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The Potential of Ultrasound Treatment for Sludge Reduction

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PhD Thesis
The Potential of Ultrasound Treatment for Sludge Reduction

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Abstract

The potential of ultrasound treatment for sludge reduction during the activated sludge process was assessed. Batch and dynamic disintegration studies were completed using an activated sludge pilot-scale plant fed with settled sewage that was comprised of two 1.2 m³ lanes, operated as a test and control. A 1 kW ultrasound system was integrated into the test lane of the pilot-scale plant to allow continuous in-line ultrasound treatment of the return activated sludge (RAS). Seven dynamic trials were completed over an 8-month period, treating from 1.7 to 12.5% of the RAS with energy densities between 42 and 168 kJ L⁻¹.

During the batch disintegration studies, it was observed that ultrasound treatment at 42 to 186 kJ L⁻¹ caused floc breakage and sludge solubilisation. Floc size was reduced by 88% at 42 kJ L⁻¹ while the degree of soluble COD release increased almost linearly from 11 to 36% between 42 to 168 kJ L⁻¹. A change in the biological activity was observed only at 168 kJ L⁻¹ with an 8.5% increase in the specific oxygen consumption of the treated RAS samples in comparison to the untreated ones.

During the dynamic studies, a 20% degree of sludge reduction was observed treating 10% of the RAS at 42 kJ L⁻¹. At these operational conditions, there was no significant difference in the total COD and nitrogen removal between the control and test lanes. However, a 5.5-fold increase in the capillary suction time and a 3.6-fold increase in the specific resistance to filtration in the RAS from the test lane indicated a detrimental impact on dewaterability. Increasing the energy input, by treating 12.5% of the RAS at 84 kJ L⁻¹, did not result in a significant increase in sludge reduction, which indicates that there might be limits to the degree of reduction achievable with the ultrasound treatment.

Results from the dynamic studies suggested that lysis-cryptic growth was the main mechanism behind the observed sludge reduction. Based on modelling, lysis-cryptic growth could justify 98% of the sludge reduction observed. Cost analysis results indicated that sludge reduction by ultrasound disintegration was not currently economically viable unless the cost for sludge treatment and reuse increased to £961 from the reference price of £423 per tonne of dry solids.
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To the memory of my father
Consider ye the seed from which ye sprang;
Ye were not made to live like unto brutes,
But for pursuit of virtue and of knowledge.

Dante Alighieri (1265-1321)
The Divine Comedy (Inferno, Canto XXVI 118-120)
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Abbreviations and notations

Abbreviations

ASP  Activated sludge process
ASPS  Advanced sonic processing systems
BOD  Biochemical oxygen demand
CAS  Conventional activated sludge
COD  Chemical oxygen demand
DD\textsubscript{COD}  Degree of SCOD release or Degree of disintegration
DS  Dried solids
DSVI  Diluted sludge volume index
ML  Mixed liquor
nbVSS  Non-biodegradable volatile suspended solids
OE  Oxygen equivalent
OU  Oxygen uptake
OUR  Oxygen uptake rate
PE  Population equivalent
PSP  Pilot-scale plant
RAS  Return activated sludge
RSP  Reduction in sludge production or Degree of sludge reduction
SAS  Surplus activated sludge
SCOD  Soluble chemical oxygen demand
SQI  Sludge quality index
SSVI  Stirred sludge volume index
SSVI\textsubscript{3.5}  Stirred specific volume index
SVI  Sludge volume index
TSS  Total suspended solids
VSS  Volatile suspended solids
WAS  Waste activated sludge
WWTP  Wastewater treatment plant
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1.1 Project background

1.1.1 The activated sludge process

The activated sludge process (Figure 1-1) is the most common wastewater treatment in Europe and US (Tchobanoglous et al., 2003). It is a biological method of wastewater treatment based on the metabolic activity of a variable and mixed community of microorganisms (i.e. the activated sludge) in an aquatic environment.

![Figure 1-1: The activated sludge process](image)

After primary treatment, the settled wastewater reaches the aeration tank where it mixes with the activated sludge. The combination of wastewater and activated sludge in the aeration tank is called mixed liquor and is kept agitated by mechanical stirrers or by the same air diffused in the aeration tank to sustain aerobic biological processes. In the aeration tank, the microorganisms present in the mixed liquor are able to remove the carbonaceous organic pollution in the wastewater by converting it into...
new biomass and into compounds that contain lower energy, such as carbon dioxide and water. Part of the organic substrate is oxidised during the respiration process using oxygen. The other part of the organic substrate is assimilated into new cellular components necessary during the growth process. Biomass growth is made possible using the energy stored during respiration. The remaining part of this energy is used to satisfy the microbial maintenance requirements.

Microorganisms can also remove ammonia, by converting it into nitrate in aerobic conditions, and nitrogen and phosphorus, using specific plant configurations and operational conditions (Low and Chase, 1999a; Gray, 2004).

After 4 to 8 hours of hydraulic retention time (Tchobanoglous et al., 2003), the influent wastewater has received sufficient treatment in the aeration tank and the mixed liquor flows into the secondary settling tank, also called final settler or clarifier. In the final clarifier, activated sludge flocs are separated by the surrounding wastewater by gravity sedimentation. This separation leads to the formation of a supernatant clear from suspended solids in the upper part of the clarifier and a settled thickened sludge at its bottom. The settled thickened sludge at the bottom of the clarifier is in part recycled back into the aeration tank (the return activated sludge, RAS), while the remaining part must be removed from the system (the waste or surplus activated sludge, WAS or SAS). SAS removal is needed to keep the process in steady and optimal operational conditions, with a constant ratio between the amount of organic substrate and biomass present in the system (Tchobanoglous et al., 2003).

The overall goal of the activated sludge process is to remove substances that have a demand for oxygen and to produce an effluent that can be discharged in the watercourse and surrounding ecosystem without damaging it or causing public health problems (Fytili and Zabaniotou, 2008). Modern wastewater treatment plant can achieve this goal reasonably effectively in less than 24 hours (Spinosa and Vesilind, 2001).

1.1.2 Sludge production

SAS production is an unavoidable by-product of biological wastewater treatment plant because it is generated by the growth of microorganisms that remove the pollution in the aeration tank (Shiota et al., 2002). The ratio of biomass produced to
the amount of substrates removed, i.e. the partition ratio between the organic substrate assimilated during the growth process and oxidised during the respiration process, gives the biomass synthesis yield. For aerobic microorganisms, biomass synthesis yields are very high and vary from 0.4 to 0.8 gram of biomass per gram of biochemical oxygen demand (BOD) removed, with a typical value of 0.6 for conventional activated sludge processes (Tchobanoglous et al., 2003). This means that more than half of the original pollution load in the incoming wastewater is transformed into new biomass (Henze et al., 2002). In addition to viable biomass, the removed SAS contains other organic and inorganic solids, due to either microbial decay or present in the influent wastewater. The ratio between the overall amount of solids and the amount of substrates removed gives the observed yield and can be up to 0.8 gram of total solids per gram of BOD removed (Stensel and Strand, 2004). In Europe, the overall amount of sludge generated from primary, secondary and tertiary treatment is up to 90 grams of dry solids (DS) per capita per day. The progressive implementation of the Urban Waste Water Treatment Directive 91/271/EEC (amended by 98/15/EC) in all member states of the European Community (EC) has increased the overall sludge production from 8 million tonnes of dried solids (DS) in 1998 to more than 10 at the end of 2005 (Fytili and Zabaniotou, 2008).

1.1.3 Sludge treatment and reuse

SAS is a suspension of organisms (Gray, 2004) with less than 1% DS, 70-85% of which is organic matter. It contains both compounds of agricultural value (including organic matter, nitrogen and phosphorus), and pollutants (e.g. heavy metals, pesticides, organic pollutants and pathogens) which can be transmitted to plants, livestock and humans (Spinosa and Vesilind, 2001). Nowadays the emphasis is more on the beneficial reuse of the removed SAS rather than its disposal (Campbell, 2000), thus turning a water pollution control problem into a solid waste reuse problem (Weemaes and Verstraete, 1998). In order to enable a sustainable and environmentally safe reuse, SAS must undergo several post-treatments to reduce its water content, its fermentation propensity and the presence of pathogens. A typical, complete post-treatment chain consists of: thickening; stabilisation; conditioning; dewatering; drying; storage; transportation and final reuse (Fytili and Zabaniotou, 2008). One of
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the most common stabilisation processes is anaerobic digestion; prior to this treatment and after thickening, SAS is generally mixed with primary sludge (Tchobanoglous et al., 2003). Sludge post-treatment costs vary between £225 and more than £900 pounds per tonne DS, depending on local conditions and type of treatment (Ginestet, 2006). As a consequence of this, even if the volume of liquid SAS produced at a wastewater treatment plant (WWTP) usually represents only 1-2% of the total flow of sewage, its treatment can account up to 30-60% of the total running cost of the plant (Canales et al., 1994; CIWEM, 1995; Wei et al., 2003). In Europe, main disposal and recycling routes for SAS are (EC, 2002; Ginestet, 2006; Fytili and Zabaniotou, 2008):

- Landfilling: up to 38% of sludge was still disposed of in landfills until 2005. After 2005, in accordance with the EU Directive 99/31, it is not permitted to deposit organic waste to landfill
- Landspreading: up to 47% of sludge is reused in agriculture as fertiliser. The Sewage Sludge Directive 86/278/EEC by regulating its safe application has also increased its costs. The problem is that some pollutants can be present in the sludge even after treatment, therefore there are growing concerns about their long term impact on the soil and, through the food chain, on human health
- Incineration: up to 17% is reused for energy recovery. It is the most expensive option because it requires intensive dewatering and 30% of the sludge solids remain as ash, which requires landfilling. There is great controversy about incineration due to the potential toxicity of gas emissions and heavy metal content of the remaining ash
- Reuse of sludge in other areas (e.g. forestry, silviculture, land reclamation) is up to 12%, but, again, there are concerns regarding the fate of pollutants present in sludge and their long-term impact of those environments.

1.1.4 Sludge reduction strategies

The current legal constraints, the rising costs and public sensitivity towards the various ways of sewage sludge disposal (i.e. landfilling) and reuse (i.e. landspreading and incineration with energy recovery) have provided considerable impetus to explore and develop strategies and technologies for the minimization of sludge production. Post-treatment processes like anaerobic digestion have been designed to stabilise and
also achieve 30-40 % reduction in dry solids through self-consumption, but their implementation involves high initial capital investment and is considered cost-effective only in WWTPs with capacity above 40,000 population equivalent (PE) (Ginestet, 2006). An ideal way to solve sludge-associated problems is the introduction of new integrated strategies for sludge management based on the reduction of sludge production during the wastewater treatment itself rather than the post-treatment of the sludge produced (Déléris et al., 2002; Ødegaard, 2004). The goal of reducing sludge production must be achieved without affecting the overall efficiency of the activated sludge process. The same quality criteria should be retained for the final effluent (e.g. carbon, nutrients and solids removal), and the residual sludge (e.g. settleability, dewaterability, level of pollutants). The main current strategies for reducing SAS production are based on the implementation of different sludge reduction mechanisms that are able to reduce the observed yield by:

- Reducing the part of biodegradable organic substrate assimilated into new biomass and/or increasing the part mineralised into end products such as carbon dioxide and water, thus decreasing the biomass synthesis yield; or
- Enhancing the biodegradability of organic compounds that would not be otherwise affected by the biological treatment, thus increasing the overall degree of mineralisation by decreasing the amount of slowly biodegradable or recalcitrant organic solids

The partial disintegration of the return activated sludge (RAS) has been proposed as a possible sludge reduction strategy. Sludge disintegration can be achieved using ultrasound or other mechanical, chemical, thermal, biological and hybrid technologies. The application of these technologies involves high capital and operational costs and can have some potential drawbacks on the overall activated sludge process. Therefore, RAS disintegration as a strategy for sludge reduction should be evaluated and chosen for practical application using costs analysis and assessment of environmental impact (Wei et al., 2003).
1.1.5 Ultrasound potential for sludge reduction

Ultrasound treatment can be used for the disintegration of sludge energy-intensive: up to 25% of cell lysis can be achieved with energy densities around 50 kJ L⁻¹ (Bougrier et al., 2005). It is generally considered more energy intensive than other technologies: ~3 and ~2 times more than high pressure homogenisers based on hydrodynamic cavitation (Müller, 2000b) and ozone (Boehler and Siegrist, 2006), respectively. However, ultrasound treatment has already reached an advanced stage of development and optimisation for full-scale application at WWTPs and good operational stability. So far, most of studies have focused on its use to disintegrate SAS as a pre-treatment to enhance anaerobic digestion (Nickel, 1999; Müller, 2000a; Tiehm et al., 2001). Few studies have addressed the potential of ultrasound for sludge reduction. Its underlying mechanisms and impact on process performance are still not clear. The few studies available (Cao et al., 2006; Ginestet, 2006; Strünkmann et al., 2006; Zhang et al., 2007a) have often been performed using lab-scale ultrasound systems, small lab-scale plants fed with synthetic sewage under operational conditions that make it difficult to evaluate their scale-up. Furthermore, they generally provide little or no insight on the role of different reduction mechanisms triggered by ultrasound action. The wide range of sludge reduction detected using ultrasound during these studies, from -28 to 91%, highlights: (1) the risk of increasing sludge production, if the wrong operational conditions are used; (2) the lack of agreement and consistency among studies and (3) the poor information provided to water utilities. As a consequence there is a strong need to assess the potential for sludge reduction of ultrasound technology using more realistic equipment (e.g. pilot-scale plants of reasonable size, ultrasound systems designed for full-scale applications) and methodologies (e.g. real sewage as feed, in-line disintegration, energy densities comparable with those used at full-scale (i.e. ~15 kJ L⁻¹)) to allow:

- A better understanding of the type and role of reduction mechanisms implemented by ultrasound treatment
- A relatively straightforward scale-up of the performance observed at pilot-scale
- A proper economic evaluation to investigate whether sludge reduction by ultrasound disintegration is not only technically feasible, but also economically viable.
Chapter 1: Introduction

1.2 Project development

The Centre for Water Science at Cranfield University has undertaken a research project into the use of ultrasound for optimising the activated sludge process in terms of sludge production and process performance. The project was sponsored by Anglian Water. Initially, during the batch studies, the project compared the performance of three full-scale ultrasound systems with different designs, investigated the best operational conditions during sludge disintegration and evaluated the impact of ultrasound on activated sludge biomass from different types of WWTP. Then, during the dynamic studies, the impact of ultrasound on sludge production and process performance was monitored on a daily basis for a period of 8 months, using an activated sludge pilot-scale plant fed with real settled sewage. The pilot-scale plant was comprised of two 1.2 m³ lanes, operated as a test and control. The RAS in the test lane was exposed to continuous in-line ultrasound disintegration during six disintegration trials using different energy inputs and densities. Particular attention was paid to replicate as much as possible full-scale operations during the dynamic studies and to assess reduction mechanisms using respirometry and modelling. The selection and integration of full-scale ultrasound equipment in the pilot-scale plant and the disintegration procedure were carefully designed to allow a reliable scale-up of the results and provide the basis to make a proper assessment of the economics of the overall sludge reduction process.

