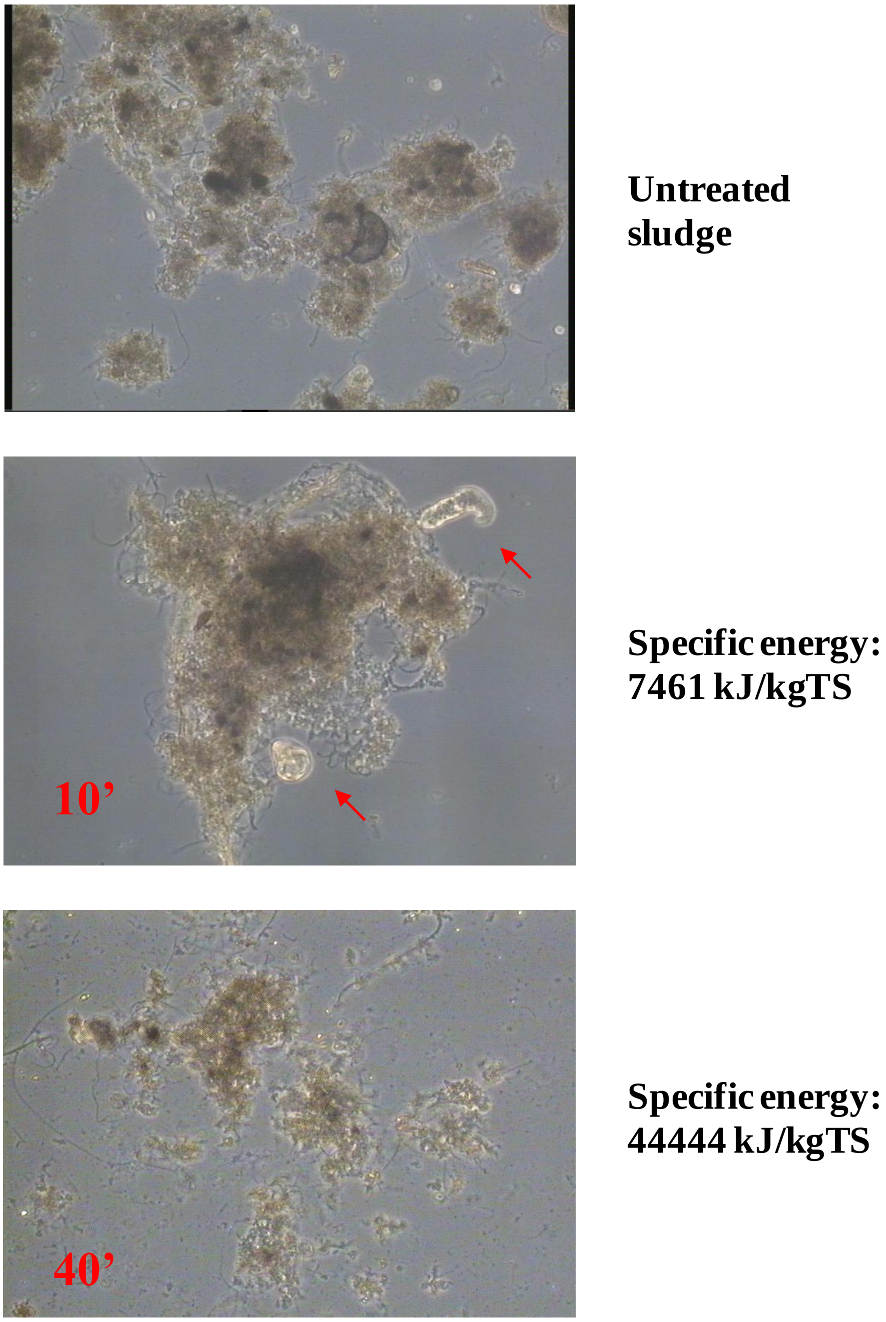

Fig. 21. Microscopic photographs of untreated and sonicted sludge

Ultrasonication disintegrates both cellular and extracellular matter, organic debris and extracellular polymeric substances (EPS) of the sludge, thus leading to a transfer of organic substances from the sludge solids to the liquid phase, as it was demonstrated by an increase of soluble COD after sonication (Fig 18 in chapter 4.1.4.1). Soluble COD represents a measure of sludge disintegration efficiency. However, sludge disintegration depends on various factors like sludge type and characteristics, TS content, power supply, frequency, ultrasonic density and duration, temperature. Almost all literature dealing with 20 kHz ultrasound pretreatment (Tiehm, 2001; Rai, 2004; Bougrier, 2005, Nickel and Neis, 2007) employed the degree of disintegration (DD_{COD}) modified by

Muller for evaluating the disintegration efficiency (see chapter 4.1.1). It has been reported that the disintegration of sludge is proportional to the provided energy (Müller and Pelletier, 1998, cited in Khanal, 2007; Lehne et al., 2001). The energy supplied is also proportional to sonication power and sonication time (Khanal, 2007). Bougrier et al. (Bougrier, 2005) found out that the minimum energy required to destruct the cells is about 1000kJ/kg TS, that is about 20 kJ/L sludge which agrees with the Gonze et al. (Gonze, 2003) who have found a minimum energy of 30 kJ/L sludge and Lehne et al. (Lehne, 2001) found a higher value which is 3000 kJ/kg TS.

Fig 22 shows DD_{COD} trend as function of the specific energy applied to different sludge samples. For specific energy higher than 20'000 kJ/kg TS, the disintegration degree steadily increased with increasing energy. The maximum destruction degree obtained was about 65%, with a specific energy input of 44'667 kJ/kgTS. This value was much higher than that reported by Tiehm (DD_{COD} of about 40%) for sludge sonication at 207 kHz (Tiehm et al., 2001).

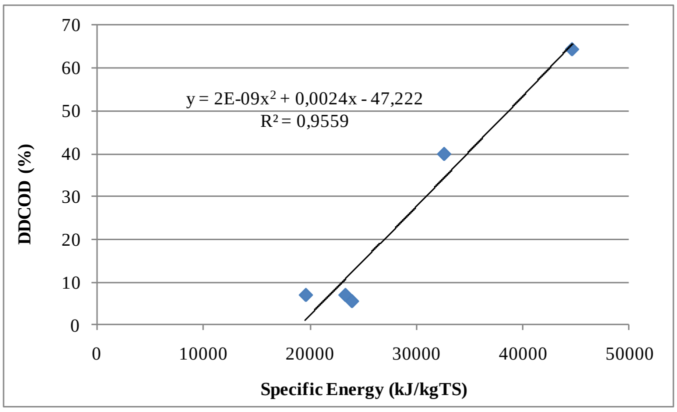

Fig. 22. DD_{COD} as function of the specific energy

The efficiency of destruction increased with the total solids content and this is consistent with previous literature findings (Onyeche, 2002). In fact, the presence of more solids enhances cavitation, as the solids particles can act as cavitation nuclei. Actually, the effect of the number of solid particles on the formation of cavitation bubbles in the sludge matrix is still unknown. It can be hypothesized that at higher TS content, the violent collapsing of micro-bubbles might accelerate the particles in the vicinity of the bubbles, which bombarded the adjacent particles. It is most likely that the abundance of particles at higher TS content could facilitate the sludge disruption due to particles-to-particles collision (Khanal, 2007).

The DD_{COD} trend as function of the sludge total solids showed a maximum value (65%) corresponding to the total solids concentration of 23.3 g/L (Fig.23). At higher TS content, the obtained disintegration degrees were lower. This decrement can be caused by the increasing sludge viscosity: in fact, high viscosity may reduce cavitation because of the scattering of the ultrasonic waves by the solids particles and by the adsorption by the fluid to generate heat rather than creating cavitation bubbles (Dewil, 2006).

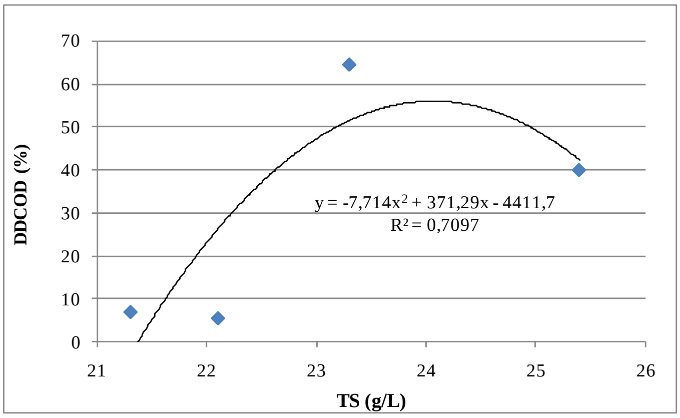

Fig. 23. DD_{COD} as function of the sludge TS content

EPS (mainly proteins and carbohydrates) represent a considerable fraction of the organic material in waste activated sludge and can be released into the liquid phase after ultrasound pretreatment.

Fig. 24 shows the increment of soluble proteins and carbohydrates concentration after the ultrasonic treatment at 200 kHz. Despite the very low proteins content in the untreated sludge (due probably to the high sludge age of the activated sludge plant) the increase of soluble protein in the liquid phase was significantly higher (50 *vs* 13 times) than that of carbohydrates, as also reported by Wang (Wang, 2006).

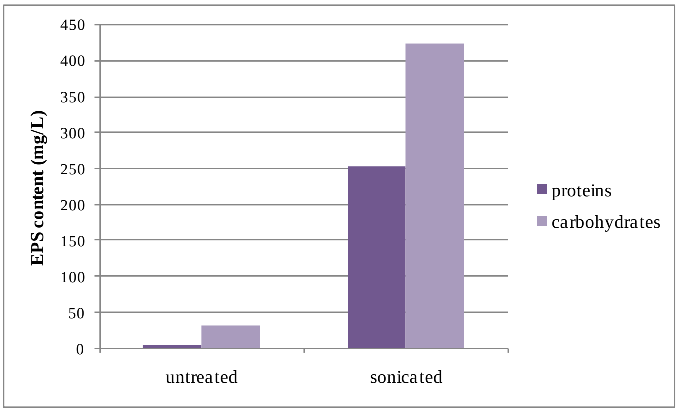

Fig. 24 EPS content of the untreated and sonicated sludge

To assess and compare the ultrasound disintegration efficiency, also the proteic COD was calculate starting from proteins concentration (Fig. 25):

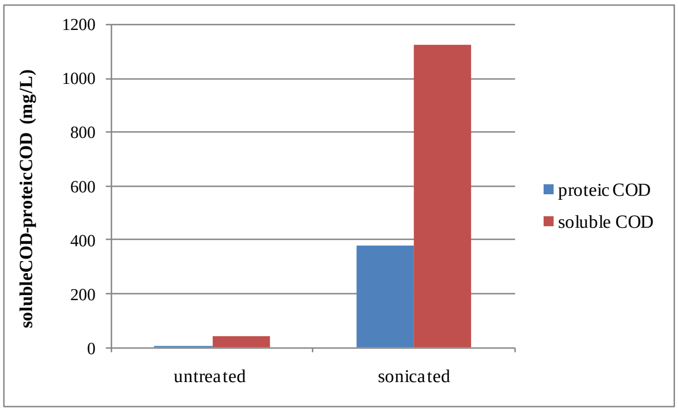

Fig. 25 Soluble and proteic COD of the untreated and sonicated sludge

In the untreated sludge, the proteic COD was 18% of the soluble COD, and after the ultrasound treatment at a specific energy of 23894 kJ/kgTS, the proteic COD incresead to 34%.

The surface charge density of sludge sample increased after the ultrasound treatment (see Fig. 19 in chapter 4.1.4.1.): this result was due to a strong particle "disintegration" with relative increase of the specific particle surface and consequently of the specific surface charge.

4.1.5 Comparison of the ultrasound treatment at 200 kHz and at 20 kHz

Ultrasound pretreatment represents the most innovative and efficient mechanical disintegration treatment, in order to improve the biodegradability of sludge. The high shear forces generated during the ultrasound pretreatment break down the microrganisms cell walls and release the intracellular materials into the liquid phase. This will accelerate the subsequent degradation and reduce the retention time needed during sludge digestion (Pavlostathis, 1986). Sludge disintegration also disrupts other organic particles into low molecular weight compounds. Frequency is an important parameter affecting the disintegration degree obtained through ultrasound pretreatment. It has been reported that the resonance bubbles radius is inversely proportional to frequency (Tiehm, 2001); thus low frequencies generate larger cavitation bubbles and higher hydrodynamic shear forces with respect to higher frequency. Better sludge disintegration has been reported at a low frequency range of 20 to 40 kHz (Tiehm, 2001), and almost all previous researches dealing with sludge pretreatment have been performed at 20 kHz.

In this chapter, the results of ultrasound sludge treatment at the "conventional" frequency (20 kHz) and at the "new" 200 kHz were compared and discussed.

For both treatments, a noticeable soluble COD increase has been observed in the sludge sample after the sonication in all the experiments. The efficacy of sludge pretreatment was assessed as disintegration degree, i.e. the ratio between the increment of soluble COD after sonication and the total soluble COD obtained by chemical disintegration.

For ultrasound treatment at 20 kHz, disintegration degree (DD_{COD}) steadily increased to 22 % with increasing specific energy input up to energy around 20’000 kJ/kgTS; beyond this energy level, DD_{COD} slightly increased reaching a plateau value. DD_{COD} was dependent also strictly on the total solids content.

Contrary to what stated in literature (Tiehm, 2001), also the ultrasound treatment with 200 kHz gave good disintegarion results. Fig. 26 shows the DD_{COD} trend for both types of treatment as function of the specific energy. For specific energy > 20000 kJ/kgTS, ultrasound pretreatment at 200 kHz permit to reach higher DD_{COD} with respect to the low frequency sonication. The maximum DD_{COD} obtained in the sonication experiments at 200 kHz was 65%, indicating also cell lyses besides the release of extracellular organic matter.

The disrupting effect of sound waves at 200 kHz was also confirmed by the increase of the proteic COD and surface charge density in sludge after the ultrasonic treatment.

By pretreating the sludge with 20 kHz ultrasound the fraction of the proteic COD increased from 29% of the soluble COD to 64% applying 11’516 kJ/kg TS, whereas if the sludge is pretreated with 200 kHz ultrasounds, the proteic COD increased “only” from 18 to 34% of the soluble COD despite of a major energy input of about 24000 kJ/kg TS.

In addition, microscope analyses on 200 kHz pretreated sludge sample showed that, after 40 min of sonication, the floc integrity is almost completely broken and only dead microorganisms were observed in the surrounding liquid phase.

In conclusion, these results provided that satisfactory sludge disintegration, in terms of flocs destruction and solubilisation of organic matter, can be obtained with ultrasound treatment at 200 kHz.

It must be pointed out that 200 kHz was chosen as working frequency for sludge treatment because was a suitable frequency for the sonolysis of the organic micropollutants, thus leading to sludge decontamination.

Further research is needed to evaluate the economic convenience of this treatment, considering both benefits, decontamination and increased stabilization, which can be achieved with 200 kHz sludge pretreatment.

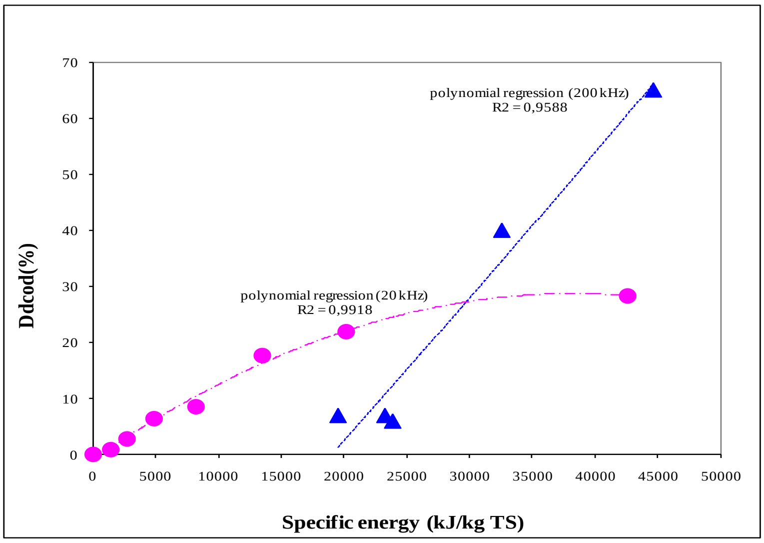

Fig. 26. Comparison of $DD_{COD\ 20kHz}$ and $DD_{COD\ 200kHz}$ pattern as function of the specific energy

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4.2 Effects of ultrasound pretreatment on anaerobic digestion performances

4.2.1 Introduction

Anaerobic digestion process is widely used for sewage sludge stabilization, resulting in the reduction of sludge and biogas production. However, the anaerobic digestion is a slow process; in particular, hydrolysis, the initial digestion phase, is the rate-limiting step of the process. This results in a long residence time in the digestion tanks and the requirement of a large tank volume. Therefore, sludge pretreatment methods have been developed to accelerate the initial hydrolysis phase; they include mechanical (Chio, 1997; Tiehm, 2001), chemical (Sakai, 1997; Saby, 2002) and thermal disintegration (Camacho, 2002; Dewil, 2007). Among them, sludge pretreatment with ultrasound is the most innovative process. It has been shown that ultrasound at low frequency facilitates sludge disintegration, due to the high mechanical shear forces produced by the implosion of the cavitation bubbles (Mason, 2001 and 2002). In biological wastewater and sludge treatment, US can be applied at various stages of the process: in the wastewater treatment line in order to reduce sludge production and COD concentration or in the sludge treatment line to increase sludge dewaterability, removal of volatile solids and biogas production.

In this chapter the results of anaerobic digestion tests with untreated and pretreated sludge are presented and compared. The sludge pretreatment was performed at 20 kHz, which is the worldwide applied frequency, and at 200 kHz, a frequency generally used for sonochemical reactions of organic pollutants. The effects of these two types of sludge pretreatment on the anaerobic digestion process were determined to obtain a comparison between “the classic” and the “new” sludge pretreatment.

4.2.2 Materials and methods

4.2.2.1 Sludge characterization and ultrasound pretreatment

Sludge

The anaerobic digestion experiments described in this section were performed on two different types of sludge: secondary sludge, untreated and sonicated at 20 kHz. Excess sludge was obtained from the municipal “Roma Nord” wastewater treatment plant and it was gravity thickened for 24 h at 4°C before feeding the bench scale anaerobic reactors.

The anaerobic inoculums was sampled from the full scale digester of the plant fed with primary and secondary sludge.

Ultrasound pretreatments at 20 and 200 kHz and the determination of soluble COD, total and volatile solids, EPS concentration, surface charge density and capillary suction time were performed as in chapter 4.1.2.1. The specific energy input for the ultrasound pretreatment at 200 kHz was in the range 23200-23900 kJ/kgTS.

LAS extraction and analysis

The experimental procedure of LAS extraction from sludge is based on the optimised extraction method of the Horizontal Project (Horizontal, 13.0, April 2006).

Untreated and sonicated mixtures were dried in oven at 80°C for 48 h,; 2-2,5 g of dried sludge is pulverized and mixed with 10 ml of methanol and 100 µl of C8-LAS (internal standard); the mixture is shaken at 320 strokes for 35 minutes and the methanolic extract is passed on a filter paper and injected to HPLC-MS. The separation of the four different LAS homologues was performed with a Dionex HPLC equipped with a low-pressure gradient pump with integrated vacuum degasser, autosampler and an Acclaim Surfactant (Dionex) column 5 µm 120 Å (2.1 x 150 mm).

The isocratic mobile phase was acetonitrile/100 mM ammonium acetate (65:35 v/v) flowing at 0.2 ml min^{-1}.

LAS determination was obtained with a single quadrupole mass detector PE Sciex API 165 with a Turbo IonSpray source, operating in full scan acquisition for surfactants identification and in SIM mode for LAS quantification.

4.2.2.1 Anaerobic digestion in batch

Experiments were carried out on bench scale anaerobic reactors of 0.4 L that were operated in batch mode, immersed in a temperature controlled, agitated water bath at 37˚C. The reactor were fed with a mixture of inoculum and raw sludge, either untreated or disintegrated. F/I was calculated as follows:

$$\frac{F}{I} = \frac{V_{sludge} \times VS_{sludge}}{\left(V_{tot} - V_{sludge}\right) \times VS_{inoculum}}$$

The produced biogas was collected in calibrated 1 L eudiometer tube placed on the digestion bottle via a ground-glass connection. The tube has a glass hose-coupling from which a sufficiently long hose connection leads a leveling flask. The upper end of the eudiometer tube is fitted with a conical stopcock for adjusting the zero point. The liquid contained in the tube and in the levelling flask was NaCl at pH 3 to avoid CO_2 losses by carbonate formation. The biogas was read daily. At regular time intervals, one digestion reactor containing untreated and one containing sonicated sludge were stopped and sludge analysis were performed.

4.2.3 Ultrasound pretreatment at 20 kHz

4.2.3.1 Application of low-frequency ultrasound pretreatment on sludge: state of the art

The challenge in the coming years will be to sustain agricultural use of good quality sludge, to reduce as much as possible disposal of polluted sludge especially in landfill. This strategy requires a separate treatment between primary and secondary (i.e. waste activated) sludge, using the available units mainly for secondary sludge treatment. Upgrading of activated sludge treatment might be required in order to increase digestibility minimizing the digestion residence time and maximising solid volatile destruction and its conversion to biogas. Nevertheless waste activated sludge is very difficult to digest due to the rate-limiting cell lysis (Lafitte-Trouque and Forster, 2002; Li and Noike, 1992). In the last years a renewed interest for the anaerobic digestion of sludge raised from the possibility of a significant gain in solids degradation and energy recovery by applying an appropriate sludge pre-treatment like mechanical disintegration by ultrasound,that leads to the breakage of flocs, cell walls, and bacteria membranes enhancing the hydrolysis of sludge volatile solids.

For research, ultrasounds have widely been applied as pretreatment of anaerobic digestion; main results are summarised in Table 21. . Considering energy consumption and enhancement of anaerobic digestion performance, applied specific energies are usually in the range from 1000 to 16,000 kJ kg−1 TS (the same as solubilisation threshold) although biogas production increases with the energy input (Salsabil, 2009). Taken as a whole, biogas enhancement ranges from 24% to 140% in batch systems and from 10% to 45% in continuous or semi-continuous systems.

Table 21

Mixed sludge	31 kHz 3.6 kW, 64 s	Continuous, HRT: 22 days 37 ◦C	Increase of VS removal from 45.8% to 50.3% (+9%)	(Tiehm, 1997)
Mixed sludge (25 g TS L−1)	9 kHz 200 W, 30 min	Batch, 11 days 36 ◦C	Increase of CH4 production from 210 to 345mLg−1 VSin (+64%)	(Wang, 1999)
Activated sludge (SRT: 16 days)	41 kHz 150 min	Semi-continuous, HRT: 8 days 37 ◦C	Increase of VS removal from 21.5% to 33.7% (+36%)	(Tiehm, 2001)
Activated sludge (9.38 g TS L−1)	20 kHz 0.33WmL−1, 20min	Batch, 100 days 35 ◦C	Increase of CH4 production from 143 to 292 g kg−1 TSin (+104%)	(Chu, 2002)
Sewage sludge (54 g TS L−1)	20 kHz 200 W, 30 min	Batch, 33 days 37 ◦C	Increase of biogas production (+138%)	(Onyeche, 2002)
Mixed sludge	20 kHz 180 W, 60 s	Batch, 28 days 35 ◦C	Increase of biogas production (+24%)	(Bien, 2004)
Activated sludge (27 g TS L−1)	20 kHz, 7000 and 15,000 kJ kg−1TS	Batch, 16 days 35–37 ◦C	Increase of biogas production (+40%)	(Bougrier, 2005)
Activated sludge	5000 kJ kg−1 TS	Semi-continuous HRT: 20 days	Increase of biogas production (+36%)	(Braguglia, 2008)
Activated sludge (17.1 g TS L−1)	20 kHz 108,000 kJ kg−1 TS	Batch, 50 days 37 ◦C	Increase of biogas production (+84%)	(Salsabil, 2009)
Activated sludge (2.14% TS)	20 kHz 9690 kJ kg−1 TS	Batch, 35 days 36 ◦C	Increase of biogas production (+44%)	(Erden, 2009)
Activated sludge	30kWhm−3 sludge	Batch	Increase of biogas production (+42%)	(Perez-Elvira, 2009)
Activated sludge	30kWhm−3 sludge	Continuous, HRT: 20 days	Increase of biogas production (+37%) Increase of VS removal (+25%)	
Activated sludge	Sonication of 25% of WAS	WWTP 330,000 PE	Increase of biogas production (+30%)	(Neis, 2008)
Mixed sludge	20 kHzWcm−2, 1.5 s	5000m3 egg-shape digester HRT: 22.5 days, 29–33 ◦C	Increase of biogas production (+45%)	(Xie, 2007)

Food to microorganism (F/M) ratio is the ratio of substrate amount as waste activated sludge to the inoculum amount as anaerobic digested sludge added to the reactor. It is an important digester parameter in terms of volatile solids loading performance of the system (Pranshanth et al., 2006).

According to Tanaka et al. (Tanaka, 1997), F/M ratio should be in the range of 0.45-0.50 in order to solubilize cells efficiently. Pranshanth et al. (Pranshanth, 2006) found the best value of F/M ratio between 0.57-0.68 for anaerobic digestion. In the study of Tomei et al (Tomei, 2008) kinetic data analysis showed that the sonication pre-treatment improved the hydrolysis rate for all investigated F/I, being the first order kinetic constant in the range of 0.08-0.20 d-1 for untreated sludge and 0.13-0.25 d-1 for the pre-treated sludge with ultrasounds.

Few studies reported the effect of ultrasound on the dewaterability of sludge following digestion (Hogan et al. 2004, Braguglia et al., 2009; Feng et al., 2009), hence more research is needed to assess dewaterability of pretreated sludge after anaerobic digestion (Khanal et al. 2007).

4.2.3.2 Results

The results of anaerobic digestion tests performed on the secondary sludge from Roma Nord treatment plant, either untreated or sonicated, are reported and discussed in this section. The main objective of these experiments is to determine the influence of the ultrasound pretreatment on the anaerobic digestion process, in terms of biogas production, solubilisation of organic substances and dewaterability. A second aim is to assess the influence of the feed/inoculum ration on the performances of the anaerobic digestion process.

Anaerobic digestion experiment at F/I = 0.5

Starting from the values of volatile solids of secondary sludge and inoculum, two different mixtures were prepared with F/I = 0.5: the first one containing 25% of thickened secondary sludge and 75% of inoculum, the second one with ultrasound pretreated secondary sludge (E_{spec}= 4332 kJ/kg TS) and inoculum in the same proportions. The anaerobic digestion experiments with both mixtures were stopped at 5 and 12 days. Total and volatile solids content and COD values (soluble, particulate and total) for the components of both mixtures are reported in Table 22.

Table 22 Characterization of the mixtures with untreated and sonicated sludge, and of the anaerobic inoculums (F/I=0.5)

	Untreated sludge	**Sonicated sludge**	**Inoculum**
TS (g/L)	28,26	28,23	23,20
VS (g/L)	18,84	19,98	13,53
COD_{sol} (mg/L)	67	3580	117
COD_{part} (mg/L)*	27250	23943	19501
COD_{tot} (mg/L)**	27317	27523	19618

*= COD_{part} is calculated as difference between COD_{tot} and COD_{sol}
**= COD_{tot} is calculated using the formula (COD_{tot}/VS=1,5)

In Table 23 the digestion trends, in terms of COD and of total and volatile solids content, are summarized for the untreated and sonicated mixtures.

Table 23 Anaerobic digestion trend of the untreated and sonicated mixture (F/I=0.5)

	UNTREATED			**SONICATED**		
	0	**120 h**	**288 h**	**0**	**120 h**	**288 h**
TS (g/L)	24,26	22,54	20,78	23,90	21,77	20,74
VS (g/L)	14,82	12,77	11,68	14,68	12,46	11,29
COD_{sol} (mg/L)	104	242	204	981	467	254
COD_{part} (mg/L)	21383	18267	16732	20310	17606	16119
COD_{tot} (mg/L)	21487	18509	16936	21291	18073	16373

During the anaerobic digestion process, a gradual decrease of total and volatile solids content in both mixtures can be observed. For the untreated mixture, the soluble COD increases in the first phase of the process (120 h) and then slightly drops down in the second phase. The particulate COD shows a diminishing trend during the entire process, because of progressive solubilization and degradation. On the other hands, in the digestion of sonicated mixture, both soluble and particulate COD constantly decrease during the process. Ultrasound effects on sludge are highlighted by the very high initial COD_{sol} value in the sonicated mix due to solubilization. In Table 24 are reported the volatile solids degradation efficiency and the biogas productions of both mixtures during the digestion process. The biogas produced from the digestion of the sonicated sludge is resulted higher (+16%) than the one obtained from the digestion of the same sludge, untreated.

Table 24 volatile solids degradation efficiency and biogas productions of untreated and sonicated sludge during the digestion process. (F/I=0.5)

	UNTREATED MIXTURE			**SONICATED MIXTURE**		
	SV degradation efficiency (%)	**Cumulative biogas production (cm^3)**	**Specific biogas production (Nm^3/kgVS)**	**SV degradation efficiency (%)**	**Cumulative biogas production (cm^3)**	**Specific biogas production (Nm^3/kgVS)**
120 h	13,9	259	0,315	15,1	292	0,329
288 h	21,2	290	0,231	23,1	336	0,248

Anaerobic digestion experiment at F/I = 1

Similarly as in the previous experiment, two mixtures were prepared mixing secondary sludge, untreated and sonicated, and inoculum in such proportions to obtain F/I = 1, i.e. 45% of sludge and 55% of inoculum. The digestion experiments of both mixtures were stopped at 55 h, 5 and 12 days.

The characterization of the secondary sludge and inoculum used in this experiment is reported in Table 25. The secondary sludge was sonicated at 24 kHz for 4 min (E_{spec} = 4078 kJ/kg TS; DD_{cod} = 13,9%), to obtain an energy input comparable with the one applied in the test at F/I=0.5.

Table 25 Characterization of seconadry sludge and inoculums (F/I=1)

	Untreated	**inoculum**
TS (g/L)	26	23,91
VS (g/L)	17,71	14,32
COD_{sol} (mg/L)	117	n.d.
COD_{part} (mg/L)	20653	n.d.
COD_{tot} (mg/L)	20770	n.d.

For both mixtures, the total and volatile solids and the COD trends during the anaerobic digestion process are reported in Table 26.

Table 26 Anaerobic digestion trend of the untreated and sonicated mixture (F/I=1)

	UNTREATED				**SONICATED**			
	0	**55 h**	**120 h**	**288 h**	**0**	**55 h**	**120 h**	**288 h**
TS (g/L)	24,27	22,67	21,38	20,97	24,40	23,01	21,81	20,60
VS (g/L)	15,40	13,83	12,71	12,15	15,46	13,94	12,83	11,69
COD_{sol} (mg/L)	177	301	346	408	1605	1315	788	686
COD_{part} (mg/L)	22157	19748	18089	17212	20818	18891	17820	16258
COD_{tot} (mg/L)	22333	20049	18435	17620	22423	20206	18608	16944

For the untreated mixture, the volatile solids and the COD, soluble and particulate, showed the same trend as in the digestion test carried out at higher inoculum content (F/I = 0.5). For the sonicated mixture, the high initial soluble COD underwent a considerable decrease in the first 120 hours of digestion and then remained almost constant till the end.

Table 27 summarizes the volatile solids degradation efficiency and the biogas production of untreated and sonicated mixtures during digestion process.

Table 27 volatile solids degradation efficiency and biogas productions of untreated and sonicated sludge during the digestion process. (F/I=1)

	UNTREATED			**SONICATED**		
	SV degradation efficiency (%)	**Cumulative biogas production (cm^3)**	**Specific biogas production (Nm^3/kgVS)**	**SV degradation efficiency (%)**	**Cumulative biogas production (cm^3)**	**Specific biogas production (Nm^3/kgVS)**
55 h	10,2	242	0,384	9,9	253	0,414
120 h	17,5	327	0,304	17	382	0,363
288 h	21,2	410	0,315	24,4	480	0,318

The biogas productions of both mixtures at the end of this experiment show increments with respect to that one produced in the digestion at F/I = 0,5: 30 and 15 % for the untreated and sonicated mix, respectively.

Anaerobic digestion experiment at F/I = 2

For the digestion experiment at F/I = 2, thickened secondary sludge and inoculum from Roma Nord treatment plant were used, with the properties reported below in Table 28.

The ultrasound pretreatment of secondary sludge was performed with a specific energy of 4888 kJ/kg TS, obtaining a disintegration degree of 10.6%.

Table 28 Characterization of secondary sludge an of inoculums (F/I=2)

	Secondary sludge	**inoculum**
TS (g/L)	27,52	24,38
VS (g/L)	19,57	14,52
COD_{sol} (mg/L)	85	n.d.
COD_{part} (mg/L)	28289	n.d.
COD_{tot} (mg/L)	28372	n.d.

The untreated and sonicated mixtures at F/I = 2 contained 60% of secondary sludge, untreated or sonicated, and 40% of inoculum. The batch anaerobic reactors were stopped at 55h, 5, 12 and 25 day. Table 29 reports the total and volatile solids content and the COD trends of untreated and sonicated mixtures during the anaerobic digestion.

Table 29 Anaerobic digestion trend of the untreated and sonicated mixture (F/I=2)

	UNTREATED MIXTURE					**SONICATED MIXTURE**				
	0	**55 h**	**120 h**	**288 h**	**600 h**	**0**	**55 h**	**120 h**	**288 h**	**600 h**
TS (g/L)	26,49	22,99	23,04	22,88	20,10	26,09	23,73	21,76	21,22	19,70
VS (g/L)	17,64	14,55	14,45	13,62	11,80	17,49	15,26	13,35	12,53	11,30
COD_{sol} (mg/L)	117	502	510	322	304	1890	2220	1480	800	504
COD_{part} (mg/L)	25464	20601	20442	19428	16806	23469	19912	17883	17371	15881
COD_{tot} (mg/L)	25581	21103	20952	19750	17110	25359	22132	19363	18171	16385

In the digestion of the untreated mixture, a significant degradation of the VS in the first 55h was observed, followed by an almost constant trend until 120 hours and finally a regular decrease up to the end. The soluble COD of the untreated mix considerably increased within 120 hours and then started to decrease. The same volatile solids trend was observed in the digestion of the sonicated mixture, while soluble COD showed a maximum within the first 55 hours and after decreased progressively. The volatile solids degradation and the biogas production of both mixtures are reported in Table 30.

As expected, the digestion of the untreated mixture at F/I = 2 produced more biogas (+ 40 and +15%) with respect to the digestion at F/I = 0.5 and 1. Also as regards the digestion of the sonicated mixture the production of biogas was higher (+35 and +13%) with respect to the tests at lower F/I.

Table 30 volatile solids degradation efficiency and biogas productions of untreated and sonicated sludge during the digestion process. (F/I=2)

	UNTREATED MIXTURE			**SONICATED MIXTURE**		
	SV degradation efficiency (%)	**Cumulative biogas production (cm^3)**	**Specific biogas production ($Nm^3/kgVS$)**	**SV degradation efficiency (%)**	**Cumulative biogas production (cm^3)**	**Specific biogas production ($Nm^3/kgVS$)**
55 h	17,5	277	0,224	12,7	220	0,247
120 h	18,1	383	0,300	23,6	385	0,233
288 h	22,8	482	0,299	28,3	548	0,277
600 h	33,1	550	0,235	35,4	600	0,242

4.2.3.3 Discussion

In the sewage sludge anaerobic digestion, the biological hydrolysis, which is the initial phase of the process, is identified as the rate-limiting step (Pavlostathis, 1986). The addition of an anaerobic inoculum, which contains all bacteria species involved in the anaerobic digestion process, permits to accelerate the initial phase of the digestion.

Objective of this section was to evaluate the effect of the initial ratio between raw activated sludge and anaerobic inoculum (F/I) on the hydrolysis process kinetics and to highlight the differences between the anaerobic digestion performances of the untreated and ultrasound pretreated sludge. For this purpose, VS biodegradation kinetics, biogas production and soluble COD pattern have been investigated in parallel batch tests, carried out for 12 days.

VS degradation kinetics

Table 31 shows VS degradation efficiency for both untreated and sonicated sludge: the beneficial effect of ultrasound pretreatment on the efficiency of VS degradation can be observed. Moreover, by increasing the F/I, i.e. in presence of more substrate availability, the removal efficiency increases, both for untreated and for sonicated sludge.

Table 31 VS degradation efficiencies of the untreated and sonicated sludge in the three digestion tests

F/I	**VS DEGRADATION EFFICIENCY (%)**	
	Untreated sludge	**Sonicated sludge**
0.5	21	23
1	21	24
2	23	28

The kinetic of the digestion process is strictly dependent on the hydrolysis rate of the particulate substrate, because hydrolysis is the rate-limiting step of the whole digestion process (Eastman, 1981). VS data were used for kinetic calculations.

The kinetics of the hydrolysis was assumed to follow a first-order kinetic equation:

$$\frac{dXs}{dt} = -k_x Xs$$

where k_x is the hydrolysis constant (d^{-1}), t is the time and X_S is the anaerobically degradable fraction of the activated sludge. The total volatile solids of activated sludge (X_T) are constituted of degradable fraction (X_S) and of a non-degradable fraction (X_I) which is inert material originated by endogenous biomass decay process. The active biomass involved in the hydrolysis process is assumed in first approximation equal to the VS content of the inoculum. The concentration of "digestible" substrate can be expressed as $X_S = f_x X_T$, while X_I is $(1-f_x)X_T$, where f_x is the anaerobically degradable fraction. The ratio $f_x = X_S/X_T$ is reported in literature in the range 0,3-0,4 (Engelhart, 2002) depending on the operating conditions of the treatment plant: in this sperimentation, f_x was assumed to have a low value (0,3) because the full scale plant is operated at high sludge retention time (SRT= 20 d) so a quite high non biodegradable fraction is expected in the produced sludge. Integrating the previous equation, it results:

$$\frac{X_{Sf}}{X_{S0}} = e^{-k_x t_f}$$

where the subscript 0 and f represent the initial and final time, respectively. Expressing X_{S0} as $f_x X_{T0}$, it can be written:

$$X_{Sf} = f_x X_{T0} e^{-k_x t_f}$$

As the non biodegradable fraction of the biomass was defined as $X_I = (1-f_x)X_{T0}$ and considering that the total volatile solids X_T is the sum of the inert and degradable fractions, the VS mass balance referring to the whole digestion time at the final VS concentration X_{Tf} is:

$$X_{Tf} = X_{T0}\left[1 - f_x\left(1 - e^{-k_x t_f}\right)\right]$$

The VS degradation efficiency is defined as $\eta = 1 - \frac{X_{Tf}}{X_{T0}}$, hence:

$$\eta = f_x\left(1 - e^{-k_x t_f}\right) \text{ or in the linearized form}$$

$$\ln\left(1 - \frac{\eta_x}{f_x}\right) = -k_x \times t_f \, .$$

Plotting $\ln\left(1-\frac{\eta_x}{f_x}\right)$ versus digestion time, the slope of the straight line represents the hydrolysis constant k_x. Fig 27 shows the comparison between experimental data relating to the digestion of the untreated mixture and correlation functions: R^2 are always > 0,96, confirming the hypothesis of a first-order kinetic. The hydrolysis constant are derived from the slope of the straight lines in Fig. 27:0,094, 0,076 and 0,061 d^{-1} for the digestion experiments with F/I= 0,5, 1 and 2, respectively. The hydrolysis constants of the digestions, in particular at high F/I (1 and 2), are not statistically different.

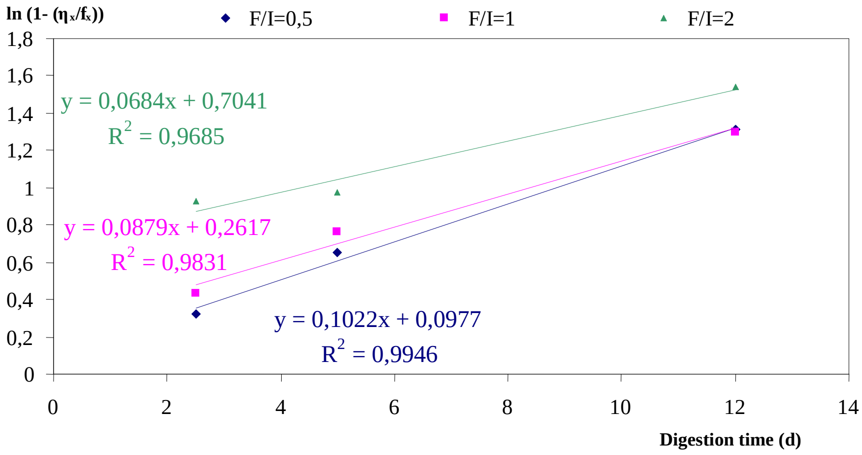

Fig. 27

Also the digestion experiments of the sonicated mixture (Fig. 28) appear to follow the first-order kinetic. The hydrolysis constants derived from Fig. 28 for the three digestion experiments are statistically different: the highest F/I experiment is characterized by the highest hydrolysis constant (0,23 d^{-1}), while lower kinetic constants (0,17 and 0,13 d^{-1}) were found for digestions at F/I = 0,5 and 1, respectively.

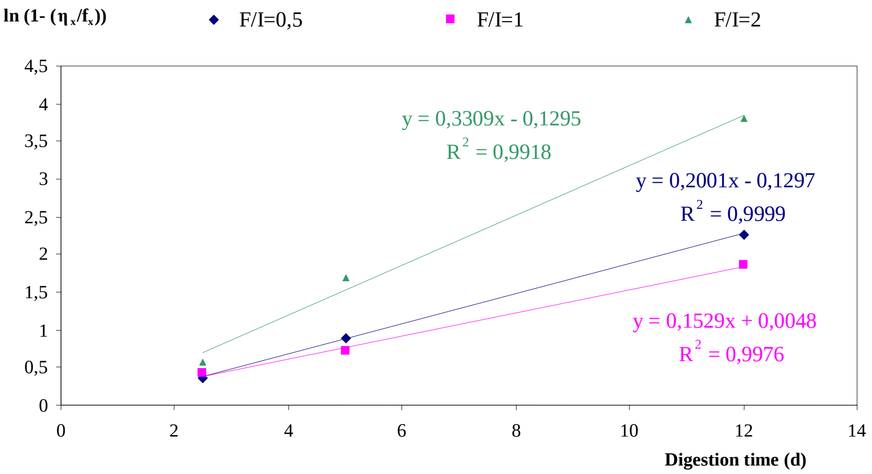

Fig. 28

Fig. 29 highlights the beneficial effect of the sonication pretreatment on the hydrolysis kinetics with increases of k_x values for all investigated F/I. The hydrolysis rate values show a decreasing trend with increasing F/I for the untreated sludge. This could be due to the fact that with increasing F/I ratio, there is an excess of substrate respect to the available hydrolytic biomass thus leading to an hydrolysis rate reduction. The same pattern is observed for the sonicated sludge, except for F/I = 2 that gives the highest k_x: this unexpected value may be due to a synergistic positive effect of high F/I and sonication (Tomei, 2008).

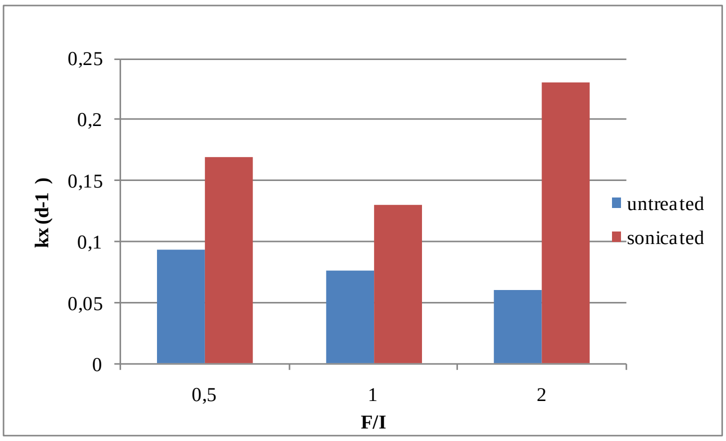

Fig. 29 Hydrolysis constants as function of the food/inoculums ratio, for the untreated and sonicated sludge

Soluble COD pattern

Table 32 shows the soluble COD variation during the digestion experiments for both untreated and sonicated mixture.

Table 32 Soluble COD variation during the digestion process, for the untreated and sonicated slugde

	UNTREATED		**SONICATED**	
F/I	*COD_s(t=0)*	*COD_s(t=12 d)*	*COD_s(t=0)*	*COD_s(t=12 d)*
0.5	104	204	981	254
1	177	408	1605	686
2	117	322	1890	800

It must be pointed out that the initial soluble COD in the sonicated mixture is considerably higher than that of the untreated sludge, demonstrating the effectiveness of the ultrasonic pretreatment.

For the untreated sludge, during the digestion at low F/I (0,5 and 1) soluble COD slightly increases in the first 55 hours of the process and it remains almost constant. At high F/I the COD increment in the first phase is higher, probably due to the lower amount of inoculum. In such a situation the activity of hydrolytic bacteria at the beginning of the digestion is likely to be prevalent respect to methanogen bacteria. The trend of the soluble COD was different for the sonicated sludge: the high initial COD is rapidly removed (Fig. 30, 31 and 32)). The initial accumulation of organic compounds in the case of the sonicated sample at F/I = 2 can be explained by different kinetics of hydrolysis and conversion, with prevalent effect of hydrolysis in the first phase of the process.

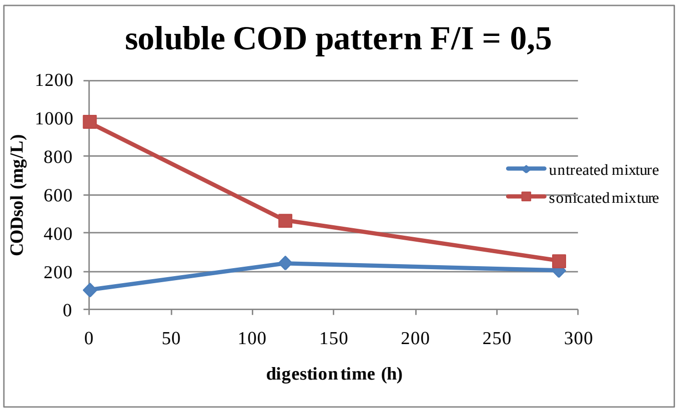

Fig. 30 Soluble COD pattern at F/I=0.5

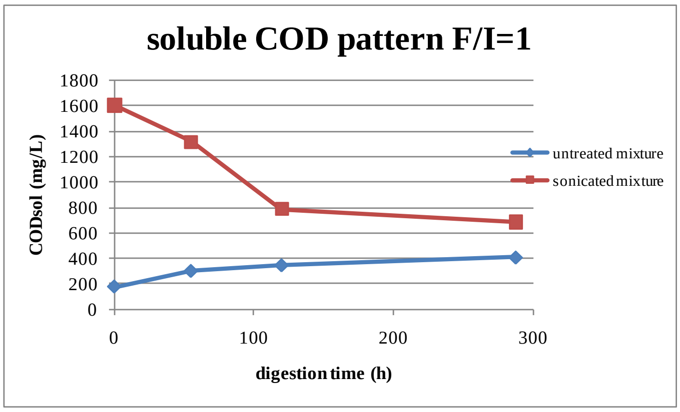

Fig. 31 Soluble COD pattern at F/I=1

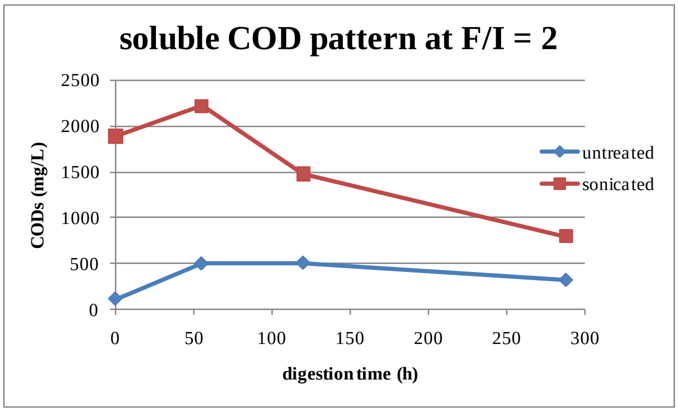

Fig. 32 Soluble COD pattern at F/I=2

Biogas production

As expected, biogas production of the untreated sludge was higher for the digestion at F/I = 2, because of the high amount of available volatile solids. Fig. 33 shows that the cumulative production decreases by decreasing F/I, but the produced biogas volume per unit of degraded substrate remains constant. At the end of the digestion (12 d), the specific biogas production was 449 Nm^3/kg VS degraded. This value results consistent with biogas productions reported in literature, which are in the range 430-650 Nm^3/kg VS degraded (Tiehm, 2001; Engelhart, 2002). The low value found in these experiments may be attributable to the high sludge age, which can be affected the stoichiometry of the anaerobic reactions in the reactors and thus the specific biogas production.

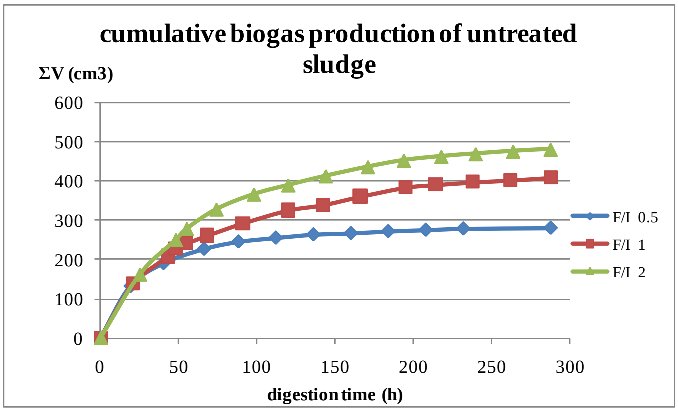

Fig. 33 Cumulative biogas production of the untreated sludge in the three digestion tests

Also in the digestions of the sonicated sludge the biogas production increases with increasing F/I ratio: the highest cumulative biogas production was obtained with the test at F/I = 2, because of the high amount of available substrate with respect to the inoculum (Fig. 34).