1.3 Project hypothesis

RAS disintegration by ultrasound can achieve sludge reduction cost-effectively.

1.4 Project aim and objectives

The project aim is:

- To evaluate the potential of ultrasound treatment for sludge reduction

Main project objectives are:

1. **Current knowledge** – Review the current knowledge on the use of ultrasound disintegration for sludge reduction in the activated sludge process and highlight gaps in research
2. **Equipment comparison** – Compare different ultrasound equipment and evaluate optimal operational conditions for sludge disintegration

3. **Impact on sludge biomass from different types of WWTP** – Evaluate the impact of ultrasound treatment on activated sludge biomass from different types of WWTP

4. **Impact on the activated sludge process** – Evaluate the impact of ultrasound on the process performance in the pilot-scale activated sludge plant

5. **Degree of sludge reduction** – Evaluate the degree of sludge reduction in the pilot-scale activated sludge plant

6. **Sludge reduction mechanisms** – Investigate the mechanisms of sludge reduction triggered by ultrasound treatment based on: (1) the effects on the activated sludge biomass; (2) the actual impact on sludge production and (3) modelling

7. **Implications for water utilities** – Outline possible strategies for ultrasound application in Anglian Water’s activated sludge processes

### 1.5 Thesis plan

The experimental work in this thesis was undertaken exclusively by the author, apart from the batch disintegration trials for equipment comparison in Chapter 4, which were performed in collaboration with another PhD student.

The main objectives of this project and how they interconnect with each other and the thesis structure is outlined in Figure 1-2.
In chapter 2, there is a review of the literature on the current knowledge about:

- Ultrasound technology and its use in wastewater and sludge treatment
- Ultrasound potential for sludge reduction based on: (1) its ability to implement different sludge reduction mechanisms by sludge disintegration and (2) its impact on sludge production and the activated sludge process performance.

The review also seeks to highlight gaps of knowledge about ultrasound application for sludge reduction.

Chapter 3 describes the materials and methods used during this research.

Chapter 4 provides the results from the batch studies. Two groups of batch disintegration trials were completed:

- Equipment comparison trials – Trials to compare three ultrasound industrial systems and a mechanical one to: (1) evaluate their performance for sludge
disintegration in terms of sludge solubilisation and energy input and (2) gain
information on the optimal operational conditions during disintegration

- Ultrasound impact trials – Trials to evaluate the impact of ultrasound on sludge
  biomass from different types of WWTP.

Chapter 5 provides the results from the dynamic studies. A combination of batch and
dynamic trials were completed:

- Ultrasound potential trials – Batch disintegration trials to investigate the potential
  of ultrasound treatment to implement different sludge reduction mechanisms
  based on its effects on the pilot-scale plant biomass

- Pilot-scale plant trials – Dynamic trials to investigate: (1) the potential of
  ultrasound treatment to implement different sludge reduction mechanisms based
  on its effects on sludge biomass and sludge production and (2) the impact of
  ultrasound treatment on the process performance. The effects of ultrasound
  treatment on sludge biomass, sludge production and process performance were
  evaluated using an activated sludge pilot-scale plant with an ultrasound system
  integrated in the test lane to provide continuous in-line ultrasound treatment of the
  return activated sludge (RAS).

Chapter 6, taking into account the results from Chapter 4 and 5, examines the output
of a model of the activated sludge pilot-scale plant and investigate the ultrasound
potential to implement different sludge reduction mechanisms.

Chapter 7 describes the implementation of a cost analysis with different scenarios for
the ultrasound disintegration process, to provide Anglian Water with information
about its economic viability.

In Chapter 8, the results from the previous chapters are discussed.

In Chapter 9, the overall conclusions reached from this research in relation to the
project objectives are presented together with the practical implications for Anglian
Water and suggestions for further work necessary to develop and enhance the studies
completed in this thesis.
Chapter 2: Literature review

2.1 Outline

In the second section of this literature review, there is an introduction to:

- Ultrasound physics and background theories
- Main applications in the environmental sector
- Characteristics of ultrasound systems used in the water sector in relation to their potential for sludge disintegration purposes.

In the third section, there is:

- An introduction to microbial metabolism to identify the main processes involved in the biological treatment of wastewater
- A description of some theoretical and operational methods to evaluate synthesis and observed yield and understand their relationship with sludge production
- An overview of the main sludge reduction mechanisms and the technologies used to implement those mechanisms

In the fourth section, there is:

- A more detailed description of the reduction mechanisms which can be triggered by disintegrating part of the RAS during the activated sludge process
- A review of the most significant batch and dynamic studies undertaken to investigate the potential of ultrasound for sludge reduction and its impact on the process performance.

The rationale of section 4 is to create a baseline that can be used for comparison with the results from the experimental work involved in this study. A summary and some final considerations are given as a conclusion of the literature review.
2.2 Ultrasound technology

2.2.1 Physics and background theories

Definition

Ultrasound is defined as sound with a frequency beyond the upper limit of human hearing. The normal range of hearing is between 16 Hz and about 18 kHz and ultrasound is generally considered as any sound with a frequency greater than 20 kHz. In practice, three ranges of frequencies are reported (Ince et al., 2001):

- High frequency, or diagnostic ultrasound (2–10 MHz)
- Medium frequency ultrasound (300–1000 kHz)
- Low frequency or conventional power ultrasound (16–100 kHz).

Sonochemistry

If a low to medium ultrasound frequency with a high power is applied to a liquid system, it is possible to produce physical and chemical changes in it through a technology that has become known as sonochemistry. These changes result from the “extreme” temperatures and pressures generated by the formation, growth and collapse of cavitation bubbles induced by sound energy, i.e. acoustic cavitation. The frequency range for sonochemistry is generally from 16 kHz to 1000 kHz, with a maximum extension up to 3 MHz.

Acoustic cavitation

The phenomenon called “acoustic cavitation” consists of at least three distinct and successive stages: nucleation, bubble growth (expansion), and under proper conditions implosive collapse (Figure 2-1) (Mason and Lorimer, 1988). Like any sound wave, ultrasound is propagated via a series of compression and rarefaction waves induced in the molecules of the medium through which it passes. At sufficiently high power, the rarefaction cycle may exceed the attractive forces of the molecules of the liquid and cavitation bubbles will form. Such bubbles grow by a process known as rectified diffusion, i.e. small amounts of vapour (or gas) from the medium enters the bubble during its expansion phase and are not fully expelled during compression. The bubbles grow over the period of a few cycles to an equilibrium size.
for the particular frequency applied which matches the frequency of bubble resonance. The acoustic field experienced by the bubble is not stable. The collapse and implosion of these bubbles in succeeding compression cycles generates the energy for chemical and mechanical effects. The final stage of cavitation, i.e. implosive collapse, occurs only if the intensity of the ultrasound wave exceeds that of the “acoustic cavitation threshold”. The cavitation threshold for activated sludge is around 0.1 W cm\(^{-2}\), for water 0.4 W cm\(^{-2}\) (Lorimer, 1990). The lower threshold in activated sludge is due to the presence of a larger number of solids acting as cavitation nuclei (Neis \textit{et al.}, 2001). It is very difficult to generate cavitation at frequencies above 1 MHz (Mason, 1999; Neis \textit{et al.}, 2000). In general, it is not easy to operate at frequencies greater than 200 kHz also because the power required for the onset of cavitation increases with an increase in the frequency of irradiation. Working at very high frequencies might make the application of ultrasound uneconomical (Mason, 1990) due to the fact that significant quantities of supplied energy will be used only in the generation of the cavities. Therefore, for practical reasons, readily available laboratory equipment often works at frequency between 20 and 40 kHz (Mason, 1992). Cavitation is a complex process that is yet not fully understood in all details. At present, no consensus exists for the physical explanation of the collapse phase, except that “extreme and non-equilibrium” conditions exist during the implosion (Neis and Tiehm, 1999).

![Figure 2-1: Scheme of a collapsing cavitation bubble](image_url)
Chapter 2: Literature review

The hot spot theory

The most highly favoured explanation for the last stage of cavitation is the “hot spot” theory, which suggests that the collapse is so rapid (in the range of microseconds according to Neis and Tiehm (1999)) that the compression of the gas and vapour inside the bubble is adiabatic. Consequently, the temperatures and pressures within a collapsing microbubble can reach values as high as 4200–5000K and 20–50MPa, respectively, just before fragmentation (Ince et al., 2001). Enormous heating and cooling rates (>10^9 K sec^{-1}) occur and strong turbulent eddies of size 5-100 µm are induced around the collapsing bubbles (Chu et al., 2001). When collapsing near a surface, fluid movements generate liquid jets targeted at the surface with speeds in excess of 100 m s^{-1} (Mason, 1999) which can lead to high levels of material erosion. The ultrasound power input can lead to a marked temperature rise in the liquid medium after a long-duration operation. Under these conditions, heating effects on the on-going process are unavoidable and, if not controlled, cannot be ignored (Chiu et al., 1997).

2.2.2 Operational parameters and mechanisms of action

Operational parameters

The application of ultrasound is extremely sensitive to operational parameters, which cannot be controlled without a good knowledge and understanding of physical and chemical phenomena (Ince et al., 2001). A reliable description of the sound field would be accomplished by a local energy density formulation which includes the distribution of sound energy in the liquid volume and takes into account the reactor geometry (Horst and Hoffmann, 1999). In practice, the ultrasound power input is quantified by calorimetric or, even more directly, by electrical measurements. The most common parameters used are defined below:

- **Ultrasound frequency**: specifies the optimal resonating frequency driving the transducer that converts the alternating current of an electric oscillator into mechanical waves that are transmitted to the suspension. It is measured in [kHz]
- **Ultrasound power intensity**: is the power supplied per transducer area, measured in [W cm^{-2}].
• **Ultrasound power density**: is the power supplied per sample volume, measured generally in \([W \, L^{-1}]\) or \([W \, cm^{-3}]\).

• **Ultrasound energy dose or energy density by volume** or, simply, **energy density**: is the energy input per sample volume, measured in \([kJ \, L^{-1}]\) or \([kWh \, L^{-1}]\).

• **Ultrasound energy density by mass** or, simply, **specific energy**: is the energy input related to the sludge dry solids content, measured in \([kJ \, kg^{-1}]\) or \([kWh \, kg^{-1}]\). It is the ratio between the ultrasound energy dose and the biomass concentration in the sample volume considered.

**Mechanisms of action**

Ultrasound can provide the driving force for a wide range of chemical and industrial processing. Its potential stems from three main types of actions: mechanical, chemical and thermal effects, related to the formation and collapse of bubbles (cavitation) in the liquid to which the ultrasound is applied (Neis and Tiehm, 1999). The specific effects that can be observed when cavitation is generated are summarised below (Portenlänger, 1999; Neis et al., 2001):

- **Mechanical action**: generation of shear stress and forces, jets and shock waves resulting in rapid mass transfer, particle size reduction and cell destruction

- **Chemical action**: radical reactions due to the generation of hydrogen atoms and hydroxyl radicals which can trigger subsequent chemical transformation of (organic) substances

- **Thermal action**: generation of high temperature with breakdown of volatile hydrophobic substances within the imploding bubbles.

The ratio of mechanical and chemical effects for ultrasound reactions in aqueous solutions depends mainly on the frequency used. For low ultrasound frequencies in the range from 20 to 400 kHz, the main effect is correlated with the mechanical forces. Applying higher frequencies in the range of 500 kHz to 1.6 MHz only radical and/or thermal reactions are responsible for the chemical effects (Portenlänger, 1999). Besides frequency, the most critical parameters for ultrasound application are intensity, solids content and temperature. The role played by power intensity, solids content and temperature will be described in more details in the section dedicated to...
the review of batch disintegration studies in the literature. The selection of suitable operating parameters, e.g. ultrasound frequency range and intensity, is essential for carrying out any chemical reaction using ultrasound because acoustic cavitation field behaviour can be significantly altered by varying those parameters in order to achieve the required conditions for the application of ultrasound treatment (Gogate and Pandit, 2000b).

2.2.3 Ultrasound applications in environmental engineering

Sludge disintegration

The potential of ultrasound for sludge reduction stems from its ability to disintegrate sludge. In theory the disintegration of sludge could be the result of both mechanical and chemical actions but experiments using different frequencies demonstrate a continuously increasing degree of cell disintegration with decreasing frequency (Neis et al., 2001). The best disintegration is achieved at low frequencies, between 20 and 40 kHz, which suggests that sludge disintegration is in fact predominantly a mechanical process (Tiehm et al., 2001). Most full-scale ultrasound systems operate at a low frequency range (20 to 30 kHz) and high intensity (~ 50 W cm\(^{-2}\)) (Nickel and Neis, 2007). The energy dose varies depending on the specific type of application and must be evaluated case by case. The application to sludge biomass of the hydromechanical forces generated by cavitation causes:

- **Floc breakage and, at higher energy inputs, cell lysis:** at low energy inputs, flocs are separated and dispersed, consequently floc size is reduced but cell membranes are not disrupted. At higher energy inputs, above 32 kJ L\(^{-1}\) according to Lehne et al. (2001), cell membranes are broken and cell lysis starts to occur
- **Sludge solubilisation:** following floc breakage and cell lysis, first extracellular and then intracellular compounds are released into the liquid phase
- **Changes in the biological activity of sludge biomass:** floc breakage, cell lysis and stress can all change the metabolic conditions of microorganisms, measured in terms of their oxygen utilisation.

These effects, triggered by the mechanical action of ultrasound, can be used to improve or control important processes during the wastewater or sludge treatment
Depending on the targeted process, sludge disintegration can occur at different stages (Figure 2-2):

- In the sludge treatment chain to:
  - Enhance the anaerobic sludge digestion process
- In the wastewater treatment chain to:
  - Improve nutrient removal
  - Control bulking sludge
  - Reduce sludge production.