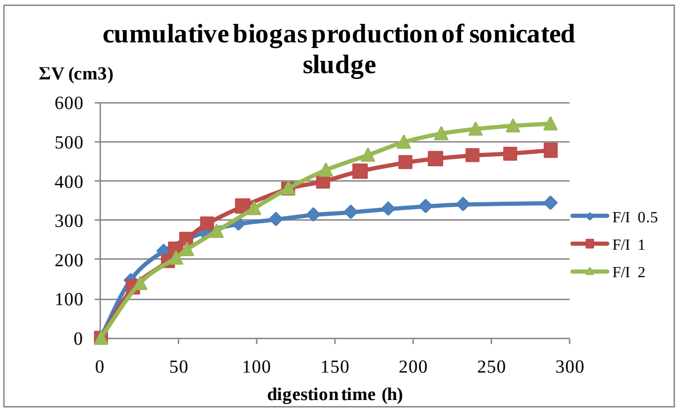

Fig. 34 Cumulative biogas production of the sonicated sludge in the three digestion tests

The biogas production of the sonicated sludge at F/I = 2 shows a slowdown in the first 55 h: this is due to the initial accumulation of soluble organic substances suggesting a kinetic decoupling between hydrolysis (i.e. solubilisation of organic substances) and methanogenesis (i.e. biogas production). Nevertheless, in the second phase the biogas production increase and overtakes the other ones. The produced biogas volume per unit of degraded substrate is 280 Nm^3/kg VS degraded, which is comparable with literature data. This low value, compared to the biogas produced by the untreated sludge, may be due to different biodegradability characteristics of COD fractions present in the sonicated sample, which can cause different fermentation pathways (Tiehm, 2001).

From the energy point of view, Fig. 35 shows that the ultrasound pretreatment has a positive effect in all investigated F/I.

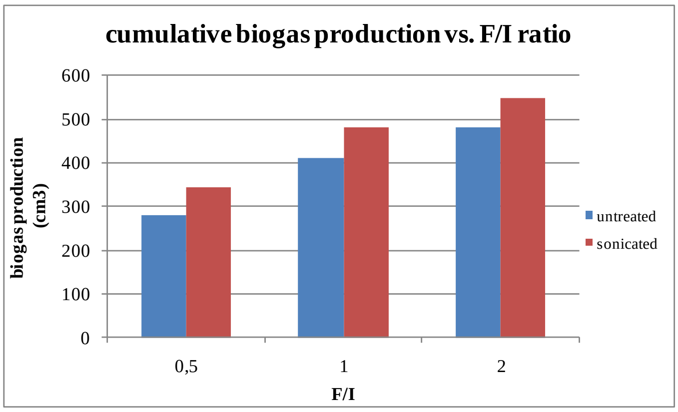

Fig. 35 Cumulative biogas production as function of the F/I ratio, for the untreated and sonicated sludge

Table 33 Biogas gain

	Untreated		Sonicated		Δbiogas**(%)
	Biogas (cm^3)	Δbiogas*(%)	Biogas (cm^3)	Δbiogas*(%)	
F/I 0,5	290	-	361	-	24.5
F/I 1	410	41.4	480	33	17
F/I 2	482	17.6	548	14.2	13.7

*= biogas increment at increasing F/I for untreated/sonicated sludge

**= biogas increment of sonicated sludge with respect to the untreated one at the same F/I

The biogas production increment of the sonicated sample respect to the untreated one decreases with increasing F/I ratio: the more convenient gain was obtained with the test at high inoculum (F/I = 0.5), as shown in Table 33.

Effect of anaerobic digestion on particle charge and dewaterability

The increase of the soluble COD during the first days of the anaerobic digestion of untreated sludge at low inoculum percentage indicated a release of organic substance (mainly EPS) due to the hydrolysis of the sludge floc. This increase confirmed a kinetic “decoupling phase” between hydrolysis and biogas production in the case of digestion at F/I=2 whereas in the case of the digestion of sludge in the presence of high percentage of inoculum this kinetic phenomenon was not so significant then the released COD is constantly transformed into biogas. During the second phase of the digestion i.e from the 5th to the 12th day, removal of the soluble organic substance was observed, particularly in the case of high F/I. In Fig. 36 the trend of the soluble proteic COD is compared with this of the soluble one for the digestion at F/I=0.5 and 2 of untreated sludge.

Protein concentration in the supernatant followed the same trend as the soluble COD in the reactor, confirming a significant release of proteins in the first days of digestion, with respect to carbohydrates (Wang et al., 1999; Novak et al., 2003).

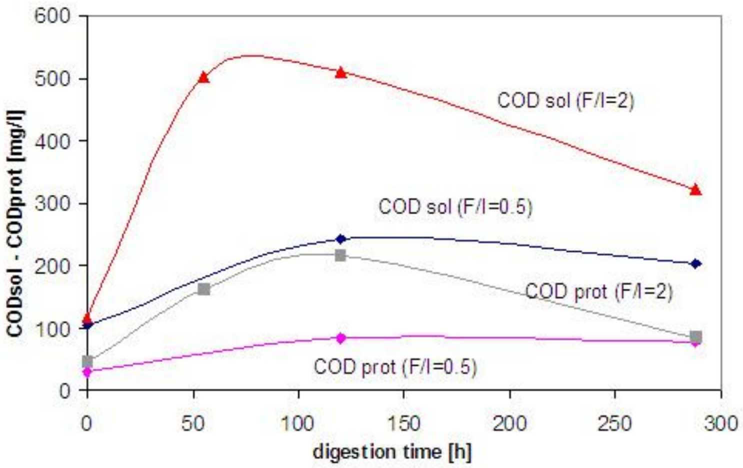

Fig. 36. Proteic and soluble COD pattern of the untreated sludge in the digestion tests at F/I=0.5 and 2

By means of sludge anaerobic digestion a release of soluble and colloidal particles (as for example proteins) was confirmed.

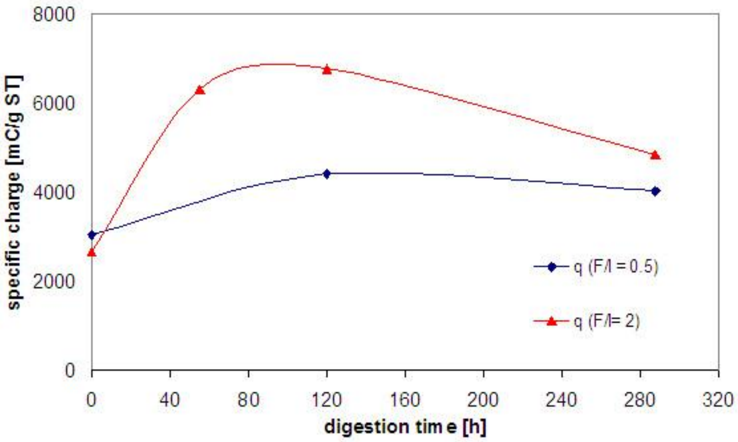

Fig. 37 Specific charge density of sonicated sludge particles in the digestion tests at F/I=0.5 and 2

Surface charge density of the sludge increased noticeably in the first days of digestion at high F/I, whereas the increase at low F/I was not so evident (Fig. 37). This result may be due to a stronger particle “disintegration” with relative increase of the specific particle surface and consequently of the specific surface charge particularly in the case of digestion at F/I=2. Particle charge density increased during the first days of digestion but during the successive phase a significant removal was observed. This confirms the initial significant release of charged biopolymers and their progressive degradation to be transformed into biogas.

Different trends have been monitored as regards the anaerobic digestion of sonicated sludge. In fact, as showed in Fig. 38, during the anaerobic digestion carried out with high content of inoculum (low F/I) already in the first days a noticeably removal of the available soluble COD was observed.

Also in this case the soluble protein concentrations followed the same trend, confirming the degradation principally of the biopolymers released in solution after the pretreatment. Also as regards the digestion in presence of low content of inoculum, a marked degradation of the soluble COD (and protein concentration) was evident (Fig. 38).

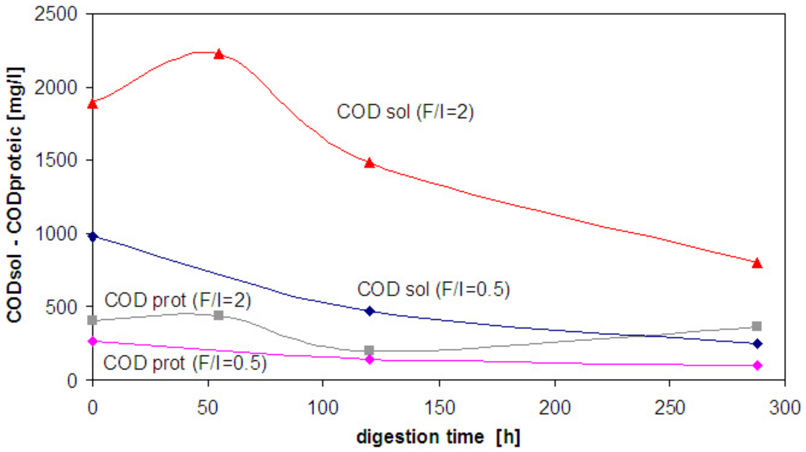

Fig. 38 Proteic and soluble COD pattern of the sonicated sludge in the digestion tests at F/I=0.5 and 2

Just in the first 55 hours there was an accumulation of the organic substance derived due to the hydrolysis of the particulate material that was not transformed immediately into biogas from the methanogens probably because of their scarcity or their inhibition due to the high substrate availability. Specific charge density of sludge particles (that in the case of sonicated sludge started from values higher than 6000 mC/gTS) decreased during the digestion process because of the abatement of the fine particles (Fig. 39).

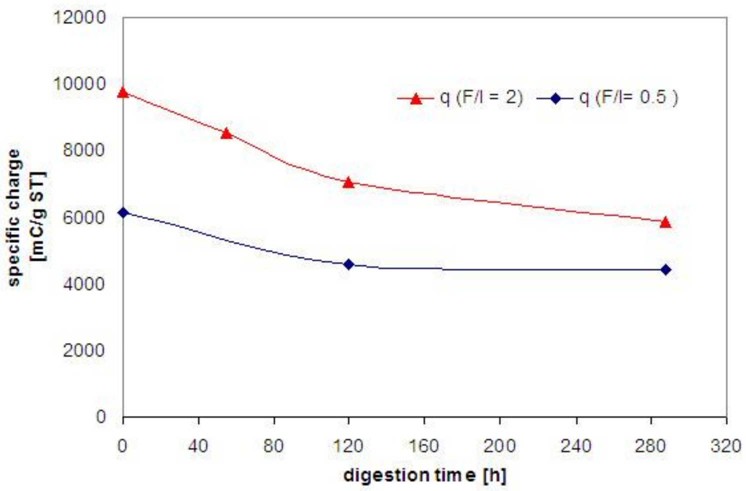

Fig. 39 Specific charge density of sonicated sludge particles in the digestion tests at F/I=0.5 and 2

The effect of the digestion process on the dewaterability was determined by means of CST (sec.L/g TS) determinations (Table 34 and 35).

Table 34 CST determination for the digestion at F/I=0.5

F/I=0.5	**UNTREATED**	**SONICATED**
start	4.3	38.5
After 5 day	4.8	12.4
After 12 days	4.4	12.1

Table 35 CST determination for the digestion at F/I=2

F/I=2	**UNTREATED**	**SONICATED**
start	4.7	115
After 55 h	9.3	68
After 5 day	14.7	44
After 12 days	10.4	46

It is important to note that the fines created by hydrolysis at the beginning of the digestion may cause deterioration of the sludge filterability and an increase of the CST. In fact, fine particles blind the sludge cake and filter medium during filtration, resulting in deterioration of sludge dewaterability.

In fact, the large release of dispersed charged fines and biocolloids observed in the case of the untreated sludge digestion had a negative impact on the filtration rates, especially for F/I=2.

On the contrary, the amelioration of the filterability (decreasing CST values) during digestion of sonicated sludge at both F/I may be due to the removal of fines and colloids already present in the pretreated feed evidencing the efficiency of the mesophlic anaerobic process in degrading rapidly (in the first 5 days) fine dispersed particles produced by mechanical pre-hydrolysis.

4.2.3.4 Conclusions

The comparison between batch anaerobic digestion tests carried out with untreated and sonicated sludge showed a beneficial effect of the pre-treatment by ultrasound, both on hydrolysis kinetics and biogas production for all the investigated F/I.

For untreated sludge, VS degradation data are well correlated by a first order kinetic equation: the hydrolysis rate values show an increasing trend with decreasing F/I. Soluble COD measured after different digestion times did not change significantly for tests with F/I = 0.5 and 1, whereas a small increase in the first 55 h was monitored in the test with F/I = 2.

In the tests with sonicated sludge, the first order kinetics of VS degradation was confirmed.

It is worth noting that ultrasound pre-treatment considerably accelerates the reaction rate (k_x values are in the range 0.13–0.23 d^{-1}) and this effect is more evident with F/I = 2. The trend of soluble COD during the digestion process was basically different from that observed for the untreated sludge. The soluble COD, initially very high (so demonstrating the effectiveness of the ultrasound treatment), was rapidly removed during the first days.

For all F/I tested, biogas production was higher for sonicated sludge, and the more convenient gain (25%) was obtained for the test at high inoculum (F/I = 0.5).

4.2.4 Ultrasound pretreatment at 200 kHz[3]

4.2.4.1 Introduction

Ultrasound at low frequencies represents one of the most effective technologies for pretreating sludge in order to improve the anaerobic digestion process. There is an extensive literature dealing with the positive effects of sludge ultrasound treatment at 20 kHz on the digestion performances (see chapter 4.1.3). On the contrary, only few studies were performed to assess the efficiency of the ultrasonic pretreatment at higher frequencies. Tiehm et al. (Tiehm, 2001) evaluated the effect of ultrasound frequency, and specific energy input on the degree of sludge disintegration. With increase in frequency the degree of disintegration decreased gradually (DD_{COD} = 80% at 41 kHz and DD_{COD} = 7% at 3217 kHz). Tiehm (Thiem, 2001) investigated the effect of different ultrasound frequencies from 41 to 3217 kHz. He pointed out that the low-frequency (i.e.41 kHz) pretreatment gave the most significant effects on sludge disintegration and anaerobic digestion improvement. This can be explained because, as the cavitation bubble radius is inversely proportional to the US frequency, low-frequency ultrasound created larger cavitation bubbles which can exert strong shear forces in the liquid. High frequencies promote oxidation by radicals, whereas low frequencies

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[3] Gianico A., Gallipoli A., Gagliano M.C., Braguglia C.M., “Effects of high-frequency ultrasound on the anaerobic digestion of sewage sludge” in preparation for *Journal of Environmental Management*

promote mechanical and physical phenomena like pressure waves (Gonze, 1999). Biosolids disruption in waste activated sludge on ultrasonic treatment was most effective at 40 kHz then 20 kHz in terms of solubilisation of COD (Sae Eun Oh , 2006). Compared with the control, the amount of methane produced from the digestion of 40 kHz pretreated sludge doubled after 24 days.

The digestion experiments discussed in this section were carried out using sludge pretreated with ultrasound at 200 kHz. This frequency was chosen on the basis of the satisfactory results obtained from the LAS sonolysis reported in chapter 4.3. In fact, besides the sonochemical degradation, the assessment of the performances of the 200 kHz ultrasound pretratment on the anaerobic digestion process may be of a crucial importance for the determination of a unique treatment enhancing both sludge stabilization and decontamination.

4.2.4.2 Results

In the anaerobic digestion tests reported in this section, ultrasound sludge pretreatment was performed at 200 kHz, frequency suitable for sonolysis reactions of organic contaminants (see chapter 4.3). The aim was to evaluate the effect of the ultrasound pretreatment at 200 kHz on the sludge anaerobic digestion performances comparing the efficiency with the “classical” 20 kHz performances.

Two digestion batch experiments were carried out at two different F/I ratios in order to determine also influence of the anaerobic biomass (inoculum) content during the process.

Anaerobic digestion experiment at F/I = 0,5

Starting from VS contents of secondary sludge and anaerobic inoculum, two mixtures were prepared with 60% of inoculum and 40% of secondary sludge, untreated and sonicated. The energy delivered during sludge sonication was 23894 kJ/kg TS, obtaining a disintegration degree of about 6%. At 2, 6 and 20 days one anaerobic reactor containing untreated mixture and one containing the sonicated one were stopped and sludge analysis were performed.

In Table 36 the main characteristics of untreated and sonicated sludge, and of the anaerobic inoculum are reported.

Table 36 Characteristics of untreated and sonicated sludge, and of the anaerobic inoculum

	Untreated sludge	**Sonicated sludge**	**Inoculum**
TS (g/L)	22.1	22.6	20.9
VS (g/L)	12.5	13	11.9
VS/TS (%)	56.6	57.6	56.9
COD_s (mg/L)	41	1125	373

The anaerobic digestion was monitored in terms of VS removal, solubilisation of organic substances (COD, protein, carbohydrates), biogas production and fate of anionic surfactants content. Table 37 and 38 show the TS and VS, soluble COD, specific surface charge and EPS content (proteins and carbohydrates) in the digestion of the untreated and sonicated sludge, respectively.

Table 37 Anaerobic digestion trend for the untreated sludge at F/I=0.5

Untreated mixture								
Digestion time (h)	**TS (g/L)**	**VS (g/L)**	**VS/TS (%)**	**COD_s (mg/L)**	**Surface charge (mC/gTS)**	**VS removal (%)**	**Proteins (mg/L)**	**Carbohydrates (mg/L)**
0	21.3	12.4	58	215	82	0	122	35
48	19.3	10.6	55	613	301	15	347	159
144	18.7	9.9	53	745	335	20	409	110
480	17.5	8.5	49	132	529	31	177	137

Table 38 Anaerobic digestion trend for the sonicated sludge at F/I=0.5

Sonicated mixture								
Digestion time (h)	**TS (g/L)**	**VS (g/L)**	**VS/TS (%)**	**COD_s (mg/L)**	**Surface charge (mC/gTS)**	**VS removal (%)**	**Proteins (mg/L)**	**Carbohydrates (mg/L)**
0	21.6	12.6	58	682	150	0	189	141
48	19.2	10.6	55	1103	299	16	355	244
144	18.8	9.8	52	1055	318	23	424	123
480	17.1	8.2	48	318	580	35	210	162

From these results it is evident that consistent hydrolysis of particulate substrate occurred in the first 2 days of the digestion, leading to the release of organic matter in solution (increase of soluble COD, of colloidal specific charge and of proteins and carbohydrates). This trend was observed both for untreated and sonicated mixture, but the increase was more marked for the untreated digestion. The high initial values of COD_s, surface charge and EPS of the sonicated mix are due to the “mechanical” hydrolysis induced on raw sludge by the ultrasound pretreatment. Soluble proteins and soluble COD showed the same pattern for both untreated and sonicated mixture, with an initial increase followed by a consistent removal during the second digestion phase. On the contrary, the fate of carbohydrates during sludge digestion seemed different, for untreated but also for sonicated sludge (Fig. 40, 41 and 42).

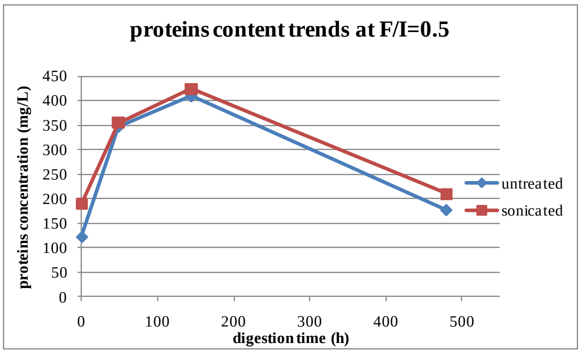


Fig. 40 Proteins content of the untreated and sonicated sludge

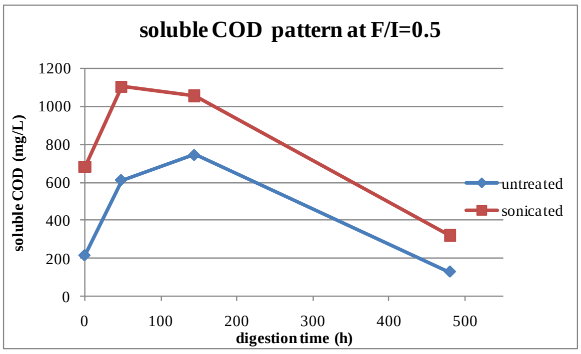


Fig. 41Soluble COD pattern of the untreated and sonicated one

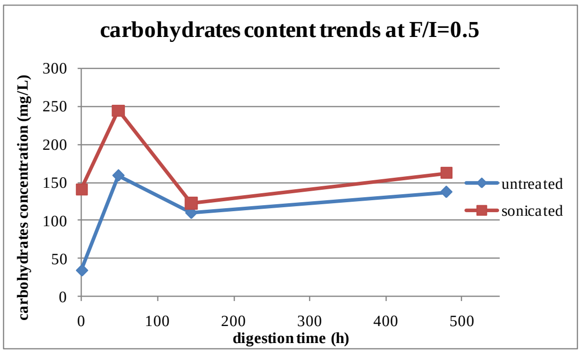


Fig. 42 Carbohydates content of the untreated and sonicated sludge

The cumulative biogas productions for untreated and sonicated mixtures are reported in Table 39.

Table 39 Cumulative biogas production of the untreated sonicated sludge

	Biogas cumulative production (cm^3)	
Digestion time (h)	**Untreated mixture**	**Sonicated mixture**
0	0	0
24	26,4	34,1
48	43,5	52,8
144	71,4	99,3
192	78,4	108,6
240	83,8	118,7
312	95,4	142,0
360	117,9	166,8
480	135,0	188,6

The sonicated mixture digestion is characterized by constantly higher biogas production with respect to the untreated one. At the end of the digestion process the biogas increment of the pretreated sludge resulted to be 40% (Fig. 43).

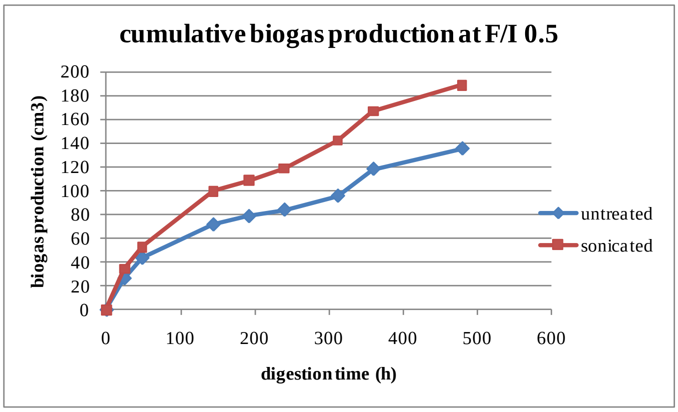


Fig. 43 Cumulative biogas production of the untretated and sonicated sludge

As regards the organic micropollutants fate during the anaerobic digestion, the trend of LAS content for both untreated and sonicated mixtures is reported Fig. 44. LAS initial concentration of the sonicated mixture was lower due to the sonolysis effect of the ultrasound pretreatment, but in the first 2 days the biological LAS degradation of the untreated sludge was so intense to achieve the same LAS concentration value as in the reactor fed with sonicated sludge.

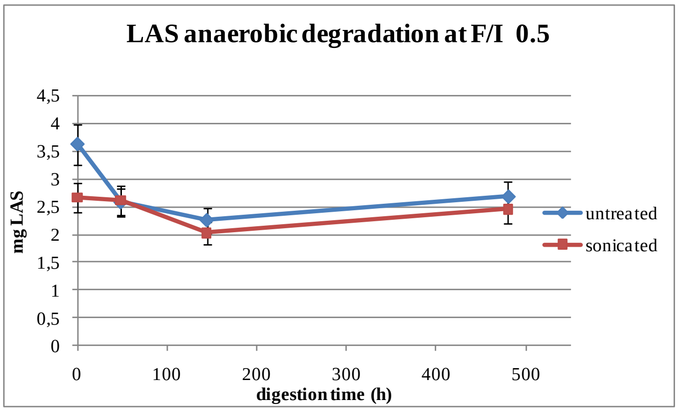


Fig. 44 LAS degradation pattern in the digestion test at F/I =0.5

Anaerobic digestion experiment at F/I = 1

For the digestion experiment at F/I = 1 two mixtures were prepared containing 50% of anaerobic inoculums and 50% of thickened secondary sludge, untreated and sonicated. The specific energy supplied during sludge ultrasound pretreatment was 23276 kJ/kgTS and the obtained disintegration degree was 7% (comparable to the one obtained in the test at F/I=0.5). The anaerobic digestion test was performed as described for the previous experiment. Table 40 summarizes the characteristics of the untreated and sonicated sludge and of the inoculum used in this digestion test.