Figure 2-2: Disintegration points in the wastewater or sludge treatment chain

**Enhancement of anaerobic digestion**

A lot of interest has been devoted to SAS disintegration as a pre-treatment to improve anaerobic sludge digestion process, which is aimed to stabilise sludge, reduce its volume and weight, and for odour control too. The main factor limiting the anaerobic digestion is the low rate of hydrolysis of particulate matter. In activated sludge, organic material is largely compartmentalised within microbial cells. Cells maintain structural integrity during digestion because cross linkage of microbial glycan with
peptide chains renders cell walls resistant to hydrolysis and biodegradation (Weemaes and Verstraete, 1998). The disintegration of thickened SAS is therefore introduced to solubilise and convert slowly biodegradable, particulate organic materials to low molecular weight, readily biodegradable compounds. Many authors have demonstrated that SAS disintegration is able to improve the efficiency of anaerobic digestion in terms of (Wang et al., 1999; Tiehm et al., 2001; Onyech et al., 2002; Hogan et al., 2004; Nickel and Neis, 2007; Elliott and Mahmood, 2007):

- Enhancement of hydrolysis rate: faster and higher degradation rates of organic matter lead to a reduction in solids retention times and final volume. Therefore the same digester can treat a higher volume of sludge
- Reduced foaming in the digester
- Increased biogas production
- Improvements in dewatering properties.

Due to the high energy demand required by ultrasound disintegration, the energy dose and volume of sludge treated must be carefully evaluated to balance cost and benefits (Winter et al., 2002). The use of low energy doses and split-stream techniques, where only a portion of SAS is treated depending on the specific needs, have been suggested to reduce problems related to high power consumption and a possible decrease in dewaterability (Friedrich, 2002). Process optimisation seems to have reached a good level and there are already dozens of full-scale applications of ultrasound treatment for improving anaerobic digestion (Enpure, 2008; Ultrawaves, 2008b). Even if some studies have been contradictory (Winter, 2002; Boehler and Siegrist, 2006), in spite of the high initial capital costs, economic analysis are reported to indicate payback times around 3 years (Friedrich, 2002; Wolff et al., 2007).

**Improvement of nutrient removal**

The release of extracellular and intracellular compounds, due to sludge solubilisation during RAS disintegration, can be used as an internal source of organic carbon to improve the denitrification process and/or the biological removal of phosphorus (Müller, 2001; Ahn et al., 2002; Saktaywin et al., 2006; Dytczak et al., 2007). Again,
the selection of the optimal energy dose and volume must be based on a compromise between cost and benefits.

**Control of bulking sludge**

Filamentous bacteria are known to be particularly susceptible to the effects of acoustic cavitation in a liquid, due to their large surface area (Jørgensen and Kristensen, 1996). By breaking up the structure of filamentous organisms, the low-density, voluminous flocs in bulking sludge are reduced to a normal size, bubbles are released, and the smaller fragments of the flocs can settle and compact better. For bulking sludge applications, RAS disintegration at low energy doses is suitable to create high local shear stresses, which are capable of breaking the filamentous structures of the sludge in a sustainable way (Müller, 2000b; Wünsch et al., 2002).

**Reduction in sludge production**

Floc breakage, sludge solubilisation and changes in biological activity can all trigger different sludge reduction mechanisms. These reduction mechanisms and ultrasound potential for sludge reduction are described in more details in the section 2.4.

**Other applications**

The use of ultrasound in the environmental field stems from:

- The potential of ultrasound technology for improving environmental processes due to the physical and chemical effects of sonochemistry
- The advantages of ultrasound as a “no touch, no chemical and no moving mechanical parts” technology with a reasonably small footprint (Neis et al., 2000).

Table 2-1 provides a general overview on current ultrasound applications in environmental engineering.
Table 2-1: Ultrasound application in environmental engineering (adapted from Mason (2005) and Neis and Tiehm (1999))

<table>
<thead>
<tr>
<th>Domain</th>
<th>Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clean Water</strong></td>
<td>Biological decontamination (disinfection by radical action)</td>
</tr>
<tr>
<td></td>
<td>Chemical decontamination (degradation of recalcitrant pollutants by radical/thermal action)</td>
</tr>
<tr>
<td></td>
<td>Improving filter regeneration</td>
</tr>
<tr>
<td></td>
<td>Removing incrustation in pipes and wells</td>
</tr>
<tr>
<td><strong>Wastewater</strong></td>
<td>Chemical decontamination (degradation of recalcitrant pollutants by radical/thermal action)</td>
</tr>
<tr>
<td></td>
<td>Decomposing bulking sludge to allow sedimentation (sludge disintegration by mechanical action)</td>
</tr>
<tr>
<td></td>
<td>Enhancing biological nutrients removal (sludge disintegration by mechanical action)</td>
</tr>
<tr>
<td></td>
<td>Enhancing aerobic degradation (sludge disintegration by mechanical action)</td>
</tr>
<tr>
<td><strong>Sludge</strong></td>
<td>Enhancing anaerobic digestion (sludge disintegration by mechanical action)</td>
</tr>
<tr>
<td></td>
<td>Improving dewatering (sludge disintegration by mechanical action)</td>
</tr>
<tr>
<td><strong>Other Applications</strong></td>
<td>Air cleaning</td>
</tr>
<tr>
<td></td>
<td>Land Remediation</td>
</tr>
</tbody>
</table>
2.2.4 Ultrasound systems

A general overview on reactor designs is given because high levels of process optimisation can be achieved by designing efficient reactors coupled with appropriate ultrasound emitters (Mason, 2003). There are three basic methods of applying acoustic power to liquids in general and sludge in particular (Figure 2-3):

- Probe systems
- Tube reactors
- Bath reactors.

**Probe systems**
- High power intensity: 50-100 W cm\(^{-2}\)
- High power density: up to 4 kW L\(^{-1}\)

**Tube reactor**
- Low power intensity: 2-18 W cm\(^{-2}\)
- High power density: up to 10 kW L\(^{-1}\)

**Bath reactor**
- Low power intensity: 2-18 W cm\(^{-2}\)
- Low power density: < 500 W L\(^{-1}\)

![Figure 2-3: Comparison of three ultrasound systems. Left: probe systems with different horn designs (modified stepped horn (left), radial horn (right)); middle: tube reactor; right: bath reactor.](image-url)
**Probe systems**

The first method is a high intensity system. Probe systems are often used for laboratory research and in the environmental field. Most of full-scale applications of ultrasound are equipped with these systems to enhance anaerobic digestion by sludge disintegration, as described by Kurth (2002), Panter (2002), Purcell (2002), Winter (2002), Enpure (2008), Wolff (2007). The most commonly used systems for sludge disintegration at full-scale are (Elliott and Mahmood, 2007):

- Sonix™ by Sonico Ltd (UK)
- Sonolyzer™ by Ultrawaves GmbH (Germany).

In probe systems, a single powerful transducer couples energy into a chemical reaction by means of a horn or velocity transformer. For sludge disintegration, the power input per transducer is generally between 1 and 6 kW (Enpure, 2005; Ultrawaves, 2008a), up to 16 kW (Hielscher, 2005). In practice, the generator, or power supply, feeds high frequency electrical energy to the transducer where it is transformed to mechanical vibration. The motion is then transmitted to a horn, generally through a booster; both are usually made of titanium alloy. The horn and the booster transmit and amplify the amplitude of this motion, i.e. they both provide a means of mechanically adjusting the amplification, or gain, provided by the transducer. For simple booster or horns, the gain is determined by their geometry (Perkins, 1986). The purpose of the horn is to focus the energy into the radiating surfaces immersed in the liquid sample. The motion of the radiating surfaces creates the sound waves that propagate through the liquid, generating microbubbles and causing cavitation. The intensity of the cavitational field is proportional to the amplitude of the motion. The amplitude is, at least within a certain range dependent on the specific probe system, proportional to the power intensity. Ultrasound is attenuated very fast and only some centimetres are sonochemically active. Using power intensities between 3 to 100 W cm\(^{-2}\), typical distances for the destructive effects are about 0 to 3 cm in front of the radiating surface (Schneider, 1999). The design of probe systems allows them to reach power intensities greater than 100 W cm\(^{-2}\) and higher than any other system. Therefore, probe systems can also achieve
Chapter 2: Literature review

higher amplitudes and more intensive cavitation fields than any other system (Horst and Hoffmann, 1999). One of the main drawbacks is that the intense cavitation causes erosion on the radiating surfaces.

Almost all lab-scale and some full-scale application systems have got an amplitude control feature which maintains a constant amplitude under all load conditions, i.e. more or less power is consumed to keep that same amplitude against varying flow rates, viscosity, temperature, pressure, etc. This feature allows reproducible operation conditions. The amplitude can be varied by changing the power input and/or by changing the type of booster and/or horn. In this way, using a generator of around 1 kW it is possible to process liquids at amplitudes from 1 to approximately 50 µm (Hielscher, 2005). Ultrasound processors designed for full-scale application and with a nominal output of 4-8 kW can generate amplitudes up to 100 µm by exploiting electronically and mechanically induced amplifications. These amplitudes correspond to power densities around 100 W cm⁻² (Kurth, 2002).

**Tube reactors**

This second method can be considered a trade-off between probe systems and bath reactors. Transducers are mounted on the outer wall of a tube in order to generate cavitation in the centre of the tubes, where the sound waves from surrounding transducers superimpose and amplify each other. In spite of this, tube reactors cannot provide the same mechanical amplification, i.e. intensity of the cavitation field, of probe systems. The section of the tube reactors can have different shapes. Main variants to the reactor designed based on a single tube are:

- Reactors with two tubes: both the inner and outer tube are equipped with transducers
- Reverberatory systems: they consist of two opposite plates, both equipped with transducers.

Treated fluids in these systems flow in a small gap between the tubes or the plates. Within this narrow reaction volume, fluids are exposed to a very homogenously distributed cavitation field. Some systems use different frequencies for the transducers attached on opposing surfaces (Horst et al., 2002; ASPS, 2005; Bandelin,
2005) because this configuration is able to amplify cavitation (Horst and Hoffmann, 1999; Feng et al., 2002; Tatake and Pandit, 2002). Power intensities are in the order of 2-18 W cm$^{-2}$ but the energy densities are very high, from a few hundreds to thousands of Watts per litre. Tube reactors are controlled by power. For a 1 kW system, the intensity over the radiating surface responds to adjustments in power by a rise in amplitude from approximately 1 to a maximum of 10-15 µm (Bandelin, 2005). Tube reactors are sometimes coupled with magnetostrictive transducers. Magnetostrictive transducers are supposed to be more reliable and durable than piezoelectric ones (Hunicke, 1990), even if there is not common agreement on this (Blackstone, 2008). However, they are also less efficient than the piezoelectric ones due to the extra conversion step required to generate the magnetic field and to magnetic hysteresis effects (Horst and Hoffmann, 1999; Keil and Swamy, 1999). The overall efficiency of magnetostrictive-based and piezoelectric-based systems is considered to be around 35-40 % and 70 % or higher, respectively (Blackstone, 2008). Tube reactors, especially when fitted with magnetostrictive transducers, have been successful implemented in fields where operational conditions are very harsh (Hunicke, 1990). Due to their robustness and smooth reactor design, some ultrasound manufacturers (e.g. Bandelin GmbH & Co. KG, Germany) propose them as a potential cheaper alternative to high intensity systems. Nevertheless, at the present time, no full-scale installation of this type of system in a WWTP is known. Most of companies providing ultrasound equipment designed for sludge disintegration (e.g. Enpure, Ultrawaves and Hielscher) have opted for high intensity probe systems. The rationale is that sludge disintegration is considered more effective at intensities around 50 W cm$^{-2}$, which are higher than those provided by tube reactors, i.e. 2-18 W cm$^{-2}$ (Nickel and Neis, 2007).

**Bath reactors**

The third method is a low intensity system. It is essentially a liquid filled tank with multiple transducers bonded around the base and/or, sometimes, walls. The working principle is the same as for tube reactors, with the base and/or walls acting as the radiating surfaces. As tube reactors, they cannot provide the high mechanical amplification boosters and horns provide in probe systems. Bath reactors generally provide power intensities in the order of 1-2 W cm$^{-2}$. The power input for a typical
transducer is roughly 50W for frequency around 20 kHz and a diameter of 6 cm (Perkins, 1986; Horst and Hoffmann, 1999). It is normal to employ a number of transducers to put enough power into liquids contained in such tanks. The transducers are generally driven with the same frequency. These systems are controlled by power, not by amplitude. The amplitude in this configuration does not play such an important role like in probe systems. The low levels of ultrasound intensity and density delivered by this type of reactors make them unsuitable for sludge disintegration purposes. In the environmental sector, they are generally used for chemical degradation (Ince et al., 2001; Gogate and Pandit, 2004b; Gogate and Pandit) and biological decontamination of water by ultrasound alone or combined with other techniques (Phull et al., 1997; Mason, 1999; Neis and Blume, 2002; Gogate and Pandit, 2004b; Gogate and Pandit, 2004a).

**Methods for optimising ultrasound systems**

Research and development in sonochemical systems exposed the significance of two basic strategies for maximizing reaction efficiencies which are valid for any application of ultrasound energy, including wastewater ones (Mason and Cordemans, 1998; Ince et al., 2001):

- Optimization of power and reactor configuration
- Enhancement of cavitation.

The knowledge required for the scale-up of ultrasound systems for efficient large scale operations is still lacking (Gogate and Pandit, 2004b; Nickel and Neis, 2007). Therefore, the first strategy, in addition to mathematical modelling, also requires an experimental approach for the:

- Selection of the most suitable transducer and generators
- Configuration and dimensioning of the reaction cell
- Optimization of the power efficiency (i.e. the effective power density delivered to the reaction medium).
The second strategy, i.e. the enhancement of cavitation, involves the addition of different gases and solids into the system to test and compare their effectiveness in increasing reaction yields and/or reaction rates.

In the wastewater sector, a lot of research is under progress in relation to the first strategy. As far as the second strategy is concerned, the presence of a high number of solids and gas bubbles, which act as cavitation nuclei in the sludge volume, already allows lower cavitation thresholds (Neis and Blume, 2002). Consequently, there is no real need of further research.

**An alternative approach: hydrodynamic cavitation**

Hydrodynamic cavitation is examined in this section to allow a comparison between acoustic and hydrodynamic cavitation systems, such as the Crown system (Froud et al., 2005). Cavitating conditions identical to acoustic cavitation, can be generated in hydrodynamic cavitation (Moholkar et al., 1999), but hydrodynamic cavitation is considered a cheaper and more efficient alternative for the cavitation-based transformation processes (Senthilkumar and Pandit, 1999). It is generated by the flow of liquid through a simple geometry, such as venture tubes or orifice plates, under controlled conditions. When the pressure at the throat falls below the vapour pressure of the liquid, the liquid flashes, generating a number of cavities, which subsequently collapse when the pressure recovers downstream of the mechanical constriction (Moholkar et al., 1999). The bubble behaviour and hence the pressure generated at the collapse of the cavity for hydrodynamic cavitation depends on the operating conditions and geometry of the mechanical constriction generating cavitation. The vapour pressure of the cavitating media and the selection of suitable operating conditions (inlet pressure and flow rate into the orifice set-up) and geometric parameters (arrangement of holes on the orifice plates) are essential for carrying out any process using the hydrodynamic cavitation set-up. Varying these conditions can significantly alter bubble behaviour in hydrodynamic cavitation, thus achieving the required conditions for the specific reaction (Gogate and Pandit, 2000a).

Cavitation is usually associated with material erosion. If properly designed, no particular erosion phenomena inside the cavitation-nozzle and inside the following pipe seem to occur in systems based on hydrodynamic cavitation. This might be
explained by the hydraulic velocity profile inside a pipe. In these systems cavitation reactions takes place within the flow rather than on the surface of any material, thus the whole system can be manufactured in stainless steel without any detriment to its performance (Gogate and Pandit, 2001). Two main factors play in favour of these systems:

- Better energy efficiency in generating the cavitation field due to their simpler mechanical operating principle
- The use of less expensive material for their construction due to their simpler mechanical operating principle, i.e. no need of titanium alloys, quartz transducers or sophisticated electronics.

These systems have not been tested extensively so far and, in general, hydrodynamic cavitation is not yet a well-established technology (Andreottola and Foladori, 2006). Nevertheless, Froud et al. (2005) reported that the Crown system has been proven to be more robust, simpler and cheaper to operate than ultrasound systems, and consequently it was selected for a full-scale trial for SAS disintegration in Germany.


2.3 Reducing sludge production during wastewater treatment

2.3.1 Bacterial metabolism in biological wastewater treatment

A basic knowledge of bacterial metabolism is necessary to understand sludge reduction mechanisms. During biological treatment of wastewater, there are thousands of chemical reactions involved in the metabolism of microorganisms. The three most important metabolic processes for heterotrophic bacteria are (Gray, 2004):

- Carbon uptake
- Respiration
- Growth.

The relationship between these major, highly integrated processes is shown as a schematic in Figure 2-4.

Figure 2-4: Schematic of the relationship between carbon uptake, respiration and growth during the heterotrophic microbial metabolism. The biomass synthesis yield $Y$ is the partition ratio between the organic carbon assimilated for growth and the one oxidised for energy production during the respiration process (Gray, 2004)
Carbon uptake, respiration and growth are linked with the most critical processes of the activated sludge process: biodegradation, aeration and sludge production, respectively.

**Carbon uptake**

The carbon uptake is divided between:

- Carbon that is broken down in respiration, to provide energy for growth and maintenance, i.e. organic carbon used for catabolic processes in Figure 2-4
- Carbon that is built up into macromolecules for storage and growth, i.e. the organic carbon used for anabolic processes in Figure 2-4.

The period needed for the biodegradation of organic compounds can vary from less than one hour (readily biodegradable organic compounds), to few hours up to few days (slowly biodegradable or recalcitrant organic compounds). This means that some complex organic carbon molecules may not be affected by the biological treatment because the available treatment time, i.e. the hydraulic retention time (HRT), is not long enough. Therefore, these compounds might be still present in the treated effluent (Tchobanoglous et al., 2003; Gray, 2004).

**Respiration**

In general, respiration can be defined as the enzyme-mediated metabolic process that is able to generate adenosine triphosphate (ATP). During respiration, either organic or inorganic compounds serve as the electron donor (i.e. the food source) and inorganic compound such as O₂, NO₃, NO₂ and SO₄ serve as the ultimate electron acceptor. ATP molecules are generated as electrons removed from the electron donor are transferred along the electron transport chain from one metabolic carrier to the next and, ultimately, to the electron acceptors (Gray, 2004). When oxygen is used as the electron acceptor, the process is called aerobic respiration. Heterotrophic bacteria use aerobic respiration to convert the energy present within the carbon substrate and store it in a chemical form, the ATP molecules. This is possible by converting the energy of intermolecular bonds in the substrate into the high-energy phosphate bonds of energy-carrying ATP molecules. This energy is then used for maintenance and for the
synthesis of the various molecular components required for cell growth (Low and Chase, 1999a; Gray, 2004). The low rate of respiration needed to provide the minimal amount of energy for all non-growth activities sustaining cell life, i.e. maintenance metabolism, is called endogenous respiration. This is the only respiration rate detected when the entire carbon source has been depleted; growth does not occur anymore and starving microorganisms start to use their storage products to provide the energy necessary for maintenance requirements.

**Growth**

Equation (2.1) describes the aerobic biological treatment of organic matter by heterotrophic bacteria:

\[
v_1(\text{organic substrate}) + v_2\text{O}_2 + v_3\text{NH}_3 + v_4\text{PO}_4^{3-} \rightarrow \text{microorganisms}\n\]

\[
v_5(\text{new biomass}) + v_6\text{CO}_2 + v_7\text{H}_2\text{O} \quad (2.1)
\]

In equation (2.1), oxygen (O\(_2\)) is the external electron acceptor of the respiratory metabolism, whereas ammonia (NH\(_3\)) and phosphate (PO\(_4^{3-}\)) are used to represent the nutrients needed for the uptake and conversion of part of the total organic carbon present in the *organic substrate* to simple end products, i.e. carbon dioxide (CO\(_2\)) and water (H\(_2\)O). The term *new biomass* represents the growth of biomass because of the uptake and conversion of the other part of the total organic carbon present into the *organic substrate*, using part of the energy generated and stored during respiration. Biomass is mostly organic material and is generally measured in terms of volatile suspended solids (VSS) while organic substrates in terms of biochemical oxygen demand (BOD) or chemical oxygen demand (COD). The ratio between the amount of new biomass produced and the amount of organic substrate consumed is defined as the synthesis yield (or true yield) \(Y\) and is generally measured in terms of \(g\) VSS \(g^{-1}\) BOD or \(g\) VSS \(g^{-1}\) COD:

\[
Y = \frac{\text{amount of new biomass produced in } [g\text{ VSS}]}{\text{amount of organic substrate consumed in } [g\text{ BOD} \text{ or } [g\text{ COD}]} \quad (2.2)
\]
Typical values for synthesis yields found during the wastewater treatment chain, i.e. the activated sludge process, and the sludge treatment chain, i.e. the anaerobic digestion, are presented in Table 2-2.

Table 2-2: Typical synthesis yields for common biological reactions in wastewater treatment (adapted from Tchobanoglous et al. (2003) and Stensel et al. (2004))

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>Electron donor</th>
<th>Electron Acceptor</th>
<th>Synthesis yield</th>
<th>End products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td>Organic compound</td>
<td>Oxygen</td>
<td>0.40 g VSS g⁻¹ COD</td>
<td>CO₂, H₂O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.65 g VSS g⁻¹ BOD</td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>Ammonia</td>
<td>Oxygen</td>
<td>0.12 g VSS g⁻¹ NH₄-N</td>
<td>NO₃, H₂O, H⁺</td>
</tr>
<tr>
<td>Anoxic</td>
<td>Organic compound</td>
<td>Nitrate</td>
<td>0.30 g VSS g⁻¹ COD</td>
<td>N₂, CO₂, H₂O</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Organic compound</td>
<td>Organic compound</td>
<td>0.06 g VSS g⁻¹ COD</td>
<td>Organic acids</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Acetate</td>
<td>Carbon dioxide</td>
<td>0.05 g VSS g⁻¹ COD</td>
<td>Methane</td>
</tr>
</tbody>
</table>

Under normal conditions, there is a balance among carbon uptake, respiration and growth. The relationship between these processes can be highlighted using respirometry. The direct registration of the respiration rate in a biological process can provide important information on the metabolism of the microorganisms in general and growth in particular. By measuring the oxygen uptake rates it is possible to predict the amount of organic carbon used for energy production (catabolic processes) (Rozich and Gaudy, 1992). Using this knowledge, when the substrate consumption is express in terms of COD removed, the total organic carbon uptake is the sum of the amount used for growth (accounted for as new biomass) and for respiration (accounted for as oxygen uptake), as stated in equation (2.3) (Rozich and Gaudy, 1992; Dircks et al., 1999):

\[
Δ\text{COD} \equiv ΔO₂ + ΔX \cdot \text{COD}_{\text{CELL}}
\]  

(2.3)

Where:

- \(Δ\text{COD}\) = total organic carbon uptake, in [g COD L⁻¹]
- \(ΔO₂\) = oxygen uptake, in [g O₂ L⁻¹]
- \(ΔX\) = amount of new biomass, in [g VSS L⁻¹]
- \(\text{COD}_{\text{CELL}}\) = COD equivalent of cell tissue: ~ 1.42 g COD g⁻¹ VSS
Considering that the new biomass can also be expressed in terms of total carbon uptake by using the synthesis yield $Y$ (equation (2.4)), it is possible to describe the relationship among synthesis yield $Y$, oxygen uptake and total carbon uptake using equation (2.5):

\[
\Delta X = Y \cdot \Delta \text{COD} \quad (2.4)
\]

\[
Y \equiv 1 - \frac{\Delta O_2}{\Delta \text{COD}} \quad (2.5)
\]

An important consequence of equation (2.5) is that any change in the carbon uptake and respiration processes will influence the biomass growth rates. In particular, any mechanism which is able to enhance the biomass respiration rate, i.e. the oxygen uptake $\Delta O_2$, will lead to a reduction in the biomass synthesis yield by increasing the amount of carbon uptake used for catabolism instead of anabolism, i.e. growth.
2.3.2 Sludge characterisation and production

Activated sludge structure and size

Activated sludge flocs are conglomerates of living and dead bacterial cells, which can also include filamentous strains, precipitated salts, trapped inorganic particles (e.g. sand) and organic fibres. They are held together by a slime matrix, comprised of polymeric compounds surrounding the cells, and by chemical bonding forces, in which divalent cations, such as Ca$^{2+}$, play an important role. Free-living bacteria, protozoa and occasionally higher organisms are present around the flocs and in the water between the flocs. The percentage of living cells depends on the plant operational conditions and influent wastewater. The difference between living and dead cells is often not distinctly visible (Eikelboom, 2000). The size of a single bacteria/microorganism may lie between 0.5 and 10 µm (Neis and Blume, 2002). Activated sludge flocs are a multi-level object (a multifractal) (Lee, 1999) and their floc sizes are well above 100 µm. According to Jorand et al. (1995), raw activated sludge has an asymmetric continuous binomial distribution (biased to the left) for particles whose apparent diameter is at least between 1.2-600 µm (the widest window analysed). Approximately, the size of 44% of the particles in raw sludge is in the range 68.3-183 µm. Jorand et al. (1995) propose the existence of four main categories of particles with a range between roughly 2.5 and 125 µm: 2.5 µm (primary particles), 13 and 51 µm (micro-flocs) and 125 µm (macroflocs). The predominating macroflocs (125 µm) are formed from 13 µm microfloc aggregates, which are made up of smaller particles (2.5 µm). Gonze et al. (2003), to model the activated sludge particle size distributions, suggested instead the contribution of five categories, each with a lognormal distribution. The values of the geometric average diameter of the five categories were: 0.8 (isolated small microorganisms, cellular fragments), 14 (micro-flocs, isolated microorganisms), 55 (flocs), 80 (flocs) and 400 (macro-flocs, large protozoans) µm. Measurements of the cell number and cell size distribution in the sludge suggests that the cell mass does not account for more than, approximately, 10-15% of the total organic fraction of the investigated sludge (Frølund et al., 1996).
Sludge production and observed yield

Biological treatment is used to remove the organic substrate and, under specific operational conditions, the nitrogen and phosphorous contained in wastewater. In addition to these compounds, it also removes (Tchobanoglous et al., 2003):

- Cell debris from biological mechanisms that involve some form of reduced growth or cell loss (e.g. maintenance metabolism and grazing). These mechanisms are generally defined as “microbial decay” (Table 2-3)
- Other organic and inorganic solids suspended in the influent wastewater.

Table 2-3: Primary mechanisms involved in microbial decay (adapted from Ødegaard (2004) and Van Loosdrecht and Henze (1999))

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
<th>Caused by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance</td>
<td>Energy consumption for maintenance of the cell integrity under the use of external primary substrate or internal stored substrate such as glycogen or PHA</td>
<td>Basic metabolic energy processes such as regulation of membrane potential, renewal of proteins etc. It can be triggered and enhanced by cell external factors such as stress, adverse environment conditions etc.</td>
</tr>
<tr>
<td>Decay</td>
<td>Processes which reduce the weight and specific activity of biomass</td>
<td>Internal and external factors</td>
</tr>
<tr>
<td>• Internal decay</td>
<td>Death and self-oxidation of cell constituents</td>
<td>Natural biological cycles, endogenous respiration</td>
</tr>
<tr>
<td>• External decay</td>
<td>Predation/grazing, lysis and solubilisation of biomass for external factors</td>
<td>Protozoa, metazoa, enzymes, pH, toxicants, viruses, adverse condition (temperature, pH), physical/mechanical stress</td>
</tr>
</tbody>
</table>

Consequently, the SAS removed from the process contains: (A) the new biomass produced by the conversion of the organic substrate; (B) cell debris from microbial decay; (C) non-biodegradable organic and (D) inorganic solids suspended in the influent wastewater. This mix of solids is generally measured in terms of total suspended solids (TSS). Under steady condition, the total solids production is given by the sum of the solids intentionally wasted, i.e. SAS, and the solids still present at the end of the treatment, in the system effluent. The total solids production, measured in g TSS d⁻¹, can be defined in terms of the wastewater characteristics and microbial kinetics parameters according to equation (2.6):
\[
P_{\text{x, TSS}} = \frac{Y(\Delta \text{COD})Q}{[1+(k_d)\text{SRT}](0.85)} + \frac{(f_d)(k_d)Y(\Delta \text{COD})Q(\text{SRT})}{[1+(k_d)\text{SRT}](0.85)} + \frac{Q(\text{nbVSS}) + Q(\text{TSS}_o - \text{VSS}_o)}{\Delta \text{COD}}
\]

(2.6)

Where:

\(P_{\text{x, TSS}}\) = solids production, in [g TSS d\(^{-1}\)]
\(A\) = biomass component, in [g VSS d\(^{-1}\)]
\(B\) = biomass cell debris component from microbial decay, in [g VSS d\(^{-1}\)]
\(C\) = influent non-biodegradable VSS component, in [g VSS d\(^{-1}\)]
\(D\) = influent inorganic solids component, in [g TSS d\(^{-1}\)]
\(Y\) = synthesis yield, in [g VSS g\(^{-1}\) COD]
\(\Delta \text{COD}\) = COD removed, in [g m\(^{-3}\)]
\(Q\) = influent wastewater flow, in [m\(^3\) d\(^{-1}\)]
\(\text{SRT}\) = solids retention time, in [days]
\(k_d\) = endogenous decay coefficient, in [g VSS g\(^{-1}\) VSS d\(^{-1}\)]
\(0.85\) = typical biomass VSS/TSS ratio, in [g VSS g\(^{-1}\) TSS]
\(f_d\) = fraction of biomass which remains as cell debris, in [g VSS g\(^{-1}\) VSS]
\(\text{nbVSS}\) = influent non-biodegradable VSS, in [g m\(^{-3}\)]
\(\text{TSS}_o\) = influent TSS concentration, in [g TSS m\(^{-3}\)]
\(\text{VSS}_o\) = influent VSS concentration, in [g VSS m\(^{-3}\)]