Table 40 Characterization of the untreated and sonicated sludge, and of the anaerobic inoculum

	Untreated sludge	**Sonicated sludge**	**Inoculum**
TS (g/L)	24.1	23.2	31.7
VS (g/L)	14.4	13.9	17.4
VS/TS (%)	59.7	59.9	54.9
COD_s (mg/L)	15	1433	271

In order to assess a comparison of the anaerobic digestion performances as function of the two different F/I ratios, the same parameters of the previous experiment were monitored during the process.

Table 41 and 42 summarize the TS and VS, soluble COD, surface charge and EPS content trends during the digestion of the untreated and sonicated mixtures.

Table 41 Anaerobic digestion trend for the untreated sludge at F/I=1

Untreated mixture								
Digestion time (h)	**TS (g/L)**	**VS (g/L)**	**VS/TS (%)**	**COD_s (mg/L)**	**Surface charge (mC/gTS)**	**VS removal (%)**	**Proteins (mg/L)**	**Carbohydrates (mg/L)**
0	27.3	15.8	58	122	104	0	121.9	33.1
48	26.6	14.3	54	1439	310	9	501.5	167.4
144	26.2	13.6	52	1419	302	14	594.4	138.6
480	23.9	12.1	51	459	225	23	426.2	131.6

Table 42 Anaerobic digestion trend for the sonicated sludge at F/I=1

Sonicated mixture								
Digestion time (h)	**TS (g/L)**	**VS (g/L)**	**VS/TS (%)**	**COD_s (mg/L)**	**Surface charge (mC/gTS)**	**VS removal (%)**	**Proteins (mg/L)**	**Carbohydrates (mg/L)**
0	27.2	15.7	58	867	267	0	295.6	128.1
48	26.2	14.1	54	1459	338	10	485.5	183.4
144	25	13.1	52	1730	319	16	544.7	147.9
480	22.5	10.8	48	582	308	31	337.5	137.4

As in the previous test, an initial hydrolysis phase can be observed for both mixtures, followed by a significant removal of the organic substances. It is important to note that in the first phase the accumulation of soluble organic material in the reactor occurred regardless of sonication pretreatment. Anaerobic bacteria attack the untreated organic substrate so intensely to reach in the reactor a very high concentration of soluble COD, surprisingly comparable (1439 *vs* 1459 mg/L) with the one of the reactor fed with sonicated sludge despite of the high content of solubilised material due to the ultrasound disintegration.

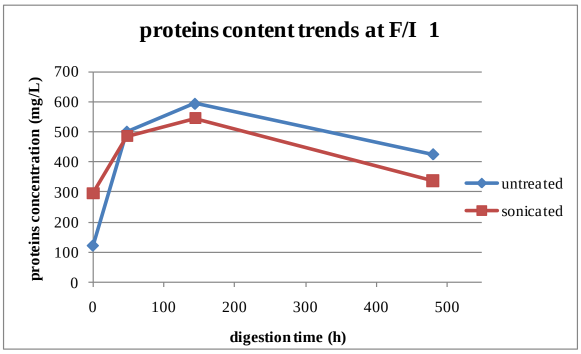


Fig. 45 Proteins content of the untreated and sonicated sludge

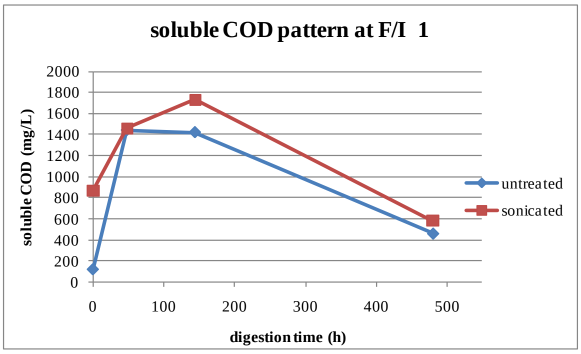


Fig. 46 soluble COD paternt of the untreated and sonicated sludge

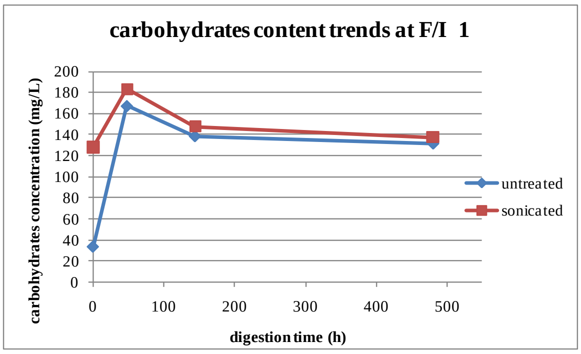


Fig. 47 Carbohydrates content of the untreated and sonicated sludge

As noted in the case of F/I = 0.5, soluble proteins and soluble COD showed the same pattern during the anaerobic digestion: an initial hydrolysis phase, followed by a noticeable removal in the second phase. On the contrary, an increase of carbohydrate concentration was observed in the first 144 h; afterwards, the carbohydrates concentration remains almost constant (Fig. 45, 46 and 47).

The cumulative biogas productions of both mixtures are reported in Table 43. The biogas produced by the untreated mixture is always higher than the other one during the entire digestion process. Only at the end of the experiment the biogas production of the pretreated mix overtakes the production of the untreated sludge. The biogas increment was 4% (Fig. 48)..

Table 43 Cumulative biogas production of the untreated and sonicated, at the digestion test at F/I=1

	Cumulative biogas production (cm^3)	
Digestion time (h)	**Untreated mix**	**Sonicated mix**
0	0	0
24	67	54
48	101	78
72	116	87
144	124	101
168	130	102
192	138	114
216	138	114
240	143	128
312	147	137
336	151	146
360	154	153
408	161	161
480	164	170

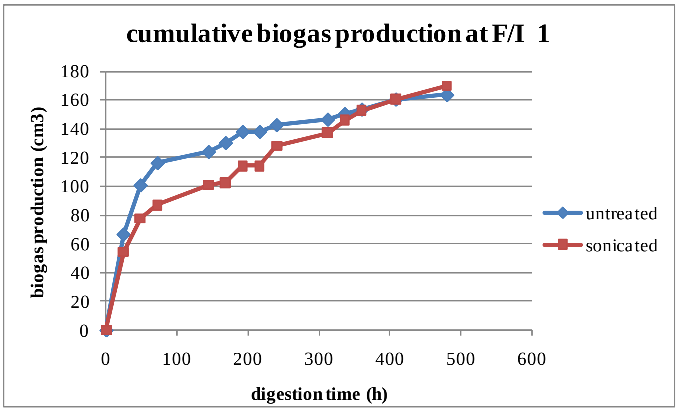


Fig. 48 Cumulative biogas production of the untreated and sonicated, at the digestion test at F/I=1

Both untreated and sonicated mixture showed LAS concentration removal at the end of the process (Fig. 49).

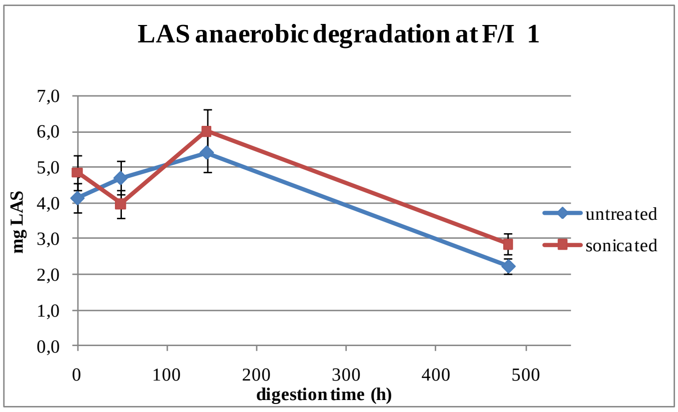


Fig. 49 LAS degradation pattern in the digestion test at F/I = 1

4.2.4.3 Discussion

In order to have a complete view of the anaerobic digestion performances of a pretreated sludge with ultrasound at 200 kHz, the digestion process was studied with monitoring both the conventional used parameters (solubilisation of the organic substance and biogas production) and the degradation of LAS, which is one of the most abundant classes of organic micropollutants in sludge.

Anaerobic LAS biodegradation

Many studies about the biodegradation of LAS have demonstrated that anionic surfactants are readily biodegradable under aerobic conditions (Swisher, 1987). There is wide evidence that the primary biodegradability of homologues of LAS (C10 to C14) in general increases as the chain length increases. On the contrary, the first researches on LAS removal in anaerobic conditions showed a negligible surfactants biodegradation (Steber, 1995, Federle, 1992, Hera, 2004, McEvoy, 1985, De Wolf and Feijtel, 1998). These earlier conclusions were supported by the findings that in anaerobically treated sludge relatively high concentrations of LAS have been found (5–10 g/kg dry solids) whereas aerobically treated sludge contain low LAS concentrations (0.1–0.8 g LAS/kg dry matter) (Berna et al., 1989; McAvoy et al., 1998; Waters and Feitjel,1995). More recently studies have been performed to improve LAS anaerobic biodegradation potential (Prats, 1999; Angelidaki, 2000; Mogenesen, 2003), since LAS are strongly adsorbed onto sludge, which is often subjected to anaerobic digestion. Their results pointed out that LAS biodegradation in anaerobic conditions can occur and also temperature seems to play an important role. Higher LAS removals are reported for the thermophilic treatment; while the maximum removal efficiency for mesophilic anaerobic digesters is in the range 20-25% (Brunner, 1988). In addition, LAS anaerobic digestion by a two-members facultative anaerobic bacterial consortium has been reported (Khleifat, 2006).

The results reported below (Table 44) indicate that acceptable LAS biodegradation occurred during the anaerobic digestion at F/I=0.5 and F/I=1 but the degradation seemed affected by the abundance of substrate available for the anaerobic biomass present in the mixtures. It must be pointed out that the LAS monitored during the digestion process are effectively the ones deriving from secondary sludge (=food) and from anaerobic sludge (=inoculum), no spike was added into the reactors. In this way we can follow the fate of the surfactants bound in the sludge, whereas the LAS added with the spike surely follow different pathways during the digestion process because not encompassed in the sludge flocs. Because of the mixing between sludge and inoculum before digestion it was then impossible to discern the LAS deriving from the substrate and the ones deriving from the inoculum (but this is a “classical” problem in sludge digestion tests, also as regards VS and TS). Nevertheless, to solve in part this problem, the anaerobic digestion of the inoculum alone was monitored. The LAS concentration in the inoculum was in the range 600-700 ppm, whereas the LAS in the sludge used as substrate in these tests was significantly lower, in the range 50-70 ppm.

Fig. 44 and 49 showed the quantity of the LAS in the reactors of both untreated and sonicated mixtures during the anaerobic digestion experiments, at the two F/I tested. The inoculum alone was analyzed at the beginning and at the end of the two digestion tests, both for F/I=0.5 and F/I=1 (in the reactors the amount of food was substituted by water). Surprisingly, 28% of the LAS in the inoculum were degraded at F/I=0.5, and 23% for the test at F/I=1.

The presence of LAS in sludge did not result inhibitory for the anaerobic digestion process. In literature is reported that LAS inhibit both acetogenesis from propionate and methanogenesis from acetate and hydrogen, with the propionate-utilising bacteria more sensitive to the presence of LAS than acetoclastic methanogens. It has been proved that inhibition intensity depends on the sludge solids concentration and the inhibitory specific LAS concentration should be around 14 mg LAS/g VS (Gavala, 2002). LAS concentrations in the sludge used for these digestion tests were always noticeably lower (around 0.1 mgLAS/g VS) thus no inhibitory effect was expected for the anaerobic digestion experiments.

The digestions of the untreated sludge showed higher LAS removal with respect to sonicated sludge in the case of F/I=0.5, whereas for F/I=1 the removal efficiency was comparable (see Table 44). Moreover, the extent of LAS degradation was function of the percentage of inoculum in the reactor: during the low inoculum test (F/I = 1) high surfactant removal (40-46%) both for untreated and ultrasound pretreated sludge was observed (while the LAS degradation due to the sole inoculum was 23%). In the case of F/I=0.5 on the contrary all the removal seemed ascribable to the LAS of the inoculum for the digestion of the untreated sludge, and no removal of the "substrate-LAS" was observed. On the contrary, assuming that the "inoculum-LAS" removal was the same independent on the type of food in the reactor (sludge or water) the increased amount of LAS detected in the reactor suggest a release of some aged LAS (available to extract only after anaerobic digestion) from the sonicated sludge influencing negatively the removal efficiency. Same results were reported in literature monitoring other hydrophobic organic pollutants during digestion (Trably, 2003).

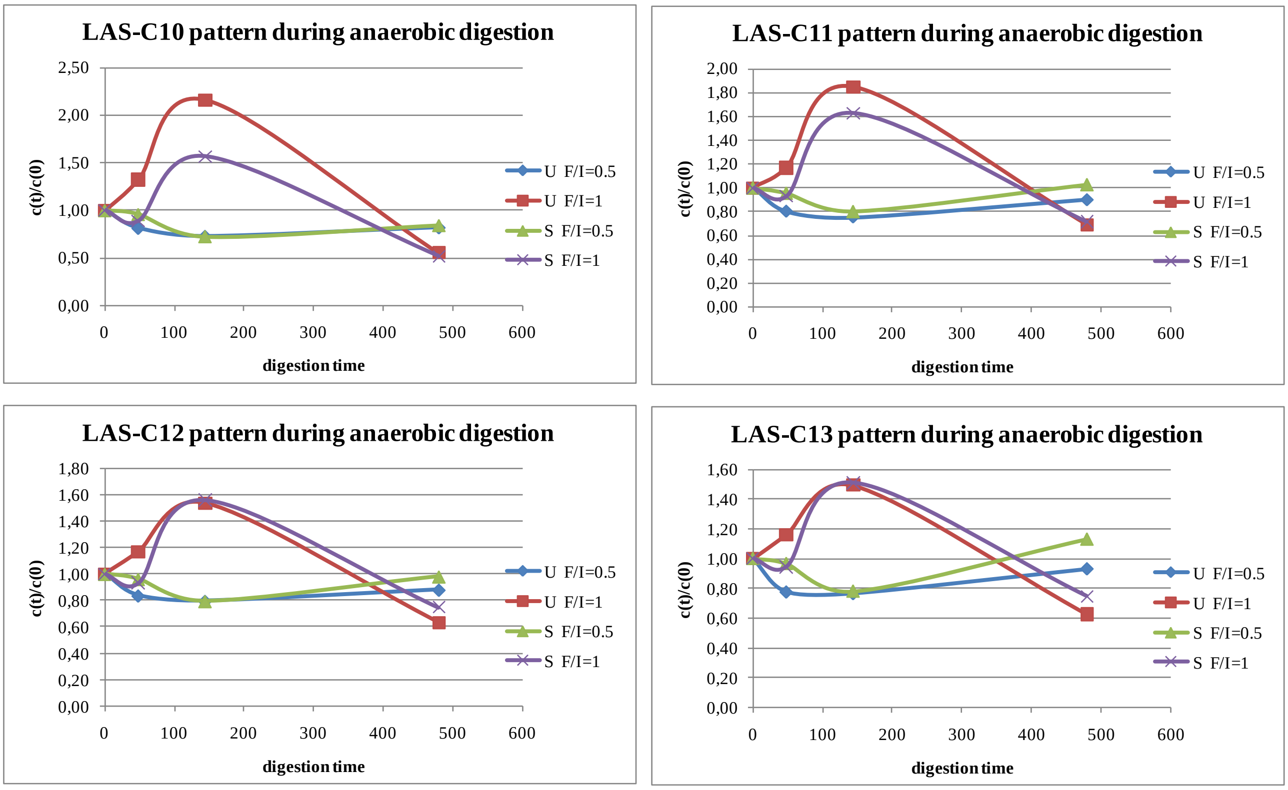
More work is needed to understand the mechanisms of degradation of these pollutants in anaerobic conditions, and the effect of the inoculum on the degradation kinetic.

Table 44 LAS biodegradation in the anaerobic batch tests at F/I==0.5 and 1

LAS anaerobic biodegradation (%)		
	Untreated mixture	**Sonicated mixture**
F/I 0.5	26±3	8±1.5
F/I 1	46±6	41±5

In Fig. 50 each LAS homologue pattern during the anaerobic digestion experiments are shown. No differences can be observed for the LAS homologues in their patterns during the anaerobic digestion. Nevertheless, LAS-C10 seemed to be the homologue which was removed more efficiently at the end of the digestion process in the two digestion tests performed, for both the untreated and sonicated sludge. LAS-C10 removal percentages were in the range 25-58%. A possible explanation of this phenomenon is that, since LAS-C10 is the less hydrophobic homologue, its concentration in the liquid phase overlying sludge is higher than the longer homologues, resulting the more available fraction for the anaerobic bacteria consortia (Garcia, 2005). In fact, biodegradation of adsorbed organic contaminants can be a function of the mass transfer rates (from adsorbed to the aqueous phase) rather than the biodegradation rates (Bouchez ,1995). However, the extent of C10 biodegradation is comparable with the removal of the other LAS homologues; as a consequence, LAS homologues distribution (C10:C11:C12:C13=5:24:34:37) in the digested sludge were comparable with the ones of the secondary sludge used as substrate.

The effect of the F/I was observed for all the homologues monitored during the digestion. As showed in Fig. 50, for the digestion at F/I=0.5 the degradation of all the homologues was progressive during the first 6 days, but at the end of the digestion the concentrations were surprisingly higher with respect to the ones detected after 6 days. This may be ascribed probably to the better availability of the aged LAS after digestion, as reported in literature. This effect in fact is more marked in the case of untreated sludge, presenting a complex structure of sludge flocs.


LAS-C10 pattern during anaerobic digestion

LAS-C11 pattern during anaerobic digestion

LAS-C12 pattern during anaerobic digestion

LAS-C13 pattern during anaerobic digestion

Fig. 50 LAS homologues pattern during anaerobic digestion tests;U=untreated mixture, S=sonicated mixture

The degradation pathway in the case of F/I=1 seemed completely different, both for untreated and sonicated, with respect to F/I=0.5. In fact, during the first 6 days a significant increase of the LAS homologues (in particular of C-10, followed progressively by C11, C12 and C13) in the digesters was noted, always due to the "extraction" of aged LAS by means of the digestion. Same trend was observed in the case of the sonicated sludge digestion, but the effect was reduced for the small chain homologues. Despite these high concentrations of surfactants in the reactors, in presence of high inoculum content, the degradation of all the homologues occurred. The effect of the pretreatment did not have positive effect on the LAS degradation rate: the removal was more intense for the untreated sludge, in particular for long chain homologues as C12 and C13.

Anaerobic digestion performances

The ultrasonic sludge pretreatment at 200 kHz was investigated to assess its positive role in terms of sludge solubilization (in terms of release of proteins, polysaccharides and COD), VS removal and biogas production thus improving the anaerobic digestion performances, with respect to an untreated sludge sample.

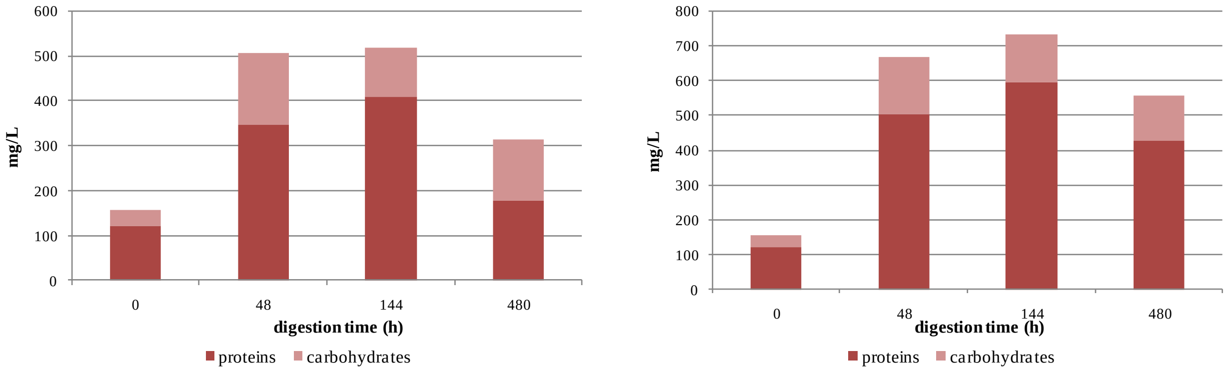
Proteins and sugars (part of the EPS) represent a considerable fraction of the particulate materials in sludge and can be released in the soluble phase because of the sludge pretreatment.

In Fig 51 and 52, the EPS content of untreated and sonicated sludge are reported during the anaerobic digestion tests at both F/I = 0.5 and 1.

For the determination of EPS, the concentration of amino acids was assumed to reflect the protein content while the concentration of neutral sugars accounts for the quantity of polysaccharide present. This is only an approximation of the composition because amino acids and mono saccharides may also originate from heteropolymers (such as lipoproteins, glycoproteins, lipopolysaccharides, polysaccharides) or complex structures (such as ligno-cellulosic complexes) but this approximation is generally assumed because of the lack of analytical techniques to discriminate between these structures. Dignac et. al. (Dignac, 1998) observed that 75.6 percent and 5.1 percent of the TOC in EPS were from protein and sugar, respectively. The remaining uncharacterized organic carbon of EPS consisted of lipids which were dominantly mono-di and triglycerides or phospholipids and sterols, nucleic acids from cell lysis and humic compounds such as fulvic and humic acids.

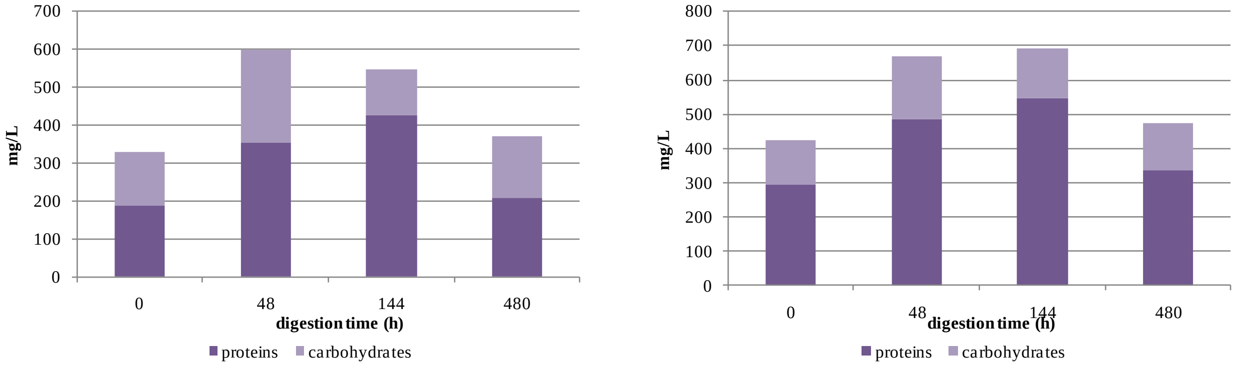
As shown in the figures below, the concentrations of the proteins are always higher than those of carbohydrates, for both untreated and sonicated sludge. Novak (Novak, 2003) stated that for anaerobic systems, the proteins concentration is 3-5 times greater than the polysaccharides concentrations. The dominance of protein in sludge could be due to the presence of a large quantity of exoenzymes in the flocs (Frolund et al., 1995).

The increase of proteins and carbohydrates in the supernatant for both untreated and sonicated sludge in the first days of digestion confirm the initial hydrolysis phase of the particulate substrate independent of the sonication. The hydrolysis was more evident in the case of the low-inoculum test, where the VS of the substrate are in the same proportion as the inoculum (F/I=1), for both untreated and sonicated sludge. After the hydrolysis step, the removal of these polymers occurred progressively up to the 20th day and better removal efficiencies were observed for the high-inoculum test, for both untreated and sonicated sludge. While the removal of proteins was evident, the polysaccharides concentration remained almost constant from the hydrolysis phase till the end. This could be explained with the results reported by Novak (Novak, 2003) where pointed out that, during anaerobic digestion, the release of proteins is relatively rapid, being complete by 20 days of batch digestion whereas the polysaccharides are released more slowly and increase at a relative steady rate over a digestion period of 50 days.



(a) **(b)**

Fig. 51 EPS content of untreated sludge during anaerobic digestion at F/I=0.5 (a) and F/I=1 (b)



(a) **(b)**

Fig. 52 EPS content of sonicated sludge during anaerobic digestion at F/I=0.5 (a) and F/I=1 (b)

The sonicated mixtures show higher initial EPS concentrations due to the ultrasonic pretreatment carried out on sludge before digestion, which acts really as “mechanical” hydrolysis step. In fact, it is important to note that protein and carbohydrates concentrations at the end of the digestion were always comparable with the ones at the beginning, on the contrary in the case of the untreated sludge, independently of the F/I ratio, the final concentrations were much more higher than those at the starting.

Soluble COD trend during anaerobic digestion process was closely related to the one of these soluble substances in the digesters. As regards the untreated sludge, soluble COD trends are similar: the hydrolysis phase occurred in the first step of the process, then the solubilised matter was removed until the end of the digestion process. In the case of low-inoculum test, as just observed for the class of EPS, the hydrolysis phase resulted more rapid and intense, reaching a very high soluble COD value after 48 h of digestion (1439 mg/L).