The observed yield \(Y_{\text{obs, TSS}}\), measured in g TSS g\(^{-1}\) COD, can be calculated dividing equation (2.6), by the substrate removal rate, which is \((\Delta \text{COD})Q\):

\[
Y_{\text{obs, TSS}} = \frac{Y}{[1+(k_d)\text{SRT}](0.85)} + \frac{(f_d)(k_d)Y(\text{SRT})}{[1+(k_d)\text{SRT}](0.85)} + \frac{\text{nbVSS}}{\Delta \text{COD}} + \frac{(\text{TSS}_o - \text{VSS}_o)}{\Delta \text{COD}}
\]

(2.7)

In some studies, the observed yield is given in terms of g VSS g\(^{-1}\) COD, according to equation (2.8):

\[
Y_{\text{obs, TSS}} = \frac{Y}{[1+(k_d)\text{SRT}]} + \frac{(f_d)(k_d)Y(\text{SRT})}{[1+(k_d)\text{SRT}]} + \frac{\text{nbVSS}}{\Delta \text{COD}}
\]

(2.8)
2.3.3 Synthesis and observed yield

A distinction is made between the synthesis yield and the observed yield. The synthesis yield is the amount of new biomass produced immediately upon consumption of the organic substrate, as in equation (2.1), or oxidation of the electron donor in case of autotrophic bacteria. In theory, synthesis yield could be evaluated with stoichiometry, using equation (2.1), or with an approach based on bioenergetics which involves the application of thermodynamic principle to biological reactions (McCarty, 1971; Tchobanoglous et al., 2003). In practice, due to the complex nature of real wastewaters, the synthesis yield and other growth kinetics parameters are generally interpreted and extrapolated from a series of specific lab-based experiments performed under different growth conditions (Grady et al., 1999). Some authors have also proposed various procedures to evaluate the synthesis yield using respirometry (Rozich and Gaudy, 1992; Dircks et al., 1999; Karahan-Gül et al., 2002; Rai et al., 2004).

The observed biomass yield, in addition to the component due to heterotrophic and autotrophic biomass production, contains other solids, as shown in equation (2.7). The observed yield values are therefore affected by (Hopwood and Downing, 1965; Grady et al., 1999; Tchobanoglous et al., 2003):

- Bacterial growth factors (e.g. species of organisms present, metabolic pathways followed, degradation of microbial cells by endogenous respiration, ingestion of bacteria by predators)
- Wastewater characteristics (e.g. biodegradability of organic pollutants, inorganic solids content)
- Operational parameters before and during the biological treatment (e.g. type of primary treatment, hydraulic and solids retention times, food to microorganism ratios, environmental factors).

Any operating variable affecting the rate of any of these processes or the time at which they occur would also be expected to affect sludge production.
2.3.4 Estimation of observed yield

The observed yield $Y_{obs}$ can be evaluated using equation (2.7) when microbial growth kinetics and wastewater characteristics are known. The knowledge of the kinetics of microbial growth is necessary when an in-depth understanding of the design and operation of the biological processes is really needed (Tchobanoglous et al., 2003). When microbial growth kinetics is not known or important, a direct method is used to determine the observed yields from data collected with full-, pilot- or lab-scale bioreactors. This method involves the calculation of the daily sludge production and substrate removal rate in terms of g TSS g$^{-1}$ COD (or BOD). Under steady condition, the daily sludge production rate at day $d$, $P_{X,TSS}(d)$, must take into account:

- The quantity of wasted sludge
- The sludge loss in the effluent
- The sludge accumulation within the biological reactor.

This mass balance can be evaluated using equation (2.9):

$$P_{X,TSS}(d) = Q_W X_W + (Q_{Inf} - Q_W) X_{Eff} + V \frac{dX}{dt}$$

Where:

- $P_{X,TSS}(d)$ = daily sludge production rate at day $d$, in [g TSS d$^{-1}$]
- $Q_W$ = waste sludge flow rate, in [m$^3$ d$^{-1}$]
- $X_W$ = concentration of wasted sludge, in [g TSS m$^{-3}$]
- $Q_{Inf}$ = influent wastewater flow rate, in [m$^3$ d$^{-1}$]
- $X_{Eff}$ = concentration of sludge in effluent, in [g TSS m$^{-3}$]
- $V$ = biological reactor volume, in [m$^3$]
- $\frac{dX}{dt}$ = sludge accumulation in reactor, in [g TSS m$^{-3}$ d$^{-1}$]

The cumulative sludge production is given by equation (2.10):

$$TSS \text{ Produced} = \sum_{d=1}^{d} P_{X,TSS}(d)$$

(2.10)
The substrate removal rate at day \(d\), \(\Delta \text{COD}(d)\), is given by equation (2.11):

\[
\Delta \text{COD}(d) = Q_{\text{inf}} (\text{COD}_{\text{inf}}) - (Q_{\text{inf}} - Q_{\text{w}}) \text{COD}_{\text{Eff}}
\]  

(2.11)

Where:
- \(\Delta \text{COD}\) = substrate removal rate at day \(d\), in [g COD d\(^{-1}\)]
- \(\text{COD}_{\text{inf}}\) = influent substrate concentration, in [g COD m\(^{-3}\)]
- \(\text{COD}_{\text{Eff}}\) = effluent substrate concentration, in [g COD m\(^{-3}\)]

The cumulative COD removal is given by equation (2.12):

\[
\text{COD Removed} = \sum_{d=1}^{d} \Delta \text{COD}(d)
\]  

(2.12)

The observed yield can be defined as the ratio between the cumulative sludge production and cumulative COD removed over a certain period of time using equation (2.13) (Déléris et al., 2002; Camacho et al., 2005):

\[
Y_{o,bs} = \frac{\sum_{d} P_{X, RSS}(d)}{\sum_{d} \Delta \text{COD}(d)}
\]  

(2.13)

Alternatively, the observed yield can be defined as the slope of the linearisation (not forced through zero) of the correlation between cumulative sludge production rate and cumulative removed COD over a certain period of time (Strünkmann et al., 2006).

Typical values of the observed yield for the activated sludge process for different wastewater characteristics, treatment and solids retention times are presented in Figure 2-5.
Figure 2-5: Examples of observed yields during the secondary activated sludge treatment for domestic wastewater without and with primary settling, and for a high strength industrial wastewater. Observed yield values were calculated in accordance with equation (2.7), as a function of SRT, influent COD and nbVSS concentrations, (adapted from Tchobanoglous et al. (2003) and Stensel et al. (2004))
2.3.5 **Sludge reduction mechanisms**

In order to reduce the production of biomass, wastewater processes must be engineered to (Low and Chase, 1999a; Wei *et al.*, 2003; Paul *et al.*, 2006):

- Divert substrate uptake from assimilation for biosynthesis to fuel exothermic, non-growth activities
- Increase mineralisation of slowly or recalcitrant biodegradable organic compounds
- Avoid any significant deterioration of the effluent quality and residual sludge.

Different technologies have been investigated to implement sludge reduction strategies. Most of these technologies are based on the exploitation and/or enhancement of the following sludge reduction mechanisms (Table 2-4) (Liu and Tay, 2001; Pérez-Elvira *et al.*, 2006):

- Lysis-cryptic growth
- Increased maintenance metabolism
- Enhanced metabolism
- Uncoupling metabolism
- Predation on bacteria or grazing.

Table 2-4 summarises examples of different technologies used to implement sludge reduction mechanisms. Even if it is often impossible to make direct comparisons between different studies because there is great variation in terms of plant size, operational conditions and wastewater characteristics, information given in Table 2-4 can be useful to make some considerations:

- Many technologies based on ultrasound, mechanical, chemical, thermal, biological treatment, or even combinations of these treatments, i.e. hybrid treatments, are available to disintegrate sludge and achieve sludge reduction by lysis-cryptic growth. The main advantages of these technologies are: (1) the treatment efficiency and performance can be achieved by regulating the amount of energy input or chemical dosage in a reasonable easy and flexible way and (2) the retrofit of these technologies in already existing WWTPs is considered reasonably
straightforward. On the other hand, phosphorus removal must be implemented using side-stream treatment because biological removal is not feasible with these treatments due the presence of the sludge disintegration step (Stensel and Strand, 2004)

- The use of ozone is the method most frequently investigated and there is common agreement on the high levels of reduction achievable with it: from 46 to almost 100%, at least during the experimental period, when synthetic sewage was used as feed, i.e. in presence of little inorganic solids

- Fewer studies are available on mechanical and ultrasound technologies, with a big variation in the range of sludge reduction achievable (from -28 to 91%), thus suggesting lack of agreement on their potential. The negative values for ultrasound technologies (between -28 and -8%) refer to increases in sludge production when using either lower energy densities (~ 5.2 instead of 103 kJ L\(^{-1}\)) or treating smaller amounts of sludge per day (5 instead of 20 % of the sludge present in the system) (Strünkmann et al., 2006)

- The degrees of reduction achievable with mechanical and ultrasound technologies are generally lower than with ozone and thermal technologies.
Table 2-4: Comparison of different technologies for reducing SAS production (adapted from Wei et al. (2003), Müller et al. (2004), Pérez-Elvira et al. (2006))

<table>
<thead>
<tr>
<th>Technology (grouped by main reduction mechanism)</th>
<th>Advantages</th>
<th>Disadvantages Environmental impact</th>
<th>Sludge reduction [%]</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis-cryptic growth</td>
<td>Successful full-scale experience No significant accumulation of inorganic solids High degree of reduction</td>
<td>High costs involved in ozonation (*) 100% was reported only when using synthetic sewage without inorganic solids</td>
<td>46-100 (*)</td>
<td>(Yasui and Shibata, 1994) (Sakai et al., 1997) (Kamiya and Hirotsuji, 1998) (Déléris et al., 2002) (Boehler and Siegrist, 2004) (Salihi et al., 2003) (Saktaywin et al., 2006) (Huysmans et al., 2001)</td>
</tr>
<tr>
<td>Ozonation</td>
<td></td>
<td>Increase of COD removal rate Bad sludge settling characteristics Formation of THM</td>
<td>65</td>
<td>(Saby et al., 2002)</td>
</tr>
<tr>
<td>Chlorination</td>
<td>Cheaper than ozonation</td>
<td>Decrease of COD removal rate Bad sludge settling characteristics</td>
<td>-</td>
<td>(Canales et al., 1994) (Déléris et al., 2003) (Rocher et al., 2001) (Paul et al., 2006)</td>
</tr>
<tr>
<td>Thermal or thermo-chemical treatment</td>
<td>Relatively simple</td>
<td>Corrosion Subsequent neutralisation Odour problems</td>
<td>37-80</td>
<td>(Strünkmann et al., 2006) (Ginestet, 2006) (Cao et al., 2006) (Zhang et al., 2007a)</td>
</tr>
<tr>
<td>Ultrasound systems</td>
<td>High degree of lysis Good operational stability.</td>
<td>Energy intensive Lower degree of reduction than ozone Decreased dewaterability</td>
<td>-28-91</td>
<td></td>
</tr>
<tr>
<td>Mechanical systems</td>
<td>Good compromise between performance and cost</td>
<td>Limited experience at full-scale Decreased dewaterability</td>
<td>0-72</td>
<td>(Camacho et al., 2002b) (Ginestet, 2006) (Strünkmann et al., 2006)</td>
</tr>
<tr>
<td>Enhanced metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increasing DO</td>
<td>Simple operation</td>
<td>High aeration cost The efficacy of the process is not clear The mechanism is not fully known.</td>
<td>26</td>
<td>(Abbassi et al., 2000)</td>
</tr>
<tr>
<td>Increased maintenance metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane bioreactors</td>
<td>Flexible operation High effluent quality Small footprint</td>
<td>High costs Membrane fouling Poor dewaterability (*) Not feasible to operate membrane bioreactors with complete solids retention in practice</td>
<td>&gt; 95 (*)</td>
<td>(Müller et al., 1995) (Rosenberger et al., 2002a) (Wagner and Rosenwinkel, 2000)</td>
</tr>
<tr>
<td>Uncoupling metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical uncoupler</td>
<td>Relatively simple</td>
<td>Bioacclimation Toxic to environment</td>
<td>40-87</td>
<td>(Chen et al., 2000) (Yang et al., 2003)</td>
</tr>
<tr>
<td>High S0/X0</td>
<td>No addition of materials and energy</td>
<td>Only suitable for high strength wastewater Increase the load of downstream wastewater treatment.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OSA</td>
<td>Only addition of an anaerobic tank</td>
<td>Sometimes high sludge production Further research is needed</td>
<td>50</td>
<td>(Chen et al., 2001) (Chudoba et al., 1992)</td>
</tr>
<tr>
<td>Predation on bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-stage system</td>
<td>Stable operation</td>
<td>High costs Nutrients release.</td>
<td>12–43</td>
<td>(Ratsak et al., 1994)</td>
</tr>
<tr>
<td>Oligochaete</td>
<td>Relatively simple</td>
<td>Unstable worm growth Nutrients release.</td>
<td>10–50</td>
<td>(Rensink and Rulkens, 1997)</td>
</tr>
</tbody>
</table>
2.4 Ultrasound potential for sludge reduction

For sludge reduction purposes, ultrasound treatment generally occurs along the RAS line. By treating part of the RAS, the disintegrated sludge is recycled back into the system where further biodegradation can occur. Sludge solubilisation is supposed to trigger lysis-cryptic growth (Wei et al., 2003; Strünkmann et al., 2006). Changes in the biological activity of the RAS biomass, due to induced stress and floc breakage, might also trigger other reduction mechanisms such as maintenance metabolism (Rai et al., 2004) and enhanced metabolism (Abbassi et al., 2000; Cao et al., 2006), respectively. For this reason, only these three categories will be presented and discussed in details. In the following two sections, ultrasound potential for sludge reduction is assessed on the base of:

- The effects triggered by ultrasound disintegration on sludge biomass. Sludge solubilisation, changes in the biological activity and floc breakage can theoretically lead to different sludge reduction mechanisms. The most significant batch disintegration studies reviewed in the first section generally investigated the disintegration of thickened SAS for the enhancement of anaerobic digestion along the sludge treatment chain. Nevertheless, the same effects, optimisations and operational parameters are valid and applicable to the disintegration of thickened RAS for sludge reduction along the wastewater treatment chain.

- The actual impact of ultrasound treatment on sludge production and process performance. The most significant dynamic studies available in the literature on the use of ultrasound for sludge reduction are reviewed in the second section.
2.4.1 Reduction mechanisms triggered by the effects of ultrasound treatment on activated sludge biomass

Lysis-cryptic growth by sludge solubilisation

During the biological treatment, part of the organic substrate present in the wastewater is assimilated into new sludge biomass, which is then disintegrated using ultrasound. Sludge floc breakage and cell lysis by sludge disintegration release extra/intracellular organic compounds into the medium. This organic autochthonous substrate is then reused in microbial metabolism. The biomass growth that subsequently occurs on this autochthonous substrate cannot be distinguished from the growth on the original organic substrate in the influent wastewater, and is therefore termed as cryptic growth. In spite of this further, cryptic growth, this form of repeated metabolism of the same carbon reduces the overall biomass production. This reduction occurs because during each metabolic process a portion of the carbon is mineralized as products of respiration, i.e. the synthesis yield is always lower than unity (Low and Chase, 1999a; Liu and Tay, 2001; Wei et al., 2003). The steps necessary for the implementation of the lysis-cryptic growth mechanism can be described using the schematics presented in Figure 2-6.
There are two stages in lysis-cryptic growth: lysis and biodegradation. The rate-limiting step of lysis-cryptic growth is the lysis stage, and an increase of the lysis efficiency can therefore lead to an overall reduction of sludge production. Ultrasound disintegration has been recognised as a powerful method for the break-up of microbial cells to extract intracellular material for a long time (Harrison, 1991; Save et al., 1994). During the disintegration process (Figure 2-7), the total COD, defined as the sum of particulate, truly soluble and colloidal COD, is constant, but the application of increasing energy inputs, leading first to floc breakage and then to cell lysis, causes the progressive release of soluble and colloidal COD, i.e. cell lysate, with a correspondent decrease in particulate COD. The sum of soluble and colloidal COD is generally referred to as cell lysate, or simply, lysate.
Chapter 2: Literature review

Bacteria

Intracellular compounds

Extracellular compounds

Inert solid

Cell membrane

Sludge floc

Untreated sludge

Floc breakage
(from 0 to ~32 kJ L\(^{-1}\))

Cell lysis
(> 32 kJ L\(^{-1}\))

Figure 2-7: Model of sludge disintegration by ultrasound with increasing energy inputs according to Lehne et al. (2001)

The release of cell lysate triggers the second stage of the lysis-cryptic growth: biodegradation. Therefore, the estimation of the biodegradability of lysate is a critical parameter to assess the potential of sludge reduction by lysis-cryptic growth. According to Rocher et al. (1999), around 75% of the carbon released after ultrasound treatment was removed after an incubation period of 24 h.

In the literature, the most common method to quantify sludge solubilisation is called the “Degree of disintegration” or “Degree of soluble SCOD release” (DD\(_{\text{COD}}\)) (Nickel, 1999; Rai et al., 2004). It involves the evaluation of soluble COD in accordance with equation (2.14):

$$DD_{\text{COD}}[\%] = \left( \frac{\text{COD}_{\text{tr}} - \text{COD}_{\text{ut}}}{\text{COD}_{\text{NaOH}} - \text{COD}_{\text{ut}}} \right) \cdot 100$$

(2.14)

Where:

- \(\text{COD}_{\text{tr}}\) = soluble COD of sludge treated with ultrasound, in [mg COD L\(^{-1}\)]
- \(\text{COD}_{\text{ut}}\) = soluble COD of untreated sludge, in [mg COD L\(^{-1}\)]
COD$_{\text{NaOH}} = $ soluble COD of sludge chemically hydrolysed, i.e. the alkaline hydrolysis of sludge (Nickel and Neis, 2007), in a 0.5 M NaOH solution at 20 °C for 22 h, in [mg COD L$^{-1}$]

The soluble COD (SCOD) is commonly defined as the sum of the truly soluble COD and the part of colloidal COD which is still present after filtration of sludge samples on a 0.45 μm membrane (Mamais et al., 1993; Andreottola and Foladori, 2006; Zhang et al., 2007b).

For a better comparison, the results from different studies on the use of ultrasound have been merged together in a single graph (Figure 2-8). The reasons for the very different outcomes must be found in the properties of the treated sludge, the operational procedure followed for the disintegration and in the kind of equipment used. The graph shows that with some configurations, level of disintegration up to 25% can be achieved with energy densities around 50 kJ L$^{-1}$ (Bougrier et al., 2005).

![Figure 2-8: Comparison of results from different studies in terms of degree of soluble COD release](image)

Figure 2-8: Comparison of results from different studies in terms of degree of soluble COD release
The degree of SCOD release has also been used to:

- Understand the influence on the disintegration process of various ultrasound operational parameters such as: intensity, solids content and temperature
- Compare the efficiency of other sludge disintegration efficiency.

**Role of power intensity during sludge disintegration**

Most of studies suggest that, at a specified energy input, the degree of SCOD release is higher when higher power intensities are used (Neis *et al.*, 2000; Grönroos *et al.*, 2005; Nickel; Zhang *et al.*, 2007b). The highest variation was found by Grönroos *et al.* (2005) who detected an almost 7-fold increase in SCOD release with a 3.5 increase in intensity, working at 27 kHz and 97 kJ L\(^{-1}\). The explanation given was that the higher mechanical shear forces produced at higher intensities cause the lysis of more microorganisms. The energy transfer efficiency is supposed to decrease after a critical value of intensity due to decoupling effects between the motion of the radiating surface and the liquid (Neis *et al.*, 2001). This critical value can only be assessed experimentally because it is very dependent on the specific equipment used. According to Lehne and Müller (1999), the decoupling effects must occur only at very high power intensities because they observed a decrease in the degree of disintegration only for intensities above 130 W cm\(^{-2}\).

**Role of solids content during sludge disintegration**

There is common agreement on the fact that effective ultrasound disintegration is obtained by treating thickened sludge, i.e. sludge with a high solids content, for the following reasons (Neis *et al.*, 2000; Lehne *et al.*, 2001; Eder and Günther, 2002; Gonze *et al.*, 2003):

- More solids present in the liquid deliver more cavitation sites and, by acting as nuclei, they decrease the threshold of cavitation and promote it
- The statistical probability that a solid is affected by the mechanical jet streams during bubble implosion increases with higher solids concentration.

Depending on the equipment used, there might be an optimum solids concentration beyond which the homogeneous distribution of the acoustic waves is disturbed by absorption effects, thus reducing cavitation efficiency (Neis *et al.*, 2001). The
optimum range of concentration must be quite high because Eder and Günthert (2002) found an almost linear increase in SCOD release when the sludge solids content was increased up to a concentration of 4% DS.

Role of temperature during sludge disintegration

The release of SCOD seems to be enhanced by an increase in temperature (Save et al., 1994; Chu et al., 2001; Grönroos et al., 2005). According to Chu et al. (2001), bulk temperature is an essential factor in achieving sludge solubilisation and found an average 1.7-fold increase in SCOD release every 10 ºC increase in temperature. Save (1994) suggested as an explanation that temperature makes bacterial cells more susceptible the cavitation forces, most likely by weakening bacteria cell walls.

Comparison with other sludge disintegration technologies

In most of studies comparing different sludge disintegration technologies, ultrasound treatment tends to be among the ones achieving the highest degree of SCOD release, but is often also the most energy intensive one (Springer et al., 1996; Kopp et al., 1997; Müller et al., 1998; Müller, 2000a; Winter, 2002; Boehler and Siegrist, 2006). At the same energy input, the degree of SCOD released by ultrasound was 2-3 times lower than the SCOD released by other mechanical technologies (Müller, 2000b) and 2 times lower than the SCOD released by ozone (Boehler and Siegrist, 2006). Among the mechanical technologies, high pressure homogenizers based on hydrodynamic cavitation are the best performing in terms of energy consumption, even at full-scale level (Froud et al., 2005). More than on its energy efficiency, the strong points about ultrasound treatment are based on (Lehne et al., 2001; Müller, 2001; Andreottola and Foladori, 2006):

- The extent of research experiences
- The advanced stage of development and optimisation for its full-scale application in WWTPs
- Good operational stability.

Increased maintenance metabolism by induced stress

Stress induced by ultrasound action on microorganisms might be able to change the biological activity of the treated microorganisms due to an increase in the energy
needed for their maintenance metabolism (Rai et al., 2004). If maintenance metabolism is increased, more organic substrate must be used to generate energy during the respiration process and less is assimilated for growth because microorganisms satisfy their maintenance energy requirements in preference to producing additional biomass. This diversion of energy from biosynthesis to non-growth activities decreases the biomass synthesis yield and, therefore, the overall sludge production (Figure 2-9) (Low and Chase, 1999b).

\[
\Delta \text{COD} = Y(1-Y) - r_S - q_m X
\]

Where:
- \( r_X \) = rate of sludge biomass production in [g VSS L\(^{-1}\) h\(^{-1}\)]
- \( Y \) = synthesis yield in [g VSS g\(^{-1}\) BOD or COD]
- \( r_S \) = rate of substrate uptake in [g BOD or COD L\(^{-1}\) h\(^{-1}\)]
- \( q_m \) = specific substrate uptake related to maintenance energy requirements in [g BOD or COD g\(^{-1}\) VSS h\(^{-1}\)]

Figure 2-9: Schematics of the increased maintenance metabolism due to stress induced by ultrasound action on sludge biomass

Low and Chase (1999b) modelled this specific metabolic behaviour using equation (2.15) to describe the mass balance on the utilization of the energy source, i.e. the organic substrate:
X = sludge biomass concentration in [g VSS L\(^{-1}\)]

The most obvious way to exploit the behaviour described by equation (2.15) to reduce the production of sludge biomass is to increase the biomass concentration X while keeping a constant supply of substrate, i.e. to decrease the food to microorganism (F/M) ratio. High biomass concentrations can be achieved by decreasing the solids retention time (SRT). These conditions enhance maintenance phenomena involving bacterial reactions like death, lysis and endogenous metabolism (Canales et al., 1994; Henze et al., 2002) and can lead to a situation in which the amount of energy provided by the supply of substrate equals the maintenance demand. Similar conditions can be achieved with membrane bioreactors (MBRs) operated at high SRTs and mixed liquor concentrations. Using these systems, when the F/M ratio becomes low enough, little or no SAS is produced because microorganisms utilize a growing proportion of feed for maintenance purposes and, consequently, less for growth (Canales et al., 1994; Wagner and Rosenwinkel, 2000; Cicek et al., 2001; Yoon et al., 2004). MBRs can be operated with long SRTs because SRT can be controlled completely independently from hydraulic retention time (HRT) by using membranes instead of relying on clarifiers for the separation of sludge and effluent. Typical SRTs for MBRs are between 5 to 20 days (Tchobanoglous et al., 2003). However, during studies for sludge reduction the SRTs applied varied from more than 50 days (Choo and Stensel, 2000; Strünkmann et al., 2006) to situations of complete solids retention for periods longer than one year (Rosenberger et al., 2002b; Pollice et al., 2004; Yoon et al., 2004). In practice, there are limits to the solids retention times applicable in order to keep an optimal biomass concentration range and avoid excessive aeration costs and membrane foulings (Wei et al., 2003; Pérez-Elvira et al., 2006).

Unfortunately, it is not possible to increase the sludge concentration significantly in conventional activated sludge (CAS) processes where separation of sludge from the effluent is based on sedimentation. Nevertheless, as Rai et al. (2004) suggested, any form of stress induced on microorganisms should be able to increase the portion of substrate used for fuelling maintenance metabolism, i.e. to increase \( q_{m} \) in equation (2.15). Stress can cause lysis or damage to microorganisms, which results in complete or partial loss of their ability to consume oxygen, i.e. biological inactivation. Sludge
inactivation followed by recovery of biological activity (Figure 2-10) is considered a sign of increased maintenance metabolism that could eventually lead to some form of sludge reduction (Camacho et al., 2002a; Rai et al., 2004), while persistent inactivation is considered a sign of cell lysis (Neis et al., 2001; Camacho et al., 2002b).

Figure 2-10: Example of oxygen uptake rates of treated and untreated sludge under the hypothesis of enhanced maintenance metabolism due to induced stress by ultrasound action

By measuring the maximum oxygen uptake rate (OUR), in [mg O₂ min⁻¹], of the treated (OURₜᵣ) and untreated sludge (OURᵤᵣ), the degree of inactivation can be calculated as follows (Camacho et al., 2002b):

\[
\text{DD}_0[\%] = 1 - \left[ \frac{\max(\text{OUR}_\text{tr})}{\max(\text{OUR}_\text{uᵣ})} \right] \cdot 100
\]  

The best two examples of evaluation of maintenance metabolism by assessing changes in the oxygen uptake can be found in the work of Camacho et al. (2002b) and Rai (2004). Camacho et al. (2002b), instead of an ultrasound system used a high pressure homogenizer. They found no sign of maintenance metabolism even working at high pressure (700 bars). The biological inactivation detected at high energy
densities (> 80% at around 162 kJ L⁻¹) was permanent and was attributed to cell lysis. Rai et al. (2004) investigated the presence of increased maintenance needs in sludge treated with ultrasound using respirometry. They added a known amount of synthetic sewage to both untreated and treated sludge samples and used the OUR curves to evaluate their overall oxygen uptake, i.e. the areas below the OUR curves between substrate addition at time \( t_1 \) and the end of its consumption at time \( t_2 \) (Figure 2-10). They estimated the synthesis yield of untreated and treated sludge samples in accordance with equation (2.17):

\[
Y = 1 - \frac{\int_{t_1}^{t_2} (\text{OUR}_{\text{total}} - \text{OUR}_{\text{endog}}) \, dt}{\text{COD}_{\text{removed}}}
\]  

(2.17)

Where:

- \( \text{OUR}_{\text{endog}} \) = sludge endogenous respiration rate after 12h starvation in aerated conditions, in [mg O₂ L⁻¹ min⁻¹]
- \( \text{OUR}_{\text{total}} \) = respiration rate of sludge under test, in [mg O₂ L⁻¹ min⁻¹]
- \( t_1 \) = time at which the synthetic sewage was added, in [s]
- \( t_2 \) = time at which all the synthetic sewage was biodegraded, in [s]
- \( \text{COD}_{\text{removed}} \) = amount of COD removed between \( t_1 \) and \( t_2 \), in [g COD L⁻¹]

Using equation (2.17), an increase in oxygen uptake was considered a sign of enhanced maintenance needs and translated into a lower yield. After estimating the synthesis yield for untreated and treated sample with equation (2.17), they calculated the yield reduction with equation (2.18):

\[
\Delta Y[\%] = \left[ 1 - \frac{Y_{\text{tr}}}{Y_{\text{ut}}} \right] \cdot 100
\]  

(2.18)

Following this procedure they found an interesting comparison between specific energy inputs, degree of COD release (DD\textsubscript{COD}), degree of inactivation (DD\textsubscript{O}), synthesis yield (Y) and sludge grow reduction (\( \Delta Y \)) (Table 2-5). According to their results, a 14 % degree of sludge reduction was found at around 32 kJ L⁻¹, this
reduction went up to ~29% at 256 kJ L\(^{-1}\). These results are difficult to scale-up because they are only based on batch tests. Nevertheless, they suggest that there might be a limit to the degree of sludge reduction achievable by sludge disintegration with ultrasound without incurring in excessive costs due to energy consumption: an 8-fold increase in the energy input (from 32 to 256 kJ L\(^{-1}\)) resulted in a 2-fold increase in the potential sludge reduction (from 14 to 28.7%).