In the case of sonicated sludge digestion, the initial soluble COD in the reactors was much more higher (because of the effectiveness of the ultrasound sludge pretreatment) but hydrolytic bacteria attack in any case the “less disintegrated” particulate material to solubilize more compounds, and the solubilization was more intense for F/I=1 (Fig. 53).

The efficiencies of COD_S removal at the end of the process for the sonicated sludge were 53 and 33% for the digestion at F/I =0.5 and 1, respectively. In the case of the untreated sludge the removal of soluble COD at F/I=0.5 was 39 %, while for the test at F/I=1 the final CODs in the reactor was slightly higher than the initial one. This can be ascribed to the intense hydrolysis in the first phase and to a scarce consume of these substances by the methanogens.

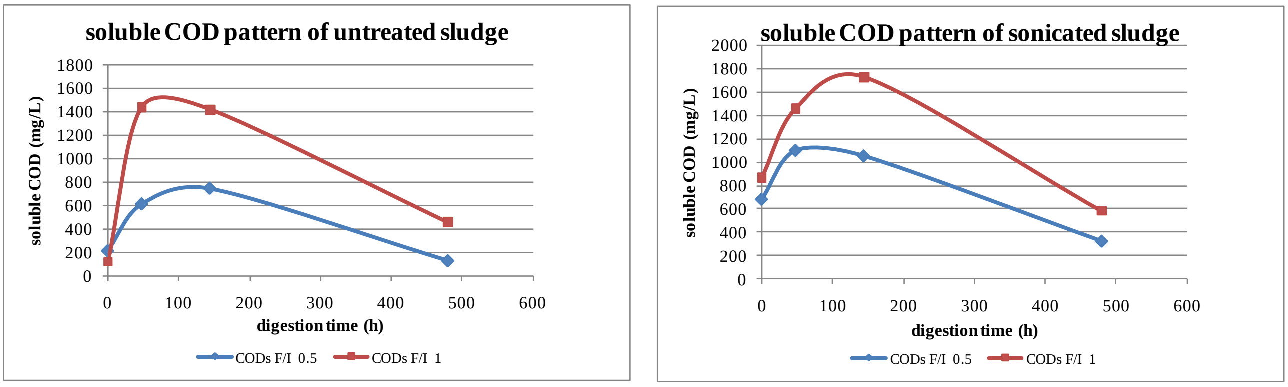


Fig. 53 Soluble COD pattern of the untreated and sonicated sludge

Fig. 54 shows the variation of the specific surface charge during the digestion tests. In the digesters with sonicated sludge, at the beginning of the process, the surface charge was always higher with respect to the untreated ones, because of the high content of colloidal charged particles deriving from the sludge disintegration due to ultrasound pretreatment. The initial hydrolysis phase of the digestion (up to 48 h) was confirmed by the initial increase of the specific surface charge both for untreated and sonicated sludge, because fine particles are generated. In the case of the high-inoculum tests (F/I=0.5) a continuous increasing trend of the specific surface charge was observed independently of the sonication. This may be explained probably because at this F/I ratio could not assure sufficient substrate for the anaerobic bacteria growth during the entire digestion process. In this situation, bacteria took the required energy from the cell protoplasm autoxidation by means of endogenous respiration and the consequence was an increase of fine particles and of the specific charge in the supernatant.

On the contrary, at low-inoculum test, after the initial increase, surface charge was slightly removed because of colloidal material degradation and conversion.

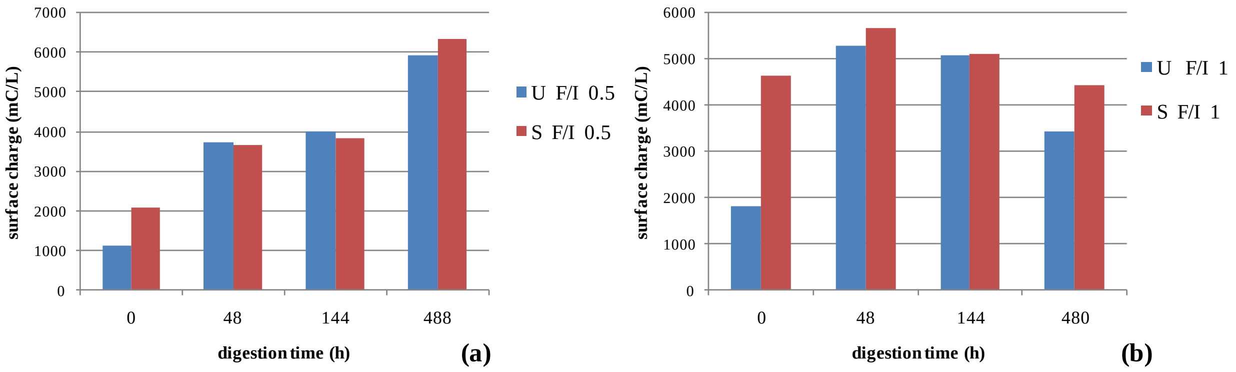


Fig. 54 Surface charge trend during anaerobic digestion tests; U=untreated sludge S=sonicated sludge

Table 45 reports the VS removal (%) for untreated and sonicated sludge in the two digestion experiments performed. Pretreatment with ultrasound gave a great advantage in VS removal improvement compared to non-pretreatment. For the test at F/I=1 the beneficial effect of ultrasound was more marked and the gain in VS removal was +35%, while for F/I=0.5 was 31%. The extent of VS removal was therefore greatly dependent on the amount of anaerobic biomass (i.e. F/I): the VS removal increases by decreasing F/I, both for untreated and sonicated sludge digestion.

Table 45 VS removal of the untreated and sonicated sludge

VS REMOVAL (%)				
	UNTREATED SLUDGE		***SONICATED SLUDGE***	
	F/I = 0.5	*F/I = 1*	*F/I = 0.5*	*F/I = 1*
0	0	0	0	0
48 h	15	9	16	10
144 h	20	14	23	16
480 h	31	23	35	31

VS reduction is direct related to biogas production during the anaerobic digestion process. Fig. 55 shows the cumulative biogas productions obtained from the digestion of the untreated and sonicated sludge, in the tests at different F/I ratios. Biogas production of the untreated sludge was, as expected, higher (+21%) for the test with F/I=1, being higher the total amount of available volatile solids. Moreover, the specific biogas productions ($Nm^3/kgVS_{fed}$) of the untreated sludge were consistent between the two F/I ratios.

From an energetical point of view, the ultrasound pretreatment had a positive effect on both F/I tested, but the improvement (+40%) was more marked for F/I=0.5 (Table 46), despite of the higher COD loading in the low-inoculum test. On the other hand as the COD loading increases, the biogas yield may decrease. According to Show et al. (Show, 2006), the reason was explained that because there is a large amount of organics degradation due to high COD loading, volatile fatty acid (VFA) formations will increase. The increase in VFA will adversely affect the biogas formation due to acidic conditions.

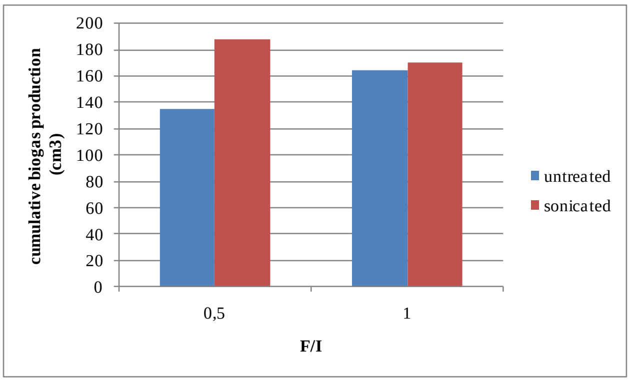


Fig. 55 Cumulative biogas production of the untreated and sonicated sludge at F/I=0.5 and 1

Table 46 Biogas gain

	Untreated		Sonicated		**Δbiogas**(%)**
	Biogas (cm^3)	**Δbiogas*(%)**	**Biogas (cm^3)**	**Δbiogas*(%)**	
F/I 0,5	135	-	188	-	40
F/I 1	164	21	170	-10	4

*= biogas increment at increasing F/I for untreated/sonicated sludge
**= biogas increment of sonicated sludge with respect to the untreated one at the same F/I

In conclusion, the reported results demonstrate that the ultrasonic sludge pretreatment at 200 kHz leads to a global improvement of the anaerobic digestion performances, in terms of the removal of the organic substance and biogas production, as compared to the untreated sludge.

4.2.5 Comparison of the ultrasound pretreatment at 200 kHz and at conventional frequency (20 kHz).

The efficacy of the ultrasound sludge pretreatment for improving the performances of the anaerobic digestion process has been extensively proved. The worldwide used frequency for sludge treatement is 20 kHz, as low frequencies provide larger cavitation bubbles and thus high shear forces in sludge. Higher frequencies, in the range 100 kHz-1MHz, are successfully employed for the sonochemical degradation of organic contaminants.

In this section the results of the digestion experiments with sludge pretreated at 20 and 200 kHz have been compared. It must be pointed out that these experiments have been carried out during two different periods of this work and for this reason the sludge characteristics are different. Moreover, the first series of tests was stopped after 12 days (when the biogas production was completed) while the second one after 20 days.

As regards the organic substance solubilisation, both the ultrasound pretreatement at 20 kHz and at 200 kHz enhanced the first hydrolysis phase of the digestion, disrupting the sludge flocs and causing the release of the organic substances in the surrounding liquid phase. This was highlighted by the considerable increase of protein content, soluble COD and surface charge in the first hours of the digestion process. Afterwards, the organic matter was rapidly removed till the end of the digestion; in particular, the removal of the high soluble COD content in the reactors with sonicated sludge was so intensive to reach comparable values as those obtained with anaerobic digestion of untreated sludge (Table 47).

The significant increase of the soluble COD at F/I=1 for the untreated sludge was confirmed by the two series of experiments and highlight the intense hydrolysis in these conditions, with respect to the other tests at F/I=0.5. Moreover, similar soluble COD trend was observed for the sonicated samples. In the case of the 20 kHz sonication the removal of the soluble COD was 73% at F/I=0.5 (while for 200 kHz was 53%) and 57% at F/I=1 (33% for 200 kHz), in spite of a slight lower disintegration degree of the 20 kHz sonicated samples.

Table 47 soluble COD pattern

Sonication at 20 kHz				
	Untreated		Sonicated	
F/I	CODs(t=0)	CODs(t=12d)	CODs(t=0)	CODs (t=12d)
0.5	104	204	981	254
1	177	408	1605	686
Sonication at 200 kHz				
	Untreated		Sonicated	
F/I	CODs(t=0)	CODs(t=20d)	CODs(t=0)	CODs (t=20d)
0.5	215	132	682	318
1	122	459	867	582

In Table 48 the improvement in the VS removal due to the 20 and 200 kHz pretreatment of sludge are reported. These are calculated considering the VS removals of the same sludge not sonicated, and digested in the same conditions. The ultrasound treatment at higher frequency provided good gains in VS removals, and surprisingly at F/I=1 the improvement was significantly higher than the one obtained at the conventional 20 kHz.

Table 48 VS degradation efficiency of the sonicated with respect to the untreated one

F/I	**VS DEGRADATION EFFICIENCY (%)**	
	US 20 kHz	**US 200 kHz**
0.5	+10	+13
1	+14	+35

Generally, a great part of the removed volatile solids during the anaerobic digestion are converted into biogas. Comparing the biogas gain obtained from the different digestion tests (Fig, 22), the results are very interesting. In fact, while in the case of sonication at 20 kHz the gain seemed not strictly dependent on the F/I, in the case of 200 kHz sonication, as just noted in the previous chapters, the biogas improvement was very high (about 50%) at F/I=0.5, whereas at F/I=1 was comparable with the biogas gained pretreating the sludge at 20 kHz (Fig. 56).

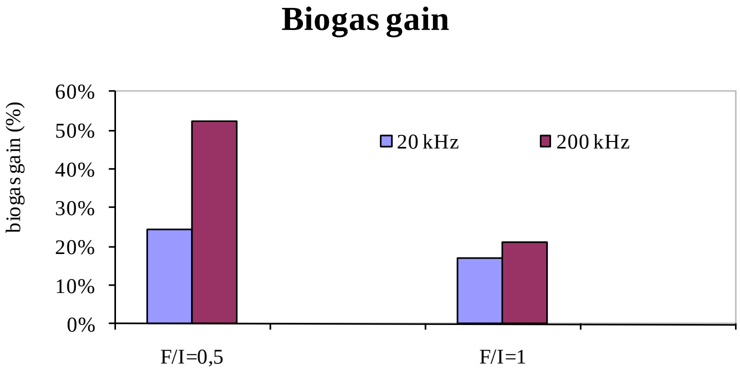


Fig. 56 Biogas gain of the 20 and 200 kHz pretreated sludge at F/I =0.5 and 1

The reported results demonstrate that the ultrasound sludge pretreatment at 200 kHz can be considered a valid option of the classic treatment at 20 kHz, since it permits to obtain better anaerobic digestion performances, as regards to solubilisation and removal of organic substances and biogas production. Moreover, the digestion test at F/I=1 resulted ideal to increase the stabilization of the sludge (higher VS removals), and at the same time, to decontaminate the sludge from organic pollutants (high LAS removals), but was not convenient form an energetical pointy of view because of the low biogas productions. The slowdown in the biogas production occurred probably because of the observed accumulation of soluble organic substances suggesting a kinetic uncoupling between hydrolysis (i.e. solubilisation of organics) and methanogenesis (i.e. biogas production). Future work is needed to understand the mechanisms in order to optimize this process, also as regards the energy gain.

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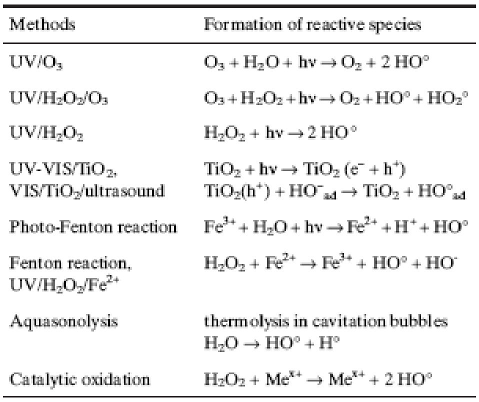
4.3 Sonolysis of organic micropollutants in sludge: LAS degradation[4]

4.3.1 Introduction

In conventional wastewater treatment plants, biological processes are the most commonly used method for wastewater treatment, due to their low costs compared to chemical or physicochemical treatments. However, they are less efficient because various bacteriotoxic or persistent pollutants can inhibit these processes. In order to achieve better degradation of the persistent organic pollutants (POPs), more effective methods have been developed, the so-called AOPs or advanced oxidation processes. AOPs permit to remove the organic contaminants present in wastewater by means of their oxidative degradation and they yield completely oxidized products or biologically degradable intermediates. The basic concept of these processes is the formation of very reactive species like $OH^{\cdot}$ radicals. Fig. 57 lists the main AOPs processes with the respective reaction leading to $OH^{\cdot}$ radicals formation; aquasonolysis i.e. the use of ultrasound for the degradation of contaminants in water, is assigned to the AOPs.



Methods	Formation of reactive species
UV/O_3	$O_3 + H_2O + hv \rightarrow O_2 + 2\ HO^{\circ}$
UV/H_2O_2/O_3	$O_3 + H_2O_2 + hv \rightarrow O_2 + HO^{\circ} + HO_2^{\circ}$
UV/H_2O_2	$H_2O_2 + hv \rightarrow 2\ HO^{\circ}$
UV-VIS/TiO_2, VIS/TiO_2/ultrasound	$TiO_2 + hv \rightarrow TiO_2\ (e^- + h^+)$ $TiO_2(h^+) + HO^-_{ad} \rightarrow TiO_2 + HO^{\circ}_{ad}$
Photo-Fenton reaction	$Fe^{3+} + H_2O + hv \rightarrow Fe^{2+} + H^+ + HO^{\circ}$
Fenton reaction, UV/H_2O_2/Fe^{2+}	$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^{\circ} + HO^-$
Aquasonolysis	thermolysis in cavitation bubbles $H_2O \rightarrow HO^{\circ} + H^{\circ}$
Catalytic oxidation	$H_2O_2 + Me^{x+} \rightarrow Me^{x+} + 2\ HO^{\circ}$

Fig. 57

The acoustic cavitation in water produced by ultrasound is a very effective process and makes some peculiar reactions take place:

- Sonolysis of water molecules and formation of $OH^{\cdot}$ radicals
- Oxidative degradation of contaminants by $OH^{\cdot}$ radicals
- Degradations of pollutants by pyrolytic reactions in the cavitation bubbles
- Breakage of high molecular weight pollutants by hydro-mechanical shear forces.

As a result, with the application of ultrasound, the degradation of organic pollutants can occur not only in liquid phase, but also in the gaseous one.

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4.3.1.1 Linear alkylbenzene sulfonates (LAS)

Surfactants are one of most important and ubiquitous families of anthropogenic organic compounds. Although the generic term applies to a great number of products, the most part of synthetic detergent formulations for both domestic and industrial purposes is a mixture of linear alkylbenzene sulphonates (LAS) and other additives. Because of their high consumption, the Working Document on Sludge, 3rd Draft, indicates 2600 mg/kg dw as a limit for LAS concentration on sludge for agricultural use. LAS were introduced in 1964 as the readily biodegradable replacement for highly branched alkylbenzene sulphonates (ABS).They are manufactured by reacting alkylbenzene with sulphuric acid or sulphur trioxide to give the sulphonic acid, which is then neutralised to give the sodium salt. The precursor for LAS is linear alkylbenzene (LAB). LAS are the surface-active components of the synthetic mixtures. They belong to the class of anionic surfactants and contain an aromatic ring sulfonated at the para position and attached to a linear alkyl chain at any position except the terminal ones. The commercial product is composed of a complex mixture of various homologues and isomers representing different alkyl chain length and aromatic ring positions along the linear chain as shown in the figure below (Fig. 58):

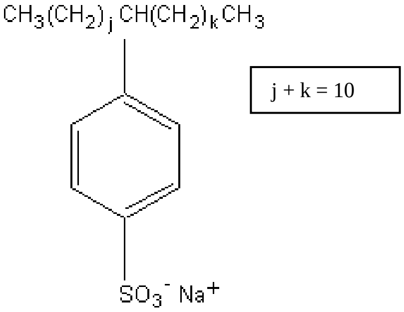


Fig. 58

The linear alkyl chain has tipically 10 to 13 C atom units, approximately in the following mole ratio C10:C11:C12:C13=13:30:33:24 with an average carbon number near 11.6. In Table 49 the main physical chemical characterises for LAS C11.6 or for the pure C12 homologue are reported (Table 49, HERA, 2004).

Table 49 LAS physical chemical characteristics

LAS	Protocol	Result
Molecular description	Solid organic acid sodium salt	
Molecular weight (g/M)	$(C_{11.6}H_{24.2})C_6H_4SO_3Na$	342.4
Vapour pressure at 25˚C (Pa)	Calculated as C12	$(3\text{-}17)\cdot 10^{-3}$
Boiling point (˚C)	Calculated as C12	637
Melting point (˚C)	Calculated as C12	277
Octanol-water partion coefficient ($\log K_{ow}$)	Calculated as C11.6	3.32
Organic carbon-water partition coefficient K_{oc} (l/kg)	Calculated as C11.6	2500
Water solubility (g/l)	Experimental	250
Sorption coefficient between soil/sediment and water, K_d, (l/kg)	Experimental	2-300
Density (kg/l)	Experimental	1.06 (relative) 0.55 (bulk)
pH (5% LAS water solution)	Experimental	7-9
Henry's constant ($Pa\cdot m^3$/mole)	Calculated as C12	$6.35\cdot 10^{-3}$

After application, LAS are usually discharged to the sewer systems and appear in municipal wastewater treatment plants. The majority of these plants are activated sludge type where the fate of LAS is determined by sorption to the sludge (and subsequent removal together with excess sludge) and by biodegradation (Temmink, 2004). LAS removal in sewers is due to a combination of biodegradation, adsorption and precipitation: in a typical activated sludge system almost 80-90% of LAS are biodegraded during wastewater treatment; the mass fraction removed via sorption to sewage sludge, mainly in the primary settlers, is in the range 10-20% and about 1% is released to surface waters. Transport of surfactants in WWTP is shown as an example in Fig. 59 (Scott , 2000). Figures may vary from one plant to another.

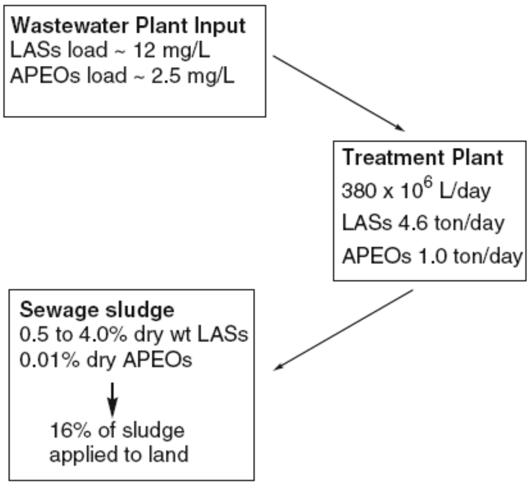


Fig. 59. Transport of surfactants in WWTP

Sorption of surfactants onto sludge or soil depends on many factors such as their physical-chemical properties, sediment nature and environmental parameters. Among surfactants characteristics, the type of LAS homologue is significant for adsorption to sludge particulate matter. LAS with longer alkyl chains are more hydrophobic than shorter homologues and this increases their adsorptive tendency. For each carbon atom added to the alkyl chain, a two- to three-fold increase in the K_a (association constant) for LAS was observed (Cirelli et al., 2008). The LAS homologues distribution in sludge is approximately in the mole ratio C10:C11:C12:C13=7:24:39:30 (Berna, 1989; Cavalli, 1999; Di Corcia 1994) with an average carbon number of C11.9 as a consequence of the preferential adsorption of higher homologues. In a monitoring work by Temmink (Temmink et al, water research, 2004) on a pilot-scale municipal activated sludge plant, the fate of C12 homologue of LAS was assessed: in sludge samples, only 2-8% was present as dissolved LAS-C12, whereas the remaining 92-98% was found to be adsorbed to the sludge. Moreover, it's was shown by Temmink that the sorption of LAS-C12 to activated sludge is an instantaneous equilibration process which can be described by a linear sorption isotherm. LAS sorption was intensively studied by Ying (Ying et al, 2006): in general, the commonly used sorption isotherm is the Freundlich equation, which defines a non-linear relationship between the amount sorbed and the equilibrium solution concentration:

$$S = K_f C^n$$

where S is the concentration of the surfactant sorbed by solid phase (mg/kg); K_f is the Freundlich sorption coefficient (L/kg); C is the equilibrium solution concentration (mg/L) and n is a power function related to the sorption mechanism. When n is equal to 1, the previous equation becomes:

$$S = K_d C$$

where K_d is the sorption coefficient (L/kg). The parameter K_d is frequently used to characterise the sorption of a chemical in sediment or soil and it represents a clear indication of the partitioning and mobility of the chemical in the environment. Because of their chemical characterises, surfactants may sorb directly on solid surfaces or may interact with sorbed molecules. The sorption mechanism is dependent on the nature of the sorbent and the surfactants concentration (Adeel, 1995; Brownawell, 1997; Ou, 1996). At low concentrations, no significant sorbate-sorbate interactions occur, whereas higher LAS concentrations may provoke the formation of monomer clusters on the surface or a second layer, build up through interactions between hydrophobic moieties of the surfactants molecules. Thus, LAS sorption on sludge and soil is a process which can be described as a combination of two stages: linear and exponentially increasing isotherms. At low LAS concentration (<90 µg/ml) the sorption isotherms are linear; at high LAS levels, cooperative sorption is observed and the sorbed LAS amount increases exponentially with the increasing of LAS concentration in solutions (Fig. 60).

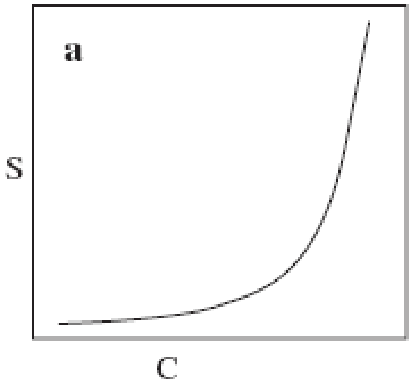


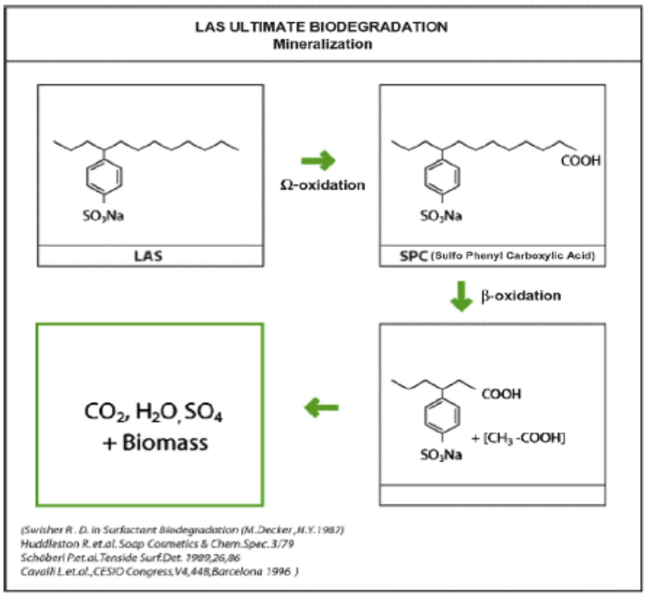
Fig. 60. Sorption isotherm for LAS.S is the sorbed surfactant concentration and C is the surfactant concentration in solution

The chemical composition of the WWTP influent may influence the adsorption of LAS: according to Berna (Berna et al, 1989) and Garcia (Garcia, 2002), water hardness is the key factor that controls the cation-brindging of Ca-LAS onto sludge. They found a correlation between LAS concentration in sludge and influent water hardness:

LAS in dry sludge (g/kg) = 0.04 × water hardness (ppm $CaCO_3$) + 2.79.

This means that waters with high Ca concentration yield sludge from primary settling tanks that contains 30-35% of the LAS concentration of the raw sewage, but relatively soft waters produce only 10-20% (Berna, 1991). Sludge removed from primary settling tanks is relatively rich in LAS, with concentrations ranging from 5 to 15 g/L (Brunner, 1988; McEvoy 1986). The presence of high concentrations of LAS in sewage sludge leaving the treatment plant is dependent on the type of treatment the sludge is subjected, in particular if stabilization is performed in aerobic or anaerobic conditions. It’s well known that many surfactants, among them LAS, can be degraded under aerobic conditions but they are persistent under anaerobic ones. Degradation of surfactants through microbial activity is the primary transformation occurring in the environment. Biodegradation is one of the main process to remove surfactants in raw sewage during sludge treatment in a plant, thus reducing their impact on biota. During biodegradation, microorganisms can utilize surfactants as substrates for energy and nutrients or cometabolize the surfactants by microbial metabolic reactions (Ying, 2006). In particular, LAS can be degraded by consortia of aerobic microorganisms, few fungi or by attached biofilms in the environment (VanGinkel, 1996). For LAS, very high levels of biodegradation (97-99%) in wastewater treatment plants with aerobic process are reported (Swisher, 1987). The mechanism of LAS breakdown starts with the degradation of the alkyl chain, then the sulphonate group and finally the benzene ring. Deeply, it can be schematized as a four-stages process (Fig. 61):

1. Oxidative conversion of one or two methyl groups of the alkyl chain into a carboxyl group (ω-oxidation) and formation of sulpho phenyl carboxylates (SPCS) as biodegradation intermediates.
2. Oxidative shortening of the alkyl chain by two carbon units (β-oxidation)
3. Oxidative ring splitting
4. Cleavage of the carbon-sulphur bond, conversion of LAS and SPCs into inorganic substances (H_2O, CO_2 and $NaSO_4$) and incorporation of LAS constituents into the biomass of microorganisms (ultimate biodegradation).



(Swisher R. D. in Surfactant Biodegradation (M.Decker, N.Y. 1987)
Huddleston R. et.al. Soap Cosmetics & Chem.Spec. 3/79
Schöberl P.et.al.Tenside Surf.Det. 1989,26,86
Cavalli L.et.al., CESIO Congress,V4,448,Barcelona 1996)

Fig. 61.LAS biodegradation

The complete biodegradation of surfactants requires a mixture of bacteria because of the limited metabolic capacities of individual microorganisms. For LAS degradation, a four member consortium is involved, with three members which oxidize the alkyl chain and a synergic mechanism of all the four members to mineralise the benzene ring (Perales, 2003). The primary LAS biodegradation is very fast and almost complete (> 99%), whereas the ultimate biodegradation, i.e. the breakdown of LAS intermediates SPCs, is slower and is generally in the range going from 80% to >95%. However, the LAS biodegradation intermediates are not persistent and their toxicity is several orders of magnitude lower than that of the parent molecule. In many field studies, performed to assess the rates of primary and ultimate LAS biodegradation, $t_{0.5} \leq 7$ and 30 days respectively have been reported (Holt, 1989; Jensen, 1999; Hera, 2004). Thus, LAS are readily biodegradable in aerobic sludge, and after application of sludge to land, they are rapidly metabolized by aerobic bacteria in sludge-amended soil and will not accumulate (Berna, 1989;

Marcomini, 1989; Holt, 1989). The rate of LAS biodegradation increase with dissolved oxygen concentration and the longer alkyl chain homologues (C12 and C13) are preferentially biodegraded. On the other hands, LAS don't show any significant biodegradation in anaerobic conditions (Steber, 1991, Federle, 1992, Hera, 2004, McEvoy, 1985,). This is mostly due to the fact that the ω-oxidation of the alkyl chain and the cleavage of the benzene ring require molecular oxygen, therefore, under anaerobic conditions, degradation via this pathway is unlikely. As anaerobic digestion is the predominant treatment of sludge from primary and secondary setting tanks and, because the amphiphilic nature of LAS promotes their adsorption to particulate matters of sludge, it seems that LAS pass through a wastewater treatment plant relatively untreated.

More recently, Angelidaki et al. (Angelidaki, 2004) and Mogensen et al. (Mogensen, 2003) demonstrated that in oxygen-limited conditions, that occur in the real world, LAS are biodegradable under methanogenic conditions, but low bioavailability in waste water treatment plant reactors would in reality prevent any biodegradation.

LAS concentrations in sludge differ dramatically depending on the influent wastewater, the treatment method and efficiency of the individual treatment plant. Sewage sludge that had been treated aerobically has LAS concentrations of 100-500 mg/kg dry weight, while those anaerobically treated sludge has much higher LAS concentrations ranging from 5000-15000 mg/kg dry weight (Jensen, 1999). There are four main reasons for the presence of LAS at relatively high concentrations in anaerobically stabilized sludge:

1. high usage per capita (1.5-4 g/capita×day), mainly as a major surfactant in household consumer goods
2. high sorption to suspended solids in primary settlers and activated sludge solids
3. precipitation as insoluble Mg^{2+}/Ca^{2+}-salts and removal in primary settlers (Berna, 1989)
4. absence of LAS biodegradation in anaerobic digesters (Gejlsbjerg 2003).

Surfactants may enter the terrestrial environment through several routes, with the use of sewage sludge as fertiliser on agricultural land being by far the most important. Because of the worldwide increasing application of sludge to agricultural land, high concentrations of surfactants and their degradation products may affect the biota. On the other hand, due to their amphiphilic nature, surfactants may interact both with inorganic as well as organic contaminants affecting their bioavailability. For this reason, decontamination with sonolysis to improve sludge quality may be an interesting and innovative research field.

4.3.1.2 Acoustic cavitation and sonochemistry

As said before, aquasonolysis, i.e. the use of ultrasound for the degradation of organic contaminants in water, today represents an advanced technology for wastewater treatment. The process is based on the phenomenon of acoustic cavitation. Ultrasound occurs at frequencies above 16 kHz, higher than the audible frequency of the human ear, and it typically comprises sound waves with frequency between 20 kHz and 10 MH. This broad frequency range can be subdivided into three main regions: low frequency, high power ultrasound (20-100 kHz); high frequency, medium power ultrasound (100 kHz-1 MHz), and high frequency, low power ultrasound (1-10 MHz). Low-frequency ultrasound is used in industry for the cleaning of surfaces, while high-frequency ultrasound is employed in sensors for materials science and for diagnostic in the field of medicine. The frequency level is inversely proportional to the power input: thus, low-intensity, high-frequency ultrasound (in the megahertz range) doesn't alter the state of the medium through which it travels, whereas high-intensity, low-frequency ultrasound does alter the state of the medium and is the frequencies range generally used for sonochemical applications. The term "sonochemistry" indicates the use of ultrasound to induce chemical effects on the reaction system. The Fig. 62 displays the sonic regions and some common applications of ultrasound.

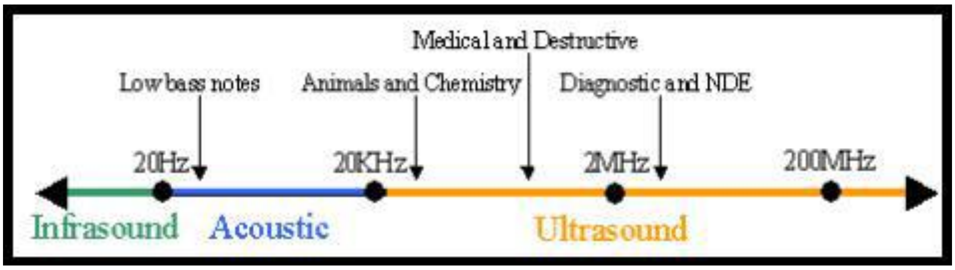


Fig. 62 Sonic regions and US applications

Ultrasound waves, like all sound waves, consist of cycles of expansion and compression. Compression cycles exert a positive pressure on the liquid, pushing the molecules together, while during expansion cycles the liquid is subjected to a negative pressure. When the negative pressure, during the expansion cycles, is so intense to overcome the tensile strength of the liquid, cavities can be generated (cavitation bubbles). Thus, for cavitation bubbles to form, a sound wave of sufficient intensity is required. The amount of negative pressure required generally depends on the type and purity of the liquid: for pure liquids, the tensile strength is so great that available ultrasound generators cannot produce enough negative pressure. In contaminated liquids, that is liquids with gas trapped in the crevices of the small suspended solids particles, the liquids' tensile strength is greatly reduced and negative pressure of only few atmospheres are sufficient to form cavitation bubbles. A bubble irradiated with ultrasound continually absorbs energy from the alternating compression and expansion cycles of the sound field and it is forced to expand and contract in a dynamic balance between the vapour inside the bubble and the liquid outside. Two forms of cavitations are known, which depend on the intensity of ultrasound: stable and transient. Transient cavitation refers to bubbles which can grow rapidly in the course of a single cycle of sound and it is produced with the application of high-intensity ultrasound. Whereas, with low-intensity sonic waves, bubbles oscillate in phase with the expansion and compression cycles. The surface area of the bubbles produced with low-intensity ultrasound is slightly greater during the expansion cycle than during compression one. Since gas diffusion is proportional to the surface area, diffusion into the bubbles during expansion cycles is greater than diffusion out of the bubbles ("rectified diffusion"). Thus, bubbles grow slowly over many cycles until they reach a critical size, where their energy absorption maximizes. The critical size depends on the frequency of applied ultrasound. At this point, bubbles can grow rapidly during a single cycle of sound and they can no more absorb energy. Without energy input, bubbles implode (Fig. 63):

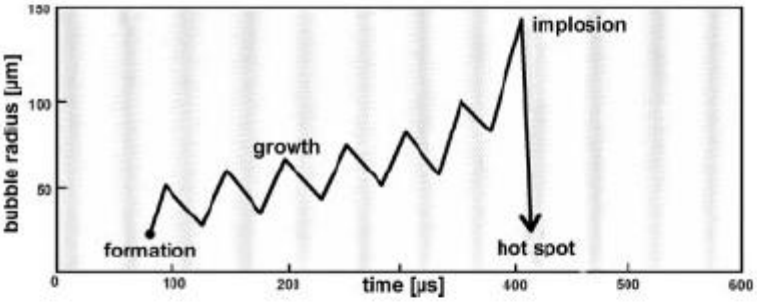


Fig. 63 Formation, growth and implosion of a cavitation bubble

The implosion of the cavitation bubbles determines an unique environment: the gas and vapour trapped in the cavities are compressed with an adiabatic process and this creates a local hot spot at very high temperatures. But, the size of these hot spot is so small that heat dissipates quickly. For a cavitation phenomenon , heating and cooling rates are of more than 10^9 K s^{-1}. By means of sophisticated mathematical models, temperature and pressure inside the cavitation bubble have been estimated: the gas phase can reach about 5500 °C and pressure is approximately 500 atm (Suslick, 1989; Lifka, 2003). In other words, through the cavitation phenomenon, sonochemistry makes available a range of energies and combinations of pressures and durations which cannot be obtained with other sources (Fig. 64).

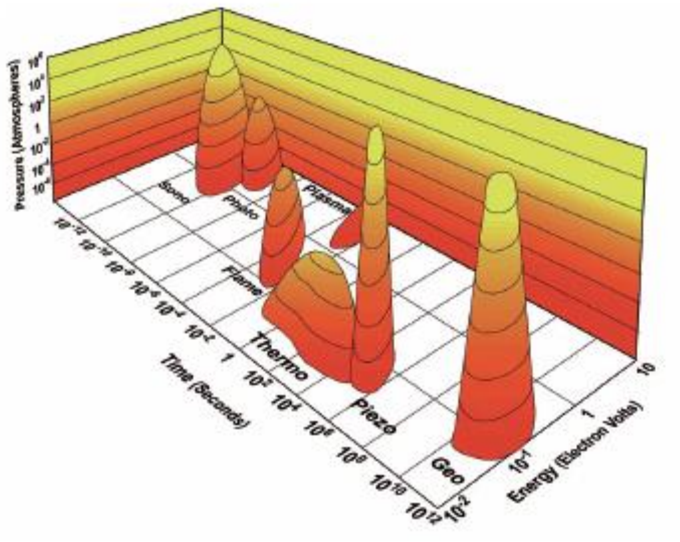


Fig. 64. Relation among time, energy and pressure for different chemistry fields

The heat from cavity implosion causes the decomposition of the surrounding liquid into radicals; for example, when cavitation bubbles collapse occurs in water, extremely reactive hydrogen atoms (H^+) and hydroxyl radicals ($OH^\cdot$) are formed. The hot spot theory is widely accepted, but also another theory exists to explain the chemical effects due to cavitation phenomenon: the electrical theory, which postulates that an electrical charge is created on the surface of the a cavitation bubble, forming enormous electrical field gradients across the bubble, which are able to break chemical bonds upon collapse (Margulis, 1992).There is a general agreement that bubble collapse and the subsequent radical formation is the origin of the sonochemical effects of ultrasound. Many parameters affect the bubble cavitation, like power intensity, frequency, the presence of dissolved gases, the nature or physicochemical properties of the solvent and the bulking solution temperature.

Intensity

Generally, sonochemical activity increases by increasing the power intensity, up to an optimal power level. Power intensity above this level gives a decrease in the reaction rate. The acoustic intensity (in W m^{-2}), in the case of progressive planar or spherical wave, is directly related to acoustic pressure:

$$I = \frac{P_a^2}{2\rho c}$$

where ρ is the density of the fluid and c is the speed of the sound in the fluid.

Another parameter, the maximum size of a cavitation bubble is dependent on the applied frequency, the acoustic pressure, the density of the liquid medium, and the hydrodynamic pressure as espressed in the following equation:

$$R_m = \frac{4}{3\omega_a}\left(P_A - P_h\right)\left(\frac{2}{\rho P_A}\right)^{1/2}\left[1 + \frac{2}{3P_h}\left(P_A - P_h\right)\right]^{1/3}$$

where ω_a is the applied acoustic frequency, P_A is the pressure amplitude and P_h is the hydrostatic (external) pressure. The maximum radius is associated to the collapse time τ of an acoustical bubble under constant pressure:

$$\tau = 0.915 R_m \left(\frac{\rho}{P_m}\right)^{1/2}\left(1 + \frac{P_{vg}}{P_m}\right)$$

where P_m is the pressure in the liquid and P_{vg} is the vapour pressure in the bubble at the beginning of the bubble collapse. The main condition in order to have an efficient cavitation is that the time of cavity collapse should be smaller than half of the ultrasonic period (T), i.e. $\tau < T/2$. Therefore, at high acoustic intensity (i.e. large P_A values), the cavitation bubbles are able to grow larger in size during a rarefaction cycle such that insufficient time is available for complete collapse during a single compression cycle.

Frequency

Frequency plays a very significant role on the cavitation processes, because it can alter the critical size of the cavitation bubbles, as shown in the following equation:

$$R_r^2 = \frac{3\kappa P_h}{\rho \omega_r^2}$$

where ω_r is the resonant circular frequency ($2\pi f$).

As the frequency increases, the critical size of the cavitation bubbles increases; moreover, changing the ultrasonic frequency, may induce a change in the local bubble population and affect the efficiency of cavitation. It is generally accepted that at lower frequencies, ultrasound produce more violent cavitation events, leading to high localized temperatures and pressures, while at higher frequencies, more acoustic cycles and hence more cavitation collapses occur, even though less violent (Thompson, 1999; Ince, 2001). At higher frequencies the cavitation efficiency is reduced because the rarefaction cycle produces a negative pressure which has duration and intensity insufficient to create cavitation, and the compression cycle is faster than the time for microbubbles to collapse (Thompson, 1999).

Nevertheless, current research indicates that in reactions such as oxidations, higher frequencies may lead to higher reaction rates. This is due to the fact that higher frequency may actually increase the number of free radicals in the system because although cavitation is less violent, there are more cavitation events and thus more opportunities for the free radicals to be produced. Francony and Petrier (Francony and Petrier, 1996) observed the ultrasonic degradation of carbon tetrachloride was enhanced and the yield of products faster when using 500 kHz ultrasound compared with 20 kHz. But at very high frequencies, the cavitation process is decreased.

Dissolved gases

Dissolved gases can diffuse into the cavitation bubbles during their growth and thus influencing temperature and pressure of the bubbles. Assuming adiabatic bubble collapse, the maximum temperature and pressure achieved in the collapsed cavitation bubble are predicted by Noltingk and Nepprias (cit) from approximate solutions of the Rayleigh-Plesset equation:

$$T_{\max} = T_0 \left[\frac{P_a(\gamma - 1)}{P_v} \right]$$

$$P_{\max} = P_v \left\{ \frac{P_a(\gamma - 1)}{P_v} \right\}^{\left(\frac{\gamma}{\gamma - 1}\right)}$$

where T_0 is the ambient temperature or temperature of the bulk solution, P_v the pressure in the bubble at the maximum size or the vapour pressure of the solution, P_a the pressure in the bubble at the transient collapse (i.e. acoustic pressure) and γ is the polytropic index (Cp/C_v) of the cavity medium. Based on these equations, higher temperatures and pressures are achieved with monoatomic gases, which have higher polytropic index than polyatomic ones. Other gases characterises are important: thermal conductivity and solubility of the gas. A gas with low thermal conductivity reduces heat dissipation from cavitation sites following adiatic collapse and should favour higher collapse temperatures. On the other hands, gas solubility acts in the opposite way: highly soluble gas reduce the cavitation threshold of a liquid but also leads to a greater amount of gas diffusing into the bubble resulting in a “cushioning” of the collapse.

Bulk solution temperature

For sonochemical reactions, temperature is a complex parameter and has contradictory effects. An increase in liquids temperature results in an increase of the amount of the vapour inside the bubble, which can promote the formation of free reactive radicals from vapour dissociation. Moreover, the increasing vapour pressure in the bubble facilitates cavitation. But on the contrary, a great amount of vapour inside the cavities may also “cushion” bubble collapse resulting in less violent cavitation events.

Surface tension

Cavitation threshold varies with surface tension, but there is not a clear relationship. However, it is demonstrated that the addition of a surfactant to the bulk solution may promote cavitation. This is due to the fact that reduced surface tension lowers the cavitation threshold and facilitates the formation of other cavitation bubbles.

The global effect of these parameters on a sonochemical system is a combination of the different mentioned effects and the optimal operating conditions are very system dependent.

4.3.1.3 Sonochemical degradation of surfactants in aqueous solutions: state of the art

As said before, ultrasound represents an efficient method for the degradation of organic pollutants in water, due to their peculiar chemical and mechanical effects.

The sonochemical reactions can occur in different reaction zones, depending on the chemical-physical properties of the compounds (Fig. 65), in particular, the substances volatility is an important parameter because it affects the diffusion of the compounds into the cavitation bubbles. This statement cannot be generalized because also the hydrophobic or hydrophilic properties of the compounds must be taken into account. (Lifka, 2003). Three sonochemical reaction regions have been identified:

1. *interior of the cavitation bubble*

Substances with high vapour pressure can reach the interior of the cavitation bubble, where very high temperatures and pressure exist. Thus, these substances are degraded mainly by pyrolysis or by oxidation reactions, if in presence of oxygen.

2. *liquid-bubble interface*

In addition to high temperatures and pressure gradient, in this boundary region also the formation of radical species at high concentration takes place. In water, the predominant specie is the OH· radical. Non-volatile compounds can accumulate at the interface between liquid and bubble and may undergo oxidation reaction by radicals. At temperatures > 667 K and pressures > 221 bar, the organic contaminants can be degraded by supercritical decomposition reactions.

3. *Bulk liquid*

In the surrounding liquid only the solvent decomposition into radical species occurs and the degradation pathway of non-volatile hydrophilic compounds is uniquely through oxidation reactions.

Thus, the sonochemical degradation of organic compounds depends on their chemical properties, i.e. their solubility, volatility and surface activity.

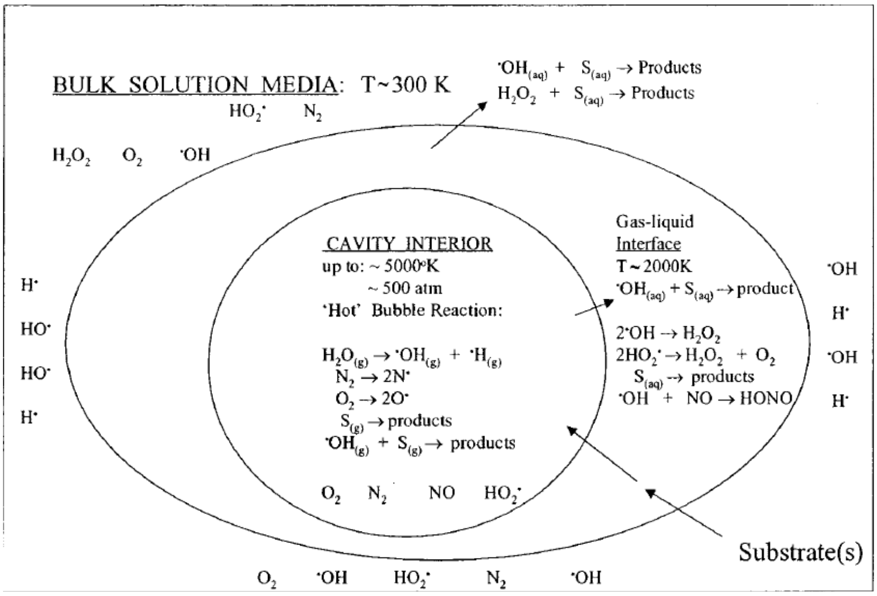


Fig. 65. The three sonochemical regions

LAS, like all surfactants, are non-volatile molecule having both hydrophilic and long-chain hydrophobic components and as a consequence they accumulate preferentially at the bubble-liquid interface. The primary effect of the surfactant adsorption at the boundary phase is to reduce the surface tension of the surrounding solution. When the surfactants start to adsorp at the gas/liquid interface, the surface tension (γ) begins to decrease until they reach an equilibrium surface tension (γ_{eq}). There is a thermodynamic relation between the amount of adsorbed surfactant at equilibrium point (Γ_{eq}) and its bulk concentration, at constant temperature and pressure; for charged surfactants with a single counter-ion, as in the case of LAS, this relation is:

$$\Gamma_{eq} = -\frac{1}{2RT}\frac{d\gamma_{eq}}{d(\ln C_i)}$$

where C_i is the bulk surfactant concentration, R the universal gas constant and T is the temperature.

After the interface is saturated, the surface tension of the solution continues to decrease with increasing surfactants concentration until a critical micelle concentration (CMC) is reached. The CMC is the point at which surfactants cluster together to form micelles and the surface tension lowers at the minimum value.

Due to the higher concentration of OH· radicals at the liquid-bubble interface than in the bulk solution, the degradation efficiency of surfactants mainly depends on their ability to partition at the bubbles surface.

Based on these promising considerations, degradation of aqueous LAS solutions under ultrasound irradiation, in particular at low frequencies, has been object of several studies in recent years.

Abu-Hassan (Abu-Hassan, 2006) showed that concentrated LAS solutions (175, 260 and 350 mg l^{-1}) can be degraded using ultrasound at 20 kHz, without obtaining a complete surfactants mineralisation, because both LAS and their degradation products are resistant to total oxidation. The degradation mechanism is firstly the attack of the $OH^{\cdot}$ radicals to the alkyl chain and then to the aromatic ring, in particular at *ortho* and *meta* positions of the benzene ring. Afterwards, the degradation intermediates can react with $OH^{\cdot}$ radicals giving soluble products and volatile compounds, which can be finally pyrolized into the cavitation bubbles. The primary reaction of $OH^{\cdot}$ radicals attack occurs always in the bubble-liquid interface because of low LAS volatility (Ashokkumar, 2003). The degradation rate (expressed as d[las]/dt) increases with increasing LAS initial concentration. This is due to the fact that a high initial surfactant concentration leads to an increase in the probability of reaction between OH radicals and LAS molecules. Moreover, high surfactant amount reduces the surface tension of the bulk liquid and consequently the energy required to form cavitation bubbles (Abu-Hassan, 2007). Also Ashokkumar highlights that the degradation rate increases with initial LAS concentration until a maximum value near the critical micelle concentration, above which the removal rate starts to decrease. Other important parameters affecting sonochemical reaction have been investigated in previous researches: LAS degradation rate increases with increasing the electric power of the ultrasound generator (Manousaki, 2004), while it is lowered with increasing the sonication time (Abu-Hassan, 2007) and the system temperature (Manousaki, 2004).