<table>
<thead>
<tr>
<th>Energy density [kJ L(^{-1})]</th>
<th>DDO [%]</th>
<th>DD(_{\text{COD}}) [%]</th>
<th>Sludge growth reduction ((\Delta Y)) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>-2.8</td>
<td>4.2</td>
<td>14</td>
</tr>
<tr>
<td>96</td>
<td>29</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>160</td>
<td>38</td>
<td>12</td>
<td>25.4</td>
</tr>
<tr>
<td>160</td>
<td>49</td>
<td>10</td>
<td>27.5</td>
</tr>
<tr>
<td>256</td>
<td>54</td>
<td>25</td>
<td>28.7</td>
</tr>
</tbody>
</table>

**Enhanced metabolism by floc breakage**

Floc breakage, followed by size reduction and release of extracellular compounds is the first effect of ultrasound disintegration on sludge biomass (Figure 2-7) (Neis and Blume, 2002). Floc breakage and size reduction due to ultrasound action has been reported to improve transfer processes and diffusion of substrate, oxygen and other nutrients within the floc matrix (Friedrich, 2002). This increased diffusion is supposed to enhance hydrolysis of dead cells inside the floc (Figure 2-11). This enhanced metabolism based on sludge self-consumption should lead to a decrease in the overall sludge production (Abbassi et al., 2000; Cao et al., 2006).
Aerobic conversion of bulk organic substrate

Aerobic conversion of lysate released by cell lysis in the anaerobic area

Anaerobic inactive area: cell lysis with little/no conversion of lysate

Enhanced aerobic conversion of lysate due to increased oxygen diffusion within the smaller flocs

Figure 2-11: Schematics of enhanced aerobic metabolism due to floc breakage

There is not common agreement on the enhanced metabolism as a viable reduction mechanism. Liu and Tay (2001) and Pérez-Elvira et al. (2006) suggested it needed further investigation because of the conflicting results in the literature regarding the effects of increased oxygen concentrations on sludge production. The degrees of sludge reduction observed during high dissolved oxygen processes varied from 0 to 66%. Furthermore, it must be noted that, to prove their hypothesis, Abbassi et al. (2000) increased the oxygen diffusion within the floc by increasing the aeration, not by reducing the floc size. Therefore, there is no direct evidence that a reduction in floc size achieved by ultrasound or any other treatment can actually trigger some form of enhanced metabolism either. In the literature, the effects of sludge disintegration on floc structure are investigated in terms of microscopy analysis and, most of time, floc size analysis. For a better comparison, the outcome of floc size analysis from different studies has been merged in a single graph (Figure 2-12). This graph shows that, regardless the initial floc size, in all studies most of the reduction in floc size, up to 80%, was reached in the first stage of ultrasound treatment, with less than 40 kJ L^{-1}. 
Tiehm et al. (2001) and Rai et al. (2004) did observe signs of enhanced metabolism in terms of increased oxygen uptake during the first stages of ultrasound disintegration. Tiehm et al. (2001) and Rai et al. (2004) reported, respectively, a 10 and 5% increase in the oxygen uptake of the treated samples in comparison to the untreated ones. Even if this increase seems in agreement with the hypothesis of Abbassi et al. (2000), another explanation was also proposed. The enhanced metabolism detected could be the consequence of the fact that, during treatment at low energy inputs, no cell lysis occurs, flocs open up and release microorganisms that previously were not contributing to the overall respiration rate. Nevertheless, it is worth investigating further the potential of achieving sludge reduction by floc breakage because this effect occurs earlier and requires lower energy inputs than those necessary to achieve the high degrees of solubilisation required to implement the lysis-cryptic growth mechanism (Friedrich, 2002; Neis and Blume, 2002).

Figure 2-12: Effect of ultrasound treatment on median floc size
Batch predictive tests for sludge reduction

It would be very important and useful to develop a series of batch predictive tests to provide estimation of the sludge reduction achievable using ultrasound or any other technology. The predictability of sludge reduction was investigated during the research and development project “Ways of Innovation for the Reduction of Excess Sludge” (WIRES) (Ginestet, 2006), a 3-year project funded by the European Commission.

The predictive tests investigated during the WIRES project were based on the two parameters that are generally used to quantify the main effects of sludge disintegration:

- The degree of sludge solubilisation
- The degree of biological inactivation.

WIRES investigation resulted in the following considerations:

- Sludge solubilisation and biological inactivation can give an important insight on the sludge disintegration process but are not representative enough to be used as parameters for the prediction of the sludge reduction achievable
- Solubilisation does not necessarily imply the biodegradation of the released compounds during the activated sludge process. A potential critical parameter could be the ability to enhance sludge biodegradability and further research should be directed towards its assessment
- Synergistic effects leading to sludge reduction during the activated sludge process might be difficult to take into account properly by simple batch tests.
2.4.2 Impact on sludge production and the activated sludge process

It is not an easy task to assess sludge reduction due to the intrinsic variability of biological systems. For example, Paul and Salhi (2003) found a 30% variation in sludge production when running for two years a lab-scale pilot plant fed with real urban wastewater. The comparison with a control system is therefore essential to provide significant information. Once the observed yield of the test system ($Y_{OBS,TEST}$) and the control system ($Y_{OBS,CTRL}$) are known, the reduction of sludge production $RSP[\%]$ can be calculated according to the equation (2.19):

$$RSP\[%\] = \left(1 - \frac{Y_{OBS,TEST}}{Y_{OBS,CTRL}} \right) \times 100$$  \hspace{1cm} (2.19)

$RSP\ [%]$, which is also referred to as the degree of sludge reduction, gives positive values when a reduction in sludge production in the test system occurs. Negative values would mean that the sludge production in the test system is actually higher than in the control one, i.e. $Y_{OBS,TEST}$ is greater than $Y_{OBS,CTRL}$.

The most significant and pertinent studies to this project are listed in Table 2-6. Considering the limited number of studies based on ultrasound, those based on hydrodynamic cavitation were also included. The mechanical action of cavitation-based technologies is supposed to trigger the same effects (Moholkar et al., 1999). Studies in Table 2-6 used a variety of operational conditions, equipment and plant size not only to evaluate the potential of acoustic and hydrodynamic cavitation for sludge reduction but also their impact on process performance. When possible the operating conditions used were reported in terms of energy input and stress factor (SF) according to the equation (2.20):

$$SF\ [d^{-1}] = \frac{\text{Amount of sludge treated per day in [g or kg d$^{-1}$]}}{\text{Amount of sludge in the system in [g or kg]}}$$  \hspace{1cm} (2.20)
Table 2-6: Summary of studies on sludge reduction using ultrasound and mechanical systems

<table>
<thead>
<tr>
<th>Technology (Reference)</th>
<th>Operational conditions</th>
<th>Sludge reduction [%]</th>
<th>Comments/Impact on process performance/Reduction mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound (UH)</td>
<td></td>
<td></td>
<td>Reduction from HPH much higher than values found by:</td>
</tr>
<tr>
<td>High pressure homogeniser (HPH) (Strünkmann et al., 2006)</td>
<td>UH: 2-47 MJ kg(^{-1}) / 5-117 kJ L(^{-1}) HPH: 650 bar HPH: SF=0.2 Lab-scale plant fed with primary settled sewage Volume: 36 L SRT varied to keep same TSS in ML</td>
<td>UH: 28-24 HPH: 72</td>
<td>• Ginestet (2006): 0 • Camacho (2002b): 24 Increased sludge production at low energy inputs with UH COD increase in effluent supernatant Nitrogen removal partly deteriorated Lysis-cryptic growth as reduction mechanism</td>
</tr>
<tr>
<td>Ultrasound (Cao et al., 2006)</td>
<td></td>
<td>44</td>
<td>Slight COD-removal decrease Little influence on NH(_4) removal Increase in effluent NO(_3) and P Decrease in SVI Decrease in ratio VSS/TSS Lysis-cryptic growth and enhanced metabolism as reduction mechanisms</td>
</tr>
<tr>
<td>Ultrasound (Zhang et al., 2007a)</td>
<td>144-216 kJ L(^{-1}) / 72-108 MJ kg(^{-1}) SF = 0.21 SBR lab-scale plant fed with synthetic sewage Volume: 7 L SRT varied to keep same TSS in ML</td>
<td>43-91</td>
<td>Very high energy densities were used to achieve 91.1% reduction: 216 kJ L(^{-1}) with an 11-fold increase in test SRT to keep same TSS in test system Increase in effluent COD, NH(_4) and P Slight increase in SVI and turbidity Stable ratio VSS/TSS</td>
</tr>
<tr>
<td>Ultrasound (UH)</td>
<td></td>
<td></td>
<td>Minimum information on experimental set-up and operational conditions was given Difficult to compare, evaluate and scale-up results Reduction from UH higher than other studies Zero reduction from HPH is in completely contrast with other studies</td>
</tr>
<tr>
<td>High pressure homogeniser (HPH) (Ginestet, 2006)</td>
<td>UH: 105 kJ L(^{-1}) HPH: 42 MJ kg(^{-1}) HPH : 600 bar HPH: SF = 0.2 Plant volume not specified Fed with settled sewage SRT not specified</td>
<td>UH: 68 HPH: 0</td>
<td>Decrease in TSS removal Slight COD-removal decrease Slight NH(_4)-removal decrease Increase in turbidity Decrease in SVI</td>
</tr>
<tr>
<td>High pressure homogeniser (Camacho et al., 2002b)</td>
<td>Energy input not clear – 30 MJ kg(^{-1}) / –45 kJ L(^{-1}) SF = 0.2 700 bar Pilot-scale plant fed with primary settled wastewater Volume: 200 L Not clear if SRT was kept constant</td>
<td>24</td>
<td>Lysis-cryptic growth as reduction mechanism Maintenance metabolism excluded as reduction mechanism</td>
</tr>
</tbody>
</table>
In relation to full-scale applications, the equipment, set-up and operational conditions used by Strünkmann et al. (2006) and Camacho et al. (2002b) are the most realistic and relevant to this work. The most significant information in their study is presented in Table 2-7.

Table 2-7: Summary of operational conditions and results found by Strünkmann et al. (2006) and Camacho et al. (2002b)

<table>
<thead>
<tr>
<th>Reference</th>
<th>(Strünkmann et al., 2006)</th>
<th>(Camacho et al., 2002b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment type</td>
<td>UH</td>
<td>UH</td>
</tr>
<tr>
<td>Disintegration trials [#]</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>SF [d(^{-1})]</td>
<td>0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Specific energy [MJ kg(^{-1})]</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>Energy density [kJ L(^{-1})]</td>
<td>5</td>
<td>117</td>
</tr>
<tr>
<td>DD(_\text{COD}) [%]</td>
<td>4.2</td>
<td>20</td>
</tr>
<tr>
<td>RSP [%]</td>
<td>-28</td>
<td>-8</td>
</tr>
</tbody>
</table>

Strünkmann et al. (2006), using a 36 L lab-scale plant fed with primary settled sewage, compared ultrasound (UH) with other mechanical devices: a high pressure homogeniser (HPH) and a stirred media mill (SMM).

- The maximum degree of sludge reduction detected was 24%, treating the mixed liquor (ML) at 103 kJ L\(^{-1}\) with a stress factor of 0.2 d\(^{-1}\) (trial 3 in Table 2-7)
- A 28% increase in sludge production was observed using a lower energy density, 5 kJ L\(^{-1}\), with a higher stress factor, 0.4 d\(^{-1}\) (trial 1 in Table 2-7)
- An 8% increase in sludge production was observed using a higher energy density, 117 kJ L\(^{-1}\), with a lower stress factor, 0.05 instead of 0.2 d\(^{-1}\) (trial 2 in Table 2-7.)

 Increases in sludge production were explained by Strünkmann et al. (2006) in terms of metabolic enhancement linked with: (1) increased diffusion of oxygen and substrate supply to the microorganisms within the floc due to floc breakage, in complete disagreement with the hypothesis suggested by Abbassi et al. (2000) and (2) the presence of lysate. However, Strünkmann et al. (2006) did not provide any experimental evidence in support for these hypothesis.
According to the results from their study, mechanical systems performed better than ultrasound: 36% and 72% reductions were achieved with a stirred media mill (SMM, data not shown) and a high pressure homogeniser (HPH, trial 5), respectively, using operational conditions similar to those used during trial 3 with ultrasound. A 68% reduction was achieved using ultrasound during trial 4 but it is not significant because during trial 4 the lab-scale plant was operated like a membrane bioreactor.

In relation to the performance of the test system, the COD removal and effluent quality were not affected because membranes were used to replace the settling tank but the COD in the effluent supernatant did increase from 284 up to 392 mg COD L⁻¹.

Camacho et al. (2002b), using a 200 L pilot-scale plant fed with primary settled sewage, also detected a 24% decrease in sludge production using a high pressure homogeniser (HPH) and treating the ML at ~25 kJ L⁻¹ with a stress factor of 0.2 d⁻¹ (Table 2-7).

Given the realistic experimental conditions used in these two dynamic studies, their results provide a good basis for highlighting some contradictions with the other dynamic studies in relation to:

- Range of sludge reduction detected
- Viability of enhanced metabolism
- Viability of increased maintenance metabolism

**Range of sludge reductions detected**

Focusing on the results from Strünkmann et al. (2006) and Ginestet (2006), a wide range of sludge reductions was observed using both ultrasound and HPHs (Table 2-7). Using ultrasound at around 110 kJ L⁻¹, the degrees of reduction observed varied from 24% for Strünkmann et al. (2006) up to 68% for Ginestet (2006). Using HPH at around 61 kJ L⁻¹, the degrees of reduction observed varied from 72% for Strünkmann et al. (2006) to zero for Ginestet (2006).