4.3.2 Aim of this study

Previous studies demonstrated that linear alkylbenzene sulphonates can be efficiently degraded by means of ultrasound at low-medium frequencies. All these researches have been performed on laboratory prepared aqueous solutions of LAS.

Aim of this study is the application of ultrasound at 200 kHz directly on sewage sludge to investigate LAS degradation in real environmental samples.

Sonochemical reactions may occur in sewage sludge because it is a complex matrix containing a high amount of water.

At the same time, sewage sludge contains particulate material and organic substances, which can act as cavitation nuclei but can also complicate the diffusion of ultrasound waves through the medium.

The removal of LAS, and other organic pollutants, from sewage sludge (i.e. decontamination of sludge) assumes considerable importance since reuse for agricultural purpose plays a relevant role in sludge management. The 3rd Draft of the Working Document on Sludge proposes 2600 mgLAS/kg dw as LAS concentration limit in sludge for agricultural utilization.

4.3.3 Materials and methods

4.3.3.1 Materials and reagents

Reagent grade sodium dodecylbenzene sulfonate (mixture of C10-C14 linear alkyl chain LAS) and sodium 4-n- octylbenzene sulfonate were purchased from Tokyo Kasei Industry LDT and used without further purification. All surfactants solutions were prepared in HPLC gradient grade methanol (VWR PROLABO). Hplc mobile phase was prepared with LC gradient grade acetonitrile (Merck LiChrosolv), ammonium acetate (VWR PROLABO GPR RECTAPUR) and distilled water. Secondary sludge was sampled from recycle stream before thickener at Roma Nord wastewater treatment plant (Fig.) and gravity thickened up to a TS concentration of 19-25 g/L. This treatment plant serves about 700,000 P.E. and is operated with a quite high sludge age (20 d) due to the unusual dilution of incoming sewage (COD average value of 200 mg/L).

4.3.3.2 Sonochemical experiments

Each sonication experiment was performed for 10 and 40 min on 300 ml of waste activated sludge. The ultrasound apparatus (Fig. 66) is constituted of an amplifier T&C Power Conversion and a sonoreactor Elac Nautik USW 51-051, operating at 200 kHz and at an average power of 90-100 W. The transducer is made up of a piezo-ceramic element with an active area of approx. 25 cm^2. The glass sonication cell is covered and water jacketed for cooling sonicated sludge mixture.

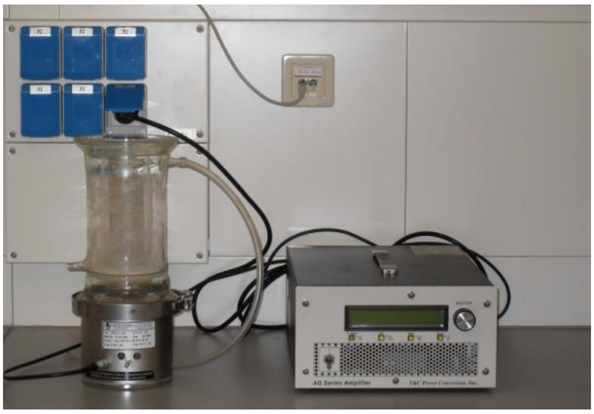


Fig. 66. US reactor and generator (200 kHz)

4.3.3.3 Analytical method

The experimental procedure of LAS extraction from sludge is based on the optimised extraction method of the Horizontal Project (Horizontal, 13.0, April 2006).

Untreated and sonicated sludge were dried in oven at 80°C for 48 h,; 2-2,5 g of dried sludge is pulverized and mixed with 10 ml of methanol and 100 µl of C8-LAS (internal standard); the mixture is shaken at 320 strokes for 35 minutes and the methanolic extract is passed on a filter paper and injected to HPLC-MS. The separation of the four different LAS homologues was performed with a Dionex HPLC equipped with a low-pressure gradient pump with integrated vacuum degasser, autosampler and an Acclaim Surfactant (Dionex) column 5 µm 120 Å (2.1 x 150 mm).

The isocratic mobile phase was acetonitrile/100 mM ammonium acetate (65:35 v/v) flowing at 0.2 ml min^{-1}.

LAS determination was obtained with a single quadrupole mass detector PE Sciex API 165 with a Turbo IonSpray source, operating in full scan acquisition for surfactants identification and in SIM mode for LAS quantification.

4.3.4 Results and discussion

4.3.4.1 Analytical method

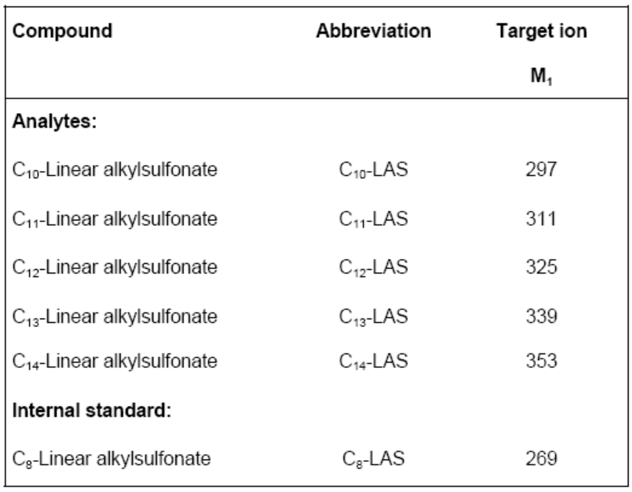
The analytical method for LAS extraction from sludge and quantification through HPLC-MS analyses is based on the procedure developed in the framework of the project “Horizontal” (Horizontal 13.0, April 2006).

Sample pre-treatment and LAS extraction:

Conventional drying at 60°C is one of the recommended sludge samples pre-treatment. As regards to LAS extraction from sludge, comparing shaking, reflux and soxhlet, the shaking technique was preferred and methanol was chosen as extraction solvent.

HPLC-MS analysis:

The identification of LAS homologues is based on the retention time of the homologues and the isomers of each homologue. Another point of identification is the pattern/fingerprint of the homologues. If a selective mass detector is used, target ions must be used for the analysis (Fig. 67).



Compound	Abbreviation	Target ion M_1
Analytes:		
C_{10}-Linear alkylsulfonate	C_{10}-LAS	297
C_{11}-Linear alkylsulfonate	C_{11}-LAS	311
C_{12}-Linear alkylsulfonate	C_{12}-LAS	325
C_{13}-Linear alkylsulfonate	C_{13}-LAS	339
C_{14}-Linear alkylsulfonate	C_{14}-LAS	353
Internal standard:		
C_8-Linear alkylsulfonate	C_8-LAS	269

Fig. 67 LAS target ion for HPLC-MS analysis

For LAS the area is determined as the sum of the peak areas of mixtures of homologues. The 4-octylbenzenesulphonate (C8-LAS) is added to the sludge samples before extraction. C8-LAS is used as internal standard to correct the losses during the analytical procedure and to calculate the concentration of the analytes. The internal standard method determines the mass concentrations and is not influenced by injection errors, the volume of water present in the sample and the matrix effect. Spike-recovery experiments of internal standard are from 90 to 98%. Six-point calibration curves (Fig. 68) were generated for each LAS homologues by injecting pooled solutions prepared from the standard mixtures. The linearity range for the LAS mixtures is 0.1-100 mg/L (as total LAS). The repeatability of the instrumental method was tested by running ten times LAS mixtures standards at two concentration levels; the variance was 4.8 and 5.3 %, respectively.

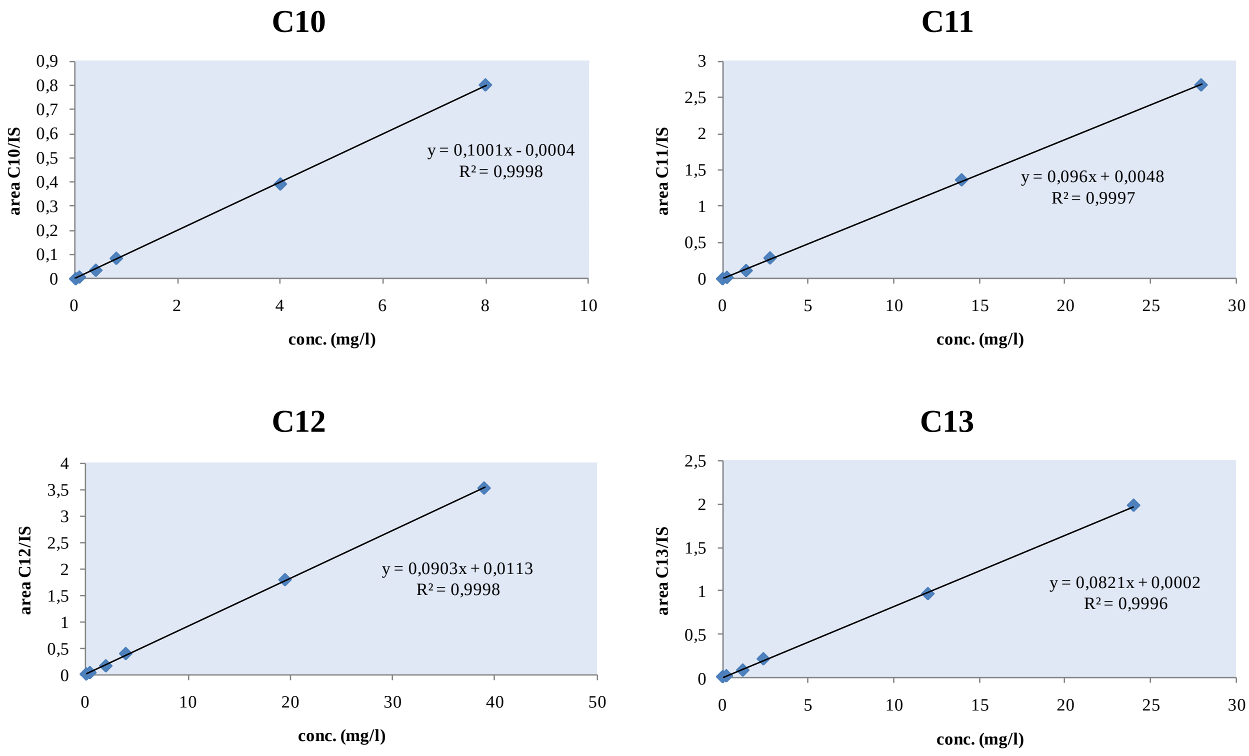


Fig. 68 Calibration curves of the four LAS homologues

Quantitative analysis was done by calculating the ratios between the peak area of each homologue and the peak area of the internal standard. The same procedure was used to plot calibration curves from the standard solutions.

Fig. 69 shows the HPLC-MS chromatograms for the untreated and sonicated sludge: a noticeable decrement of the LAS homologues peak areas can be observed for the sonicated sample (after 40 min) as compared with the peak area of the internal standard.

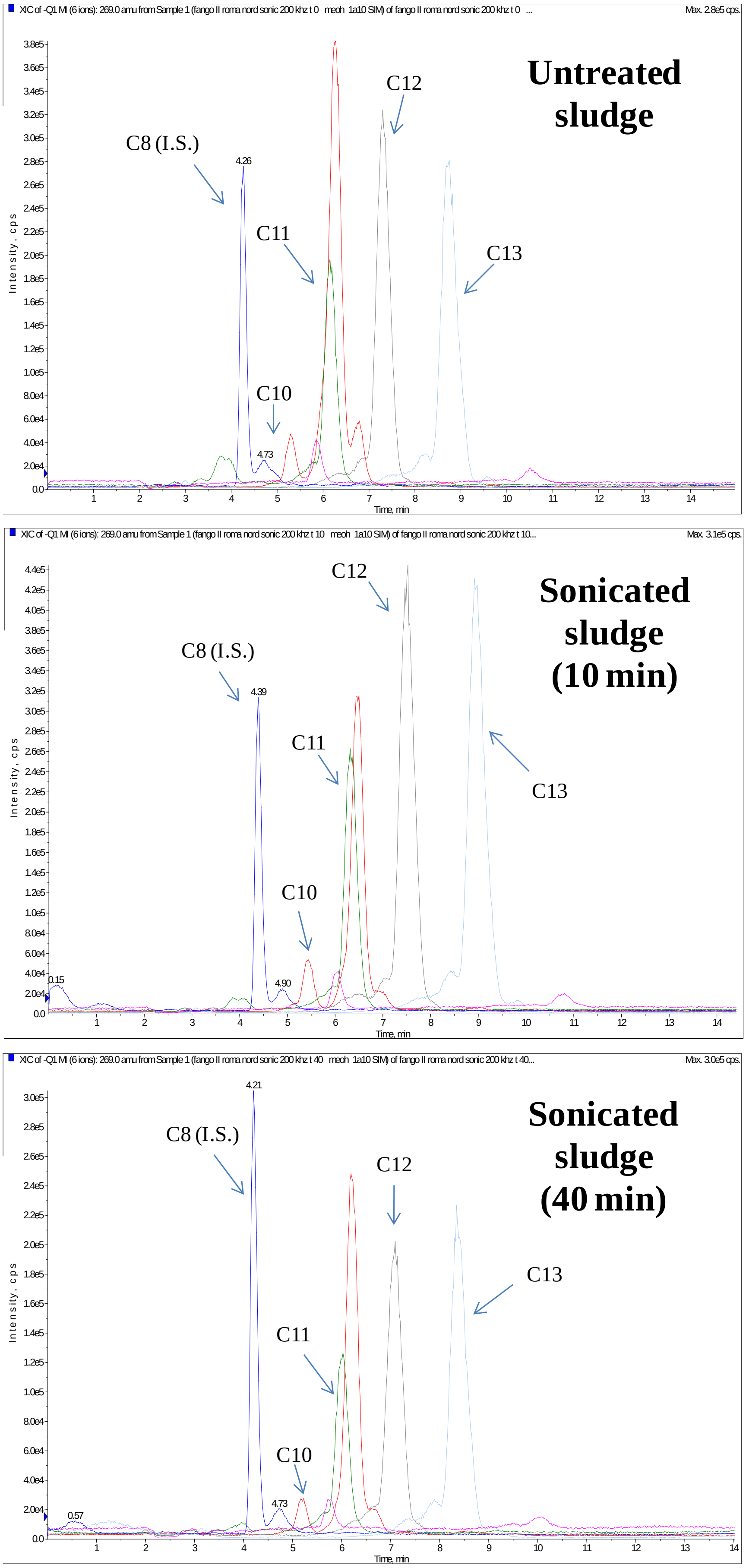


Fig. 69 HPLC-MS chromatograms of the untreated and sonicated sludge

The developed methods provide good linear responses for each LAS homologue in a wide dynamic range under isocratic conditions. With the injection volume of 25 µL, the LOD was estimated to be approximately 1 ppm (S/N = 3), and LOQ approximately 3 ppm (S/N = 10).

4.3.4.2 LAS degradation

Sonolysis experiments were performed on sludge samples from Roma Nord wastewater treatment plant at two different frequencies, 200 and 600 kHz. No LAS degradation was observed with ultrasound at 600 kHz (Fig. 70); on the contrary, the removal efficiency of sonolysis at 200 kHz is presented and discussed in this section.

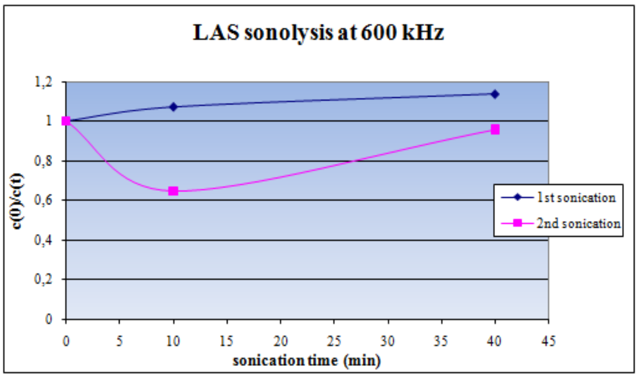


Fig. 70. LAS degradation pattern with ultrasound irradiation at 600 kHz

To understand all the parameters involved in surfactants sonolysis, six experiments were performed on real sludge samples monitoring the concentration of the different LAS homologues at different sonication time. The operating conditions of these experiments and the characteristics of the sludge samples are reported in Table 50. It’s important to note that sludge samples are quite different particularly in terms of total solids content and of the percentage of organic substance (see ratio VS/TS). This variability may be attributed to the fact that sludge sampling was performed in different periods of the year, when often influent wastewater and plant operations are not reproducible. Nevertheless, the variability permits to test the efficiency of the ultrasonic treatment on real sludge samples.

Table 50 Sluge characteristics and operating conditions of the six experiments

	SONICATION EXPERIMENTS					
	1	***2***	***3***	***4***	***5***	***6***
TS (g/l)	24.1	25.4	23.3	19.1	21.3	22.1
VS (g/l)	13.2	14.0	12.8	10.5	13.9	12.5
COD_s (mg/l)	255	331	251	490	50	41
COD_t (mg/l)	19800	21000	19500	15750	21000	18750
$^*LP_{son}$ (W)	85-100	85-110	130-134	91-100	64-71	73-90
E_{spec} (kJ/kgTS)	31040	28740	45217	42105	19541	23894

*= Load Power sonoreactor

The LAS (sum of the 4 homologues) concentrations at t = 0, 10 min and 40 min of ultrasonic treatment and the degradation percentage obtained in the sonolysis experiments are reported in Table 51. The experiments have been carried out by sonicating the secondary sludge at low energy input, to render the process more sustainable. The initial LAS concentration of the untreated sludge is quite variable, ranging from 1,2 to 1,3 mg/L_{sludge} for experiments 3, 4, 5 and 6; in the two first experiments LAS content is higher, 5,9 and 3,9 mg/L_{sludge} for experiment 1 and 2, respectively.

Table 51. LAS concentration (mg/$L_{sludge)}$ and LAS degradation (%) in the six experiments

Sonication experiments	**LAS concentration (mgLAS/L_{sludge})**			**LAS degradation (%)**
	t = 0	***t = 10 min***	***t = 40 min***	
1	5,9	8	3,8	36
2	3,9	4,2	3,3	17
3	1,3	1,1	0,8	42
4	1,3	1,3	1,1	17
5	1,3	0,8	0,9	31
6	1,2	n.d.	0,9	26

Literature on sonolysis of aqueous solution of LAS pointed out that degradation increases proportionally by increasing LAS initial concentration (Ashokkumar, 2003; Lifka et al., 2003; Abu-Hassan 2007). On the contrary in the case of LAS sonolysis directly on sludge it is impossible to determine a linear relationship between LAS degradation and initial concentration because particularly solid particles may affect the efficiency of the ultrasounds. In fact, sound attenuation due to solid particles acts to dissipate sound waves resulting in decreased ultrasonic intensity while additional active cavitation bubbles may improve sonodegradation compared to a system in the absence of particles. Moreover, solid particles affect the mass transfer of the surfactants in solution. It is important to note that just after 10 minutes of sonication a slight decrease in LAS concentration was observed for the experiments 3, 4 and 5. On the contrary, for the experiments 1 and 2, where both the initial concentration of surfactants and the solid/liquid ratio were noticeably higher, LAS concentration increased suggesting an additional LAS desorption from sludge. For hydrophobic organic carbons like linear alkylbenezene sulfonates the rate of desorption is often the rate limiting step since they are increasingly sequestrated with residence time in sludge or in soil. Any improvement in the penetration of solvent into particulate matter by means of sonication will result in the enhanced removal of soluble material that may be trapped inside the solid particles (Mason, 2004). Evidently the chemical extraction method by shaking 35 minutes is a “nonexhaustive” extraction method (Kelsey, 1997) and removes the rapid and part of slow desorbing fraction, whereas the ultrasound “leaching” may offer better recovery of the contaminant. This may result from the action of shockwaves and microjets generated during the collapse of cavitation bubbles on or in close vicinity to the sludge particles leading to enhanced link breaking between the surfactants and the biomass surface. In heterogeneous sonochemistry, where solid particles may exist in close vicinity to cavitation bubbles, the collapse of bubbles may occur asymmetrically depending on the size of the solids compared to the cavitation bubbles. Thus, the largest sludge particles are expected to result in the most contaminant desorption. However, degradation of contaminants following desorption would be expected to be highest with smaller particle sizes and particles with larger liquid– solid surface areas as they provide the most cavitation nuclei reducing the detrimental effects of scattering and attenuation of sound waves (Lu, 2002). In fact, by increasing sonication time LAS degradation occurs suggesting the formation of OH· radicals. As the sludge particle size decreases during sonication, more OH· production is in fact expected due to decreased asymmetric bubble collapses. The involvement of mainly OH· radicals during sonication of surfactant has been proved in literature.

Total LAS concentration in untreated and sonicated sludge (sonication time: 40 min) for the six experiments are shown in Fig. 71 . LAS content is expressed as sum of the four LAS homologues C10, C11, C12 and C13.

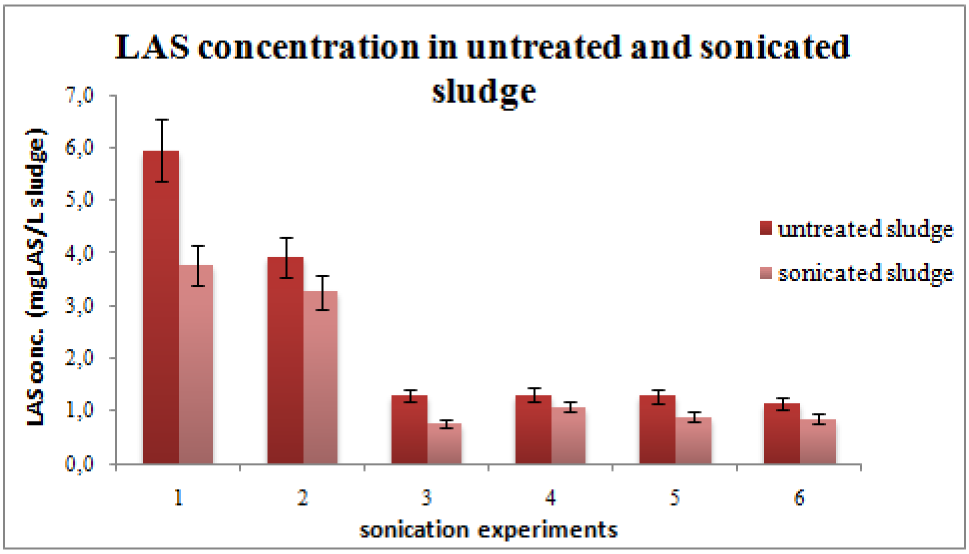


Fig. 71. LAS concentration in the untreated and sonicated sludge

LAS removal % varies from 17 to 42% after 40 minutes of sonication applying energy inputs in the range 20000-45000 kJ/kg TS. Energy and total solids are important parameters as regards ultrasound efficiency on sludge. The best removal rates have been obtained in the case of high energy and high solids content (experiments # 1 and 3) but it results clear from these data that the presence of sludge of particles may have competitive effects: the solid–liquid interface adds additional nucleation sites but also results in scattering and attenuation of sound. However, the results reported in Table 51 clearly demonstrate that LAS removal of almost 40% can be achieved with the application of ultrasound at 200 kHz to sewage sludge, in less than 1 hour and without addition of any chemical reagents, by optimizing energy and solids content.

To confirm the influence of the total solids content and the specific energy on LAS degradation efficiency, a principal component analysis (PCA) was performed (Fig. 72) with the available data from the six sonication experiments (s1.1., s1.2,..).
The variables used were: IC=initial LAS concentration; TS= total solids content; SE= specific energy; LP= sonoreactor load power, LD= LAS degradation.

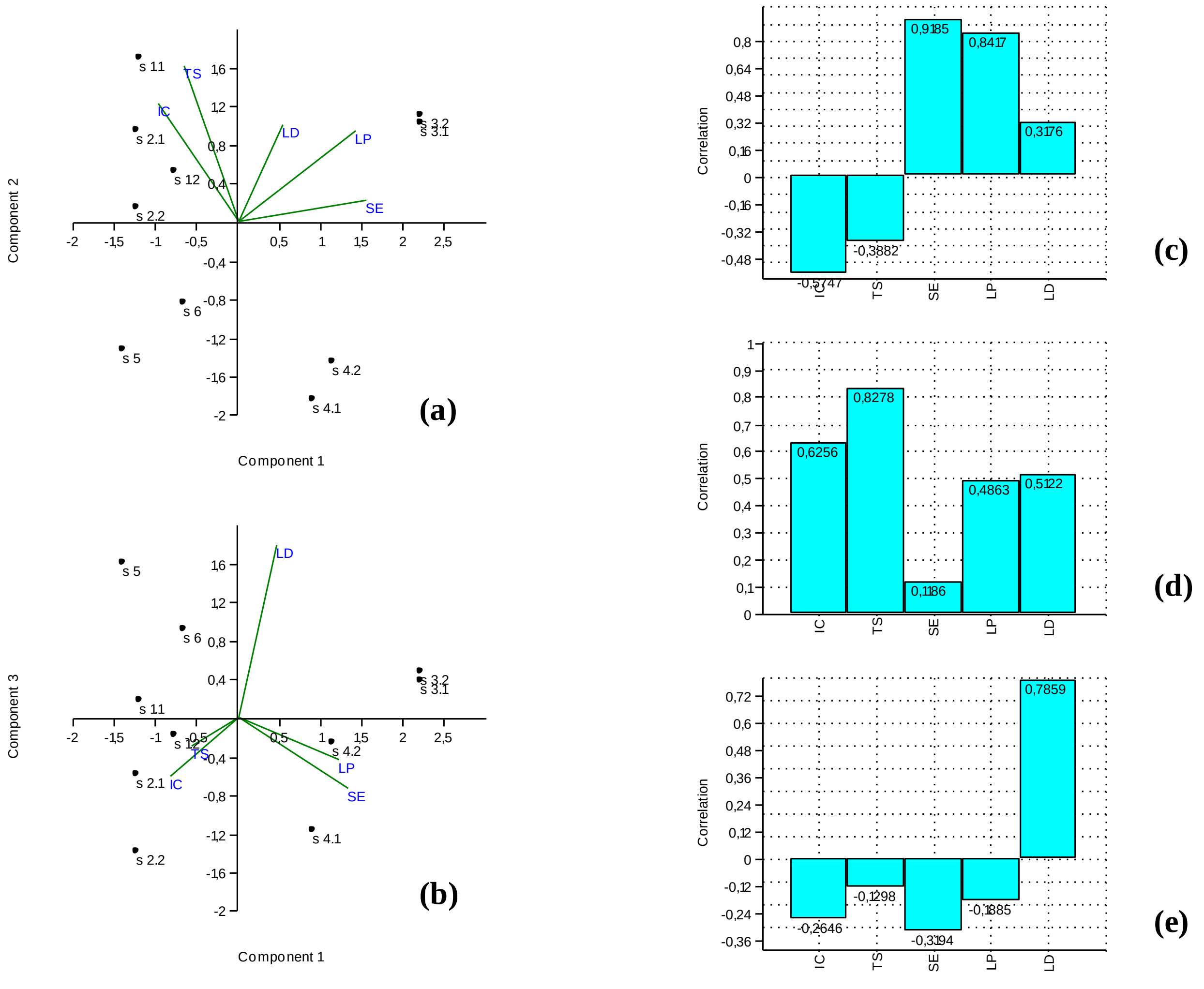


Fig. 72. PCA analyses

Table 52 reports the eigenvalues and the percentage of variance for the first three components.

Table 52. eigenvalues and the percentage of variance for the first three components

	eigenvalue	% variance
PC1	2.13408	42.682
PC2	1.58941	31.788
PC3	0.842008	16.84

Fig. 72 (a) shows the distribution of the variables in the space formed by the first two components, which together explained 74.5 % of the total variation.

This analysis suggests the presence of two different effects respectively owing to the physical chemical sludge characteristics (total solids content and LAS initial concentration) and the instrumental setup (load power of the sonoreactor and the specific energy supplied to the sludge sample).

LAS degradation is more affected by the instrumental setup rather than by the sludge chemical features: it results to be positive when instrumental parameters are positive (Fig 72 (c)).

The shift along component 1 is mainly due to high values of specific energy rather than high LAS concentration or total solids content; on component 2 the observations shift is due to the chemical sludge characteristics and LAS degradation is the most important variable for component 3, which accounts for 16.8% of the total variation (Fig. 72 (e)).

The highest LAS removal efficiency of the experiment 3 (42%) is directly related to the specific energy delivered to the sludge sample. On the contrary, high initial LAS concentration and high total solids content account for the LAS degradation efficiencies obtained in experiment 1 and 2 (36 and 17 %, respectively).

Low LAS degradation in experiment 4 (17%) is explained by Fig. 72 (b): low sludge quality reduces the surfactants percentage removal, despite of the high energy supplied to the sludge sample.

It must be pointed out that in the case of sonolysis in complex media like sludge, various radical scavengers are present, and OH· may attack indiscriminately organic matter. It is very difficult to analyze the whole oxidation sequences in the present complex system. However, a competition of OH· in reaction with the solutes and particles exists, and from literature regarding for example ozone (Cesbron, 2003), it seems that the oxidant may react first with the soluble fraction of the sludge present in the bulk solution. Probably in the case of ultrasound application, contaminants adsorbed on sludge flocs are firstly released in solution due to sludge disintegration (see chapter 4.1) and and no removal occurs while after 40 minutes of sonication up to 40% of LAS is degraded due to the formation of OH· from water lysis.

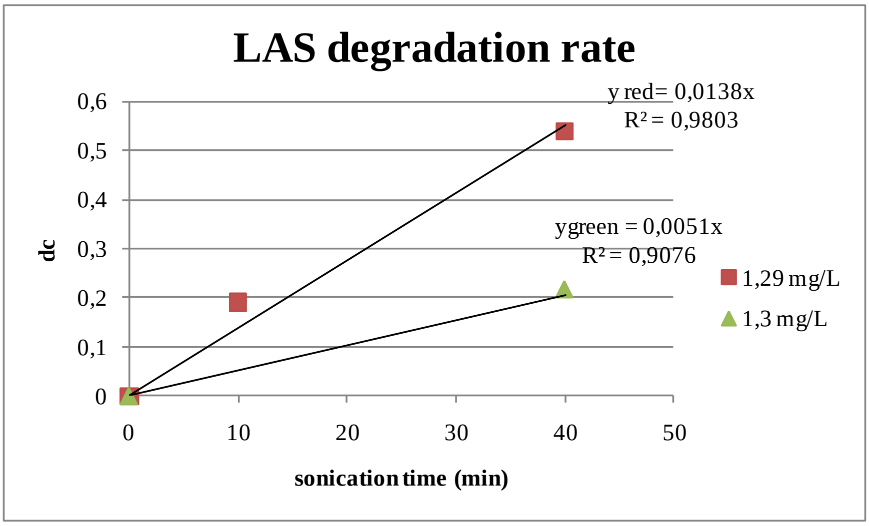


Fig. 73 LAS degradation rate

Only for two sonication experiments (experiments # 3 and 4), a linear correlation between LAS concentration variations and sonication time was observed (Fig. 73). The slope of the linear curves in figure represents LAS degradation rate.

It's important to note that LAS is not a single compound but a mixture of similar compounds.

Very interesting was the effect of of ultrasound on the degradation of the different LAS homologues, characterized by different alkyl chains. The removal profile of each LAS homologues is shown in Fig. 74: the degradation efficiency depends mainly on the type of LAS, in particular on the alkyl chain length. LAS-C10 is the homologue which can be faster and more easily removed. Based on previous studies reported in literature for sonolysis in water, LAS accumulate at the liquid-bubble interface and their degradation pathway is thermolysis at the bubble surfaces and reaction with OH· radicals either at the bubble surface or in bulk solution (Sostaric, 2001; Yang 2006). Because of the much higher concentration of hydroxyl radicals at the cavitation bubble surface than in the bulk solution, the degradation rates of LAS depend on their ability to partition at the bubble surface. Sostaric (Sostaric, 2001) demonstrated that the radical scavenging efficiency decreases with increasing alkyl chain length at plateau concentrations, suggesting that surfactants which are more surface active need more time to accumulate and equilibrate at the bubble surface. LAS-C10 is less surface active than the other longer homologues, and its degradation rate results faster due to its faster transfer rate to the cavitation bubble interface. Moreover, the existence of a linear relationship between surfactants CMCs and their maximum degradation rate has been demonstrated by Nanzai (Nanzai, 2009). For longer alkyl chain surfactants, the micelle formation occurs at lower surfactants concentration, i.e. CMCs values are estimated to be 1.0 mM (C12) < 7.1 mM (C9) < 13.8 mM (C8). The global effect of the micelle formation in the interfacial region of the cavitation bubble is to reduce the cavitation efficiency.

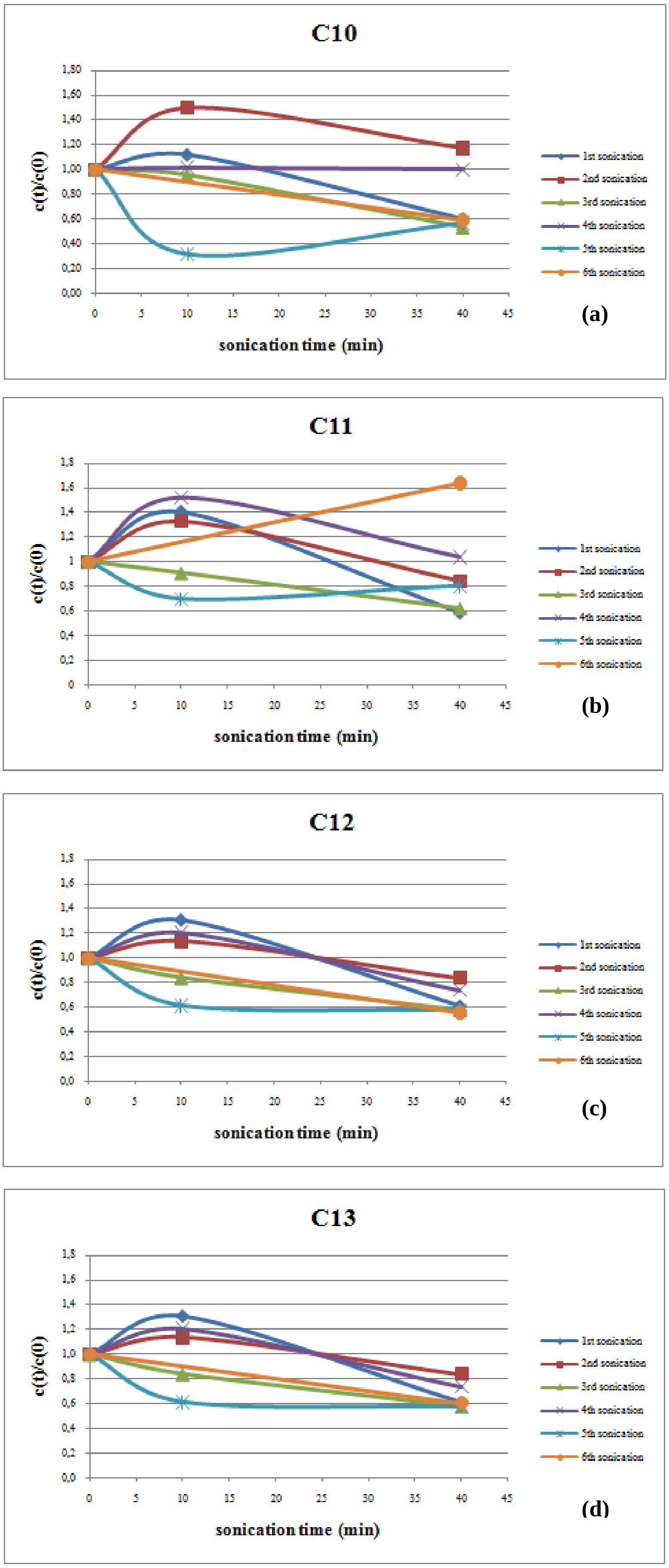


Fig. 74 LAS homologues degradation pattern

An increased concentration of all LAS homologues can be observed at t = 10 min for some sonication experiments. As just mentioned after 10 min of ultrasonic treatment, the formation of OH· radicals is negligible and an additional extraction of contaminants from sludge (Mason, 2004) may occur. The contemporary presence of various surfactants homologues in the same mixture affects the degradation rate of each component because of the competition for reaction sites at the cavitation bubble surfaces, creating an excess of more surface active homologues at the cavitation interface (Yang, 2006). Yang demonstrated that the surface tension of a binary mixture of LAS-C8 and LAS-C12 in equal molar ratio is lower than the surface tension of LAS-C8 alone but higher than the surface tension of LAS-C12 alone. Therefore, this higher surface tension of the mixture may be responsible for the slower degradation rate of LAS longer homologues in mixture rather than in single homologue solutions (Yang, 2006).

In addition, the adsorption of surfactants to bubble surfaces in mixture at concentrations below their CMCs might be slower than in single surfactant solutions (Lu, 2000). In summary, many effects like reactivity of each homologue with OH radicals, surface excess and surface tension may play a combined role in the degradation of surfactants mixture.

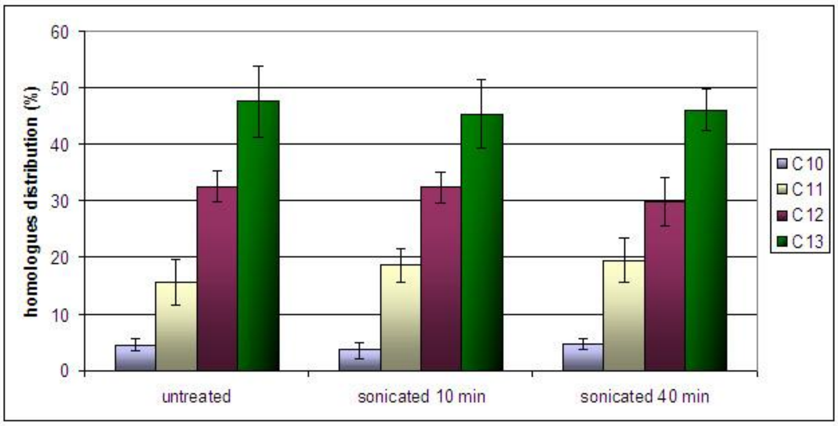


Fig. 75 Average percentage of the different homologues for untreated and sonicated samples (experiment # 5 and 6)

The average percentage of the different homologues for untreated and sonicated samples calculated for 5 of the 6 sonication experiments is reported in Fig. 75. No significant changes were observed as regards the LAS homologues distribution before and after sonication treatment. This result is confirmed also in literature where is reported that mineralization rates showed overall little variation among different chain length homologues and structural isomers (Larson, 1990). The most abundant LAS homologue in the sludge, either untreated or sonicated, resulted always the most hydrophobic one, i.e. the C13, confirming that the sorptive properties of LAS increased with increasing alkyl chain length. The sludge used for this experimentation is biological secondary sludge, aerobically "stabilized", and present a LAS homologue distribution of C10:C11:C12:C13=5:16:32:48, whereas the typical distribution for an anaerobically digested slugde is C10:C11:C12:C13=7:24:39:30 (Berna, 1989). Average linear alkyl chain carbon number was Ci=12.2, constant for untreated, and sonicated sludge, while in the case of anaerobically digested sludge the value reported in literature is Ci=11.9 . Secondary sludge is richer in LAS-C13 with respect to the digested sludge, and this may be attributed to the fact that during the anaerobic process the long alkyl chain homologue is better degraded and removed by anaerobic bacteria (see chapter 4.2)..

4.3.5 Conclusions

For the first time the application of ultrasound treatment to real sewage sludge samples was tested to determine the efficacy of linear alkylbenzene sulphonates removal. LAS degradation of almost 42% is obtained with ultrasonic treatment in less than 1 hour without addition of any chemicals.

Due to the complexity of the matrix, evaluating the effects of different parameters on the sonochemical reactions is not univocal. The specific energy delivered to the system and the sludge total solids content seem to greatly influence the ultrasound efficacy and thus LAS degradation.

Each LAS homologue presents a different degradation profile, mainly due to its physical-chemical properties, in particular to the alkyl chain length: LAS-C10 is the homologue which can be faster and more easily removed.

Also the contemporary presence of the four LAS homologues in the same sample must be considered, because of the competition for the reaction sites at the cavitation bubbles interface.

The results obtained through this research work can be further enhanced through the study of the by-products formation by chromatographic techniques, with the scope to understand the removal mechanisms. In addition, toxicity bioassays can help in the evaluation of the potential impact that these by-products may exhibit.

4.3.6 References

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5. CONCLUSIONS AND RECOMMANDATIONS FOR FUTURE WORKS

Sewage treatment plants generate quite a large amount of sludge and management of the produced sludge represents 50 % of the total costs of the plant. Because of increased environmental awareness and stringent environmental standards governing the disposal of sewage sludge, its utilization in agricultural production has been gaining increasing interest and attention in recent years. Although rich in nutrients, organic substance and microelements, in some countries sewage sludge is not accepted as agricultural fertilizer due to the awareness regarding potential health risks derived from the accumulation of heavy metals and organic compounds, as well as pathogen contamination.

Primary and secondary sludge present different characteristics. In chapter 3 the results of a monitoring campaign on three WW treatment plants in the Rome area (Co.Bis. Cesano, Roma Nord and Fregene) were presented. The monitoring campaign was performed with the aim to assess the pollution load and the nutrient contents of primary and secondary sludge. The results show that secondary sludge was richer in nitrogen and phosphorus than primary sludge. For secondary sludge the typical range of nitrogen and phosphorus concentration was 5-7% and 1.5-3% of TS. As regards heavy metals contents, negligible difference was observed between primary and secondary sludge. Extractable organic halogens (EOX), total hydrocarbons and methyl blue active substances (MBAS) were analyzed to assess sludge contamination by organic micropollutants. The concentration of these organic pollutants was always higher in primary sludge with respect to secondary one and enrichment of the above contaminants in primary sludge seemed to increase in the order hydrocarbons, EOX, MBAS. These results support the idea that separation of primary and secondary sludge could lead to more sustainable sludge management. Such an innovative concept is based on the fact that secondary sludge, poorer in pollutants than primary sludge, should be segregated and treated separately from primary sludge to increase chances of secondary sludge utilisation in agriculture, while primary sludge can be used for energy recovery and for the final incineration

To be used as agricultural fertilizer, secondary sludge must be decontaminated by organic micropollutants and stabilized, aerobically or anaerobically.

Sonolysis experiments at 200 kHz were performed directly on real sludge samples to assess the efficacy of the ultrasound treatment on the degradation of linear alkylbenzene sulphonates (LAS), one of the most abundant classes of organic micropollutants.

LAS degradation of about 42 % can be obtained in less than 1 h of ultrasonic treatment and without adding any chemicals. Evaluating the effects of the different parameters affecting the sonochemical reactions was complicated, because of the high complexity of the sludge matrix. PCA analysis on the obtained results confirmed that the specific energy delivered to the system and the sludge total solids content play the most important role in determining the ultrasound efficacy and thus LAS degradation.

Each LAS homologue presented a different degradation pattern, mainly due to its physical-chemical properties, in particular to the alkyl chain length: LAS-C10 is the homologue which can be faster and more easily removed.

Also the contemporary presence of the four LAS homologues in the same sample must taken into account, since competition for the reaction sites at the cavitation bubbles interface can occur.

Ultrasound treatment is widely applied also as sludge anaerobic digestion pretreatment, using low frequency as 20 kHz. In fact,anaerobic digestion, considered the principal process to stabilize sewage sludge, require generally sludge pretreatment to improve sludge disintegration and particulate hydrolysis, which is the rate-limiting step of the digestion process. Sonication experiments both at 20 and 200 kHz were carried out on sludge to evaluate the disintegration efficiency obtained at different frequencies.

Ultrasound treatment at 20 kHz was found to be more effective in sludge disintegration, in terms of solubilization of organic matter and flocs destruction. This is due to larger cavitation bubbles and high hydrodynamic shear forces which were generated at lower frequencies. Nevertheless, treatment with 200 kHz gave also satisfactory and encouraging disintegration results: for specific energy input > 30’000 kJ/kgTS, ultrasound pretreatment at 200 kHz permit surprisingly to reach higher disintegration degrees with respect to the low frequency sonication. The maximum disintegration degree obtained at 200 kHz was 65%, indicating possible cell lyses besides the release of extracellular organic matter.

The disrupting effect of sound waves at 200 kHz was also confirmed by the increase of the proteic COD and surface charge density in sludge after the ultrasonic treatment.

Microscope analyses on 200 kHz pretreated sludge sample showed that, after 40 min of sonication, the floc integrity is almost completely broken and only dead microorganisms were observed in the surrounding liquid phase. The same extent of flocs destruction was obtained with the ultrasound treatment at 20 kHz, but with much lower energy input.

After assessing the efficacy of ultrasound treatment both at 20 and 200 kHz for sludge disintegration, batch anaerobic tests were performed to evaluate anaerobic biodegradability of sonicated sludge with the "classical" and "new" sludge pretreatment. Also the effect of food/inoculum ratio on the digestion performances was assessed .

Ultrasound treatment, both at 20 and 200 kHz, showed to accelerate the initial hydrolysis phase. This was highlighted by the considerable increase of protein content, soluble COD and surface charge in the first hours of the digestion process indicating particulate degradation in small fines and substances release. Afterwards, the organic matter was rapidly removed till the end of the digestion; in particular, the removal of the high soluble COD content in the reactors with sonicated sludge was so intensive to reach comparable values as those obtained with anaerobic digestion of untreated sludge.

The initial accumulationof the soluble COD during untreated sludge digestion was higher if the inoculum content was comparable with the substrate one . Similar soluble COD trend was observed for the 200kHz sonicated samples.

In the case of the 20 kHz sonication the removal of the soluble COD at the end of the digestion was 73% working at F/I=0.5 (while for 200 kHz was 53%) and 57% at F/I=1 (33% for 200 kHz), in spite of a slight lower disintegration degree of the 20 kHz sonicated samples.

Surprisingly, the digestion of the 200 kHz pretreated sludge showed an higher improvement in volatile solids removal efficiency (i.e. the increment of the VS removal of the sonicated sludge with respect to the untreated control) than sludge pretreated at 20 kHz. VS degradation efficiency was found to be strictly dependent on the applied F/I: by digesting sonicated sludge, both at 20 or 200 kHz, it is not convenient to operate with high content of inoculum.

Ultrasound pretreatment enhanced biogas production both in the case of 20 and 200 kHz treatment: the biogas produced by the sonicated sludge was always higher than that produced by the untreated one. This gain seemed to be independent on the F/I ratio for the digestion of the 20 kHz pretreated sludge; while, in the case of sludge treatment at 200 kHz, the biogas improvement was very high (about 50%) at F/I=0.5, whereas at F/I=1 was comparable with the biogas gained pretreating the sludge at 20 kHz.

During the digestion of the 200 kHz pretreated sludge, also the fate of linear alkylbenzene sulphonates was monitoring during the process. The digestions of the untreated sludge showed higher LAS removal with respect to sonicated sludge in the case of F/I=0.5 (26% for the untreated sludge and 8% for the sonicated one), whereas for F/I=1 the removal efficiency was comparable (46 and 41% for the untreated and sonicated sludge, respectively). As LAS concentrations in the inoculums were much higher than those in the secondary sludge , the extent of LAS degradation was function of the percentage of inoculum in the reactor: during the low inoculum test (F/I = 1) high surfactant removal (40-46%) both for untreated and ultrasound pretreated sludge was observed (while the LAS degradation due to the sole inoculum was 23%). In the case of F/I=0.5, on the contrary, all the removal was ascribable to the LAS of the inoculum and no removal of the "substrate-LAS" was observed

LAS-C10, because of its physical chemical properties, was the homologue which was removed more efficiently at the end of the digestion process for both the untreated and sonicated sludge. LAS-C10 removal percentages were in the range 25-58%. F/I ratio had a great influence in determining the degradation pathway of LAS homologues.

Based on all these results, ultrasound treatment at 200 kHz can be considered a valid promising option to the classic treatment at 20 kHz, since, besides the sonolytical properties to remove organic pollutants permitted to obtain, satisfactory anaerobic digestion performances, as regards solubilisation and removal of organic substances and biogas production. Operating sludge digestion with a food/inoculum ratio like 1 permitted seemed in batch the ideal ratio to increase the stabilization of the sludge (higher VS removals), and at the same time, to decontaminate the sludge from organic pollutants (high LAS removals), but the convenience from the energetical point of view was low because of the scarce biogas productions. These results will be useful to optimize the future semicontinuous digestion experiments in order to find the best operating parameters to propose this new technological approach for sustainable sludge management.

Recommendations for Future Work

The development of agricultural recycling depends largely on the possibilities to improve the quality of sludge itself and increase trust in sludge quality. This requires therefore the pollution prevention at source by reducing the possibilities for heavy metals and organic compounds to enter the wastewater sewage system, improving sludge treatments as well as ensuring the monitoring of sludge quality. However, these technical solutions (as ultrasounds presented in this study) require major investment from the water companies and/or local authorities in charge of treating the wastewater.

The current state of the debate on sludge recycling and disposal routes clearly shows that the current uncertainties over possible risks for human health and for the environment play a major part in the resistance against expanding various sludge recycling routes. The areas where scientific results are the most expected by the stakeholders are possible effects of organic pollutants and pathogens in sludge. Research and communication should especially aim to promote high-quality sludge (with low levels of contaminants), which could be recognised as fertilisers (or as a component of fertiliser products) at European level.

In this prospect, future activity will be addressed to optimize new sludge treatments which are aiming at preparing sludge for agricultural utilization by producing a very clean and stabilized sludge with specific concern to organic micropollutants, hygienic aspects and properties that can have impact onsoil and crops.

On the basis of the encouraging results of this work the idea is to combine the beneficial effects of ultrasounds as regards floc disintegration and anaerobic digestion improvement, and the sonolytical removal of micropollutants directly in sludge, by optimizing energy input and output, operating with continuous fed digesters on pilot scale.