Given that both studies were performed at the Technical University of Braunschweig (Germany) as part of the WIRES research project, it is difficult to understand such a wide variations for both ultrasound and HPHs. Further understanding is prevented as
Chapter 2: Literature review

Ginestet (2006) provided little information on the experimental conditions. However, it is clear that variations between 24 and 68 % for ultrasound and, even more, between 0 and 72 % for HPHs can completely change the outcome of an economic analysis.

Viability of the enhanced metabolism

No information on the effects of low energy inputs on floc size during trials 1 and 2 (Table 2-7) was provided by Strünkmann et al. (2006). Nevertheless, considering that even low energy inputs are supposed to significantly decrease floc size, the increase in sludge production found in trials 1 and 2 can be considered in disagreement with the reduction mechanism proposed by Abbassi et al. (2000), i.e. enhanced metabolism by floc breakage.

Viability of the increased maintenance metabolism

Rai et al. (2004) proposed an increase in maintenance metabolism by induced stress as a viable reduction mechanism based on batch disintegration tests and the use of respirometry. On the contrary, Camacho et al. (2002a; 2002b), using a high pressure homogeniser and investigating a similar range of energy inputs, were quite explicit in excluding maintenance metabolism as a possible reduction mechanism when using sludge disintegration technologies based on mechanical action. Interestingly they reported signs of biological inactivation and recovery, i.e. increased maintenance metabolism, only when investigating the potential for sludge disintegration of thermal treatment (Camacho et al., 2005).
2.4.3 Drawbacks to reduced sludge production

The implementation of strategies to reduce sludge production using ultrasound technology involves an increase in capital and operational costs and has an impact on process performance, as described in more details in the following section (Weemaes and Verstraete, 1998; Low and Chase, 1999a; Müller, 2001; Wei et al., 2003).

Oxygen requirements

In conventional activated sludge processes the oxygen transfer yields range from 0.6 to 4.2 kg O\textsubscript{2} kWh\textsuperscript{-1} according to the method of aeration (Horan, 1990). Aeration typically accounts for more than 50% of total plant energy requirements (Groves et al., 1992). Strategies such as lysis-cryptic growth or enhanced maintenance metabolism increase the organic load requiring oxidation thus increasing the oxygen demand and energy costs (Low and Chase, 1999a).

Settling properties

Even if ultrasound treatment is supposed to control bulking sludge and improve settleability (Müller, 2001; Camacho et al., 2002b; Cao et al., 2006), it might happen that any strategy able to reduce biomass production also affects the growth rates of individual species differently and so alter the population dynamics. Altering the stresses on the population dynamics may in turn adversely alter the biomass settling characteristics, e.g. by changing surface chemistry and so causing poor flocculation or encouraging a proliferation of filamentous bacteria leading to sludge bulking (Low and Chase, 1999a; Zhang et al., 2007a).

Conditioning and dewatering

Dewatering is influenced by particle size distribution and extracellular polymer concentration. Optimum dewatering corresponds with lower floc fractal dimension (a more open structure) and a smaller proportion of fines and colloidal material (Yin et al., 2004). Mechanical disintegration can be used to increase the dewatering characteristics by changing the floc structure and reducing the water bound to the sludge matrix. This type of treatment requires a low energy input using any disintegration method. If the particle size has been optimized through disintegration,
the amount of coagulants needed remains almost unchanged while the solids content in the dewatered sludge can be increased (Bien et al., 1997; Kopp et al., 1997; Yin et al., 2004). On the other hand, when disintegration is performed at higher energy inputs to increase sludge biodegradability, it may result in the need of higher doses of coagulant and an overall decrease of the dewatering results (Müller, 2000a; Winter, 2002; Boehler and Siegrist, 2006; Dewil et al., 2006b). Reduced dewaterability is attributed to the reduction in the size of sludge flocs, which provides a greater surface area for the adsorption of water, increasing the bound water content of the treated sludge (Chu et al., 2001; Dewil et al., 2006a). Disruption of bacterial flocs also elicits the release of soluble anionic polymers, which promote inter-particulate repulsion necessitating increased polymer requirements to neutralise floc charges and promote coagulation of sludge solids (King and Forster, 1990). These detrimental effects on dewaterability should be less significant after anaerobic digestion.

**Nutrients removal and effluent quality**

As previously explained, in activated sludge plants, biological nutrient removal processes can be improved by sludge disintegration. Sludge solubilisation due to ultrasound treatment releases high concentrations of dissolved organic carbon that can be added, for example, to the denitrification tank. On the other hand, parts of nutrients are incorporated into sludge biomass, and then withdrawn from the system as SAS. The disintegration of biomass will release nitrogen and phosphorus compounds while the reduction in biomass production will result in less nutrients being removed from water by assimilation into biomass. Therefore, higher nutrient concentrations are expected in the final effluent (Low and Chase, 1999a; Camacho et al., 2002b; Cao et al., 2006; Zhang et al., 2007a). A solution to this problem could be the implementation of separate side stream treatments for the removal and recycling of nutrients from the disintegrated sludge, e.g. through ammonia stripping or phosphor crystallization, taking advantage of the increased nitrogen and phosphorus concentrations released during the disintegration process (Müller et al., 2004).
Other effects on effluent quality

At least a slight decrease in the general quality of the effluent should be expected due to (Müller, 2000b; Camacho et al., 2002a; Gonze et al., 2003; Cao et al., 2006; Strünkmann et al., 2006; Zhang et al., 2007a):

- An increase in SCOD concentration: caused by solubilisation of organic compounds which are not removed by further biodegradation in the aeration tank
- An increase in turbidity: caused by a decrease in TSS removal either for a reduced adsorption capacity of treated activated sludge flocs or the presence of small particles which do not settle well and enter the effluent
- An increase in the concentration of pollutants that are generally bound to sludge flocs (e.g. heavy metals) caused by floc breakage and sludge solubilisation. This increase is supposed to be slight because bound pollutants should be adsorbed again by sludge particles a few minutes after the occurrence of sludge disintegration.
2.4.4 Summary

Ultrasound potential for sludge reduction is based on the disintegration of sludge due to the hydromechanical effects of the acoustic cavitation field (Tiehm et al., 2001). During disintegration, floc breakage, cell lysis and induced stress lead to sludge solubilisation and biological inactivation. Disintegration generally occurs along the RAS line, where part of RAS is treated with ultrasound and recycled back into the activated sludge process. Although this procedure is supposed to mainly trigger lysis-cryptic growth on the organic autochthonous substrate released by sludge solubilisation (Wei et al., 2003; Strünkmann et al., 2006), other reduction mechanisms might occur, such as increased maintenance metabolism by induced stress (Rai et al., 2004) and enhanced metabolism by floc breakage (Abbassi et al., 2000; Cao et al., 2006). RAS disintegration is also supposed to improve other important processes along the wastewater treatment chain (e.g.: biological nutrients removal and bulking sludge control) and along the sludge treatment chain (e.g.: anaerobic digestion, dewaterability) (Müller, 2000b). Ultrasound treatment is considered one of the most powerful methods for the disintegration of sludge: up to 25% of cell lysis can be achieved with energy densities around 50 kJ L⁻¹ (Bougrier et al., 2005). On the other hand, it is also generally more energy intensive than other technologies: around 3 times more than high pressure homogenisers based on hydrodynamic cavitation (Müller, 2000b), and 2 times more than ozone (Boehler and Siegrist, 2006). Despite these disadvantages, process optimisation and scale-up of ultrasound treatment has been achieved, and full-scale installations of ultrasound treatment have been implemented all over the world using high intensity probe systems with power intensities around or greater than 50 W cm⁻² and frequencies around 20 kHz (Enpure, 2008; Ultrawaves, 2008a). The purpose of most of these ultrasound full-scale installations is to improve anaerobic digestion. The use of ultrasound for this application has been studied quite intensively, while few studies are available on ultrasound treatment for sludge reduction.

According to the WIRES report (Ginestet, 2006), it was not possible to predict the potential for sludge reduction on the base of the effects of the selected disintegration treatment on sludge solubilisation and biological activity during batch disintegration
trials. Therefore, sludge reduction can only be assessed during dynamic trials. Only in five studies in the literature, dynamic trials were completed to investigate the potential for sludge reduction of both acoustic and hydrodynamic cavitation. These studies investigated the effects of cavitation-based technology on sludge reduction the process performance. During these dynamic studies, the wide variation of sludge reduction detected, from -28 to 91 %, indicates:

- The risk of increasing sludge production if the wrong operational conditions are used
- The lack of agreement and consistency in assessing the potential for sludge reduction
- The limited understanding of sludge reduction mechanisms
- The overall poor information provided to water utilities.

Beside the fact that the use of different equipment and operational conditions make it more difficult to compare and scale-up results, the reasons for this wide variation among different studies can be found in the following factors:

- Lack of a proper control system or a proper baseline to assess the performance and the reliability of the experimental set-up used for the dynamic trials
- Use of small lab-scale plants fed with synthetic sewage
- Sludge disintegration performed in a batch mode, using lab-scale ultrasound systems and without temperature control
- Use of very high energy inputs, which are unlikely at full-scale and reduce the significance of the detected reductions. High levels of energy input, especially if delivered without temperature control, might also trigger reduction mechanisms that cannot be exploited at full-scale.

Investigating the potential for sludge reduction is not an easy task, also because the intrinsic variability of biological systems can lead up to a 30% variation in sludge production (Paul and Salhi, 2003). Problems and contradictions among studies investigating the potential for sludge reduction of different technologies are quite common. For example, in the report from the American Water Environment Research Foundation (WERF) (Stensel and Strand, 2004) quite a few studies on sludge
reduction based on mechanical systems (Springer et al., 1996; Springer and Higgins, 1999; Wadehra et al., 1999; Camp Dresser & McKee, 2000) were considered inconclusive. The two main reasons were the lack of a proper comparison system and/or the use of operational conditions not suitable for a proper evaluation of sludge reduction.

In relation to a full-scale scenario, among the five studies found in the literature, Strünkmann et al. (2006) and Camacho et al. (2002b) used the most realistic experimental conditions. They detected a maximum sludge reduction of 24 %, which is a relatively low degree of reduction in comparison to the other dynamic studies. Results from the work of Strünkmann et al. (2006) indicated that enhanced metabolism might not be a viable sludge reduction mechanism, while results from the work of Camacho et al. (2002b) suggested that sludge disintegration technologies based on mechanical actions, such as acoustic and hydrodynamic cavitation, might not be able to trigger an increase in maintenance metabolism.

In addition to evaluate the degree of sludge reduction achievable, important drawbacks to the recycle of disintegrated sludge, such as the increased aeration requirements and the potential detrimental effects on sludge dewaterability and effluent quality, must also be taken into account. It is therefore critical to find out if it is possible to achieve an economic viable compromise between the degree of sludge reduction and the cost/impact necessary to achieve it. The specific conditions of each treatment plant might then provide further arguments for or against the application of sludge disintegration by ultrasound. According to the WERF report (Stensel and Strand, 2004), even using mechanical equipment less expensive and energy intensive than ultrasound, benefits from sludge reduction were not able to offset equipment and energy costs unless the total sludge disposal costs were above £691 per tonne DS.

To conclude, the literature review suggests that further research is needed to overcome gaps on knowledge, limitations and contradictions found in the available literature. New studies investigating the ultrasound potential for sludge reduction should use experimental conditions as close as possible to full-scale operations (e.g. use of pilot-scale plants of reasonable size fed with real sewage, use of ultrasound systems designed for full-scale applications, application of continuous in-line disintegration at realistic energy inputs). This further research should lead to:

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• A better understanding of the type and role of the reduction mechanisms implemented by ultrasound treatment
• A relatively straightforward scale-up of the results obtained at pilot-scale
• A proper economic evaluation to investigate not only whether sludge reduction by ultrasound disintegration is technically feasible, but also economically viable.
Chapter 3: Materials and methods

3.1 Introduction

The experimental work carried out was based on batch and dynamic trials:

- **Batch trials** - As part of the batch studies, two groups of batch disintegration trials (the equipment comparison and ultrasound impact trials) were completed on RAS and SAS samples collected from different WWTPs (conventional activated sludge, oxidation ditches, biological nutrient removal) using ultrasound and mechanical disintegration systems. As part of the dynamic studies, a third group of batch disintegration trials (the ultrasound potential trials) was completed to investigate the potential of ultrasound for sludge reduction on RAS samples collected from the control and test lanes of the pilot-scale plant.

- **Dynamic trials** – As part of the dynamic studies, the RAS in the test lane of an activated sludge pilot-scale plant was exposed to continuous in-line ultrasound disintegration to monitor the impact of ultrasound on sludge production and the overall process performance by comparison with the control lane. The seven dynamic trials (the pilot-scale plant trials), comprising a baseline and six disintegration trials, were completed at different energy inputs by treating from 1.7 to 12.5% of the RAS with energy densities between 42 and 168 kJ L^{-1}.

This chapter provides a description of:

- The four disintegration systems (3 ultrasound units plus a mechanical one) used in this project
- The activated sludge pilot-scale plant used during dynamic studies
- The set-up used for the integration of the ultrasound system in the test RAS line of the pilot-scale plant to allow in-line continuous ultrasound treatment
- The batch disintegration trials, in terms of:
  - Parameters used to evaluate the effects of ultrasound treatment
  - Location, date, and type of WWTPs visited
  - Type and solids content of sludge sampled
  - Analysis performed
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- Sludge disintegration procedures for each equipment
- The pilot-scale plant trials, in terms of:
  - Operational conditions used during the ultrasound treatment of the RAS in the test lane
  - Monitoring regime for control and test lanes
  - Parameter used to evaluate impact of ultrasound treatment on sludge production and process performance
- The analytical techniques used during batch and dynamic studies.

3.2 Materials I: Sludge disintegration systems

All the systems tested in this project are used for industrial applications at full-scale. Overall, four different systems were considered for sludge disintegration:

- Three ultrasound systems:
  - Sonix probe
  - Ultrawaves probe
  - Advanced Sonic Processing Systems (ASPS) reactor
- One mechanical system
  - Pilao deflaker.

All four systems were used during the batch studies. The Ultrawaves probe was then integrated into the activated sludge pilot-scale plant test lane for the dynamic studies.

3.2.1 Ultrasound systems

During the batch studies, two high intensity systems (~50 W cm\(^2\)), i.e. the Sonix and Ultrawaves probes, and a low intensity one (~7 W cm\(^2\)), i.e. the ASPS reactor, were evaluated (Figure 3-1).
Figure 3-1: Ultrasound systems (left) and example of their full-scale configuration (right): Sonix probe (top), Ultrawaves probe (middle) and ASPS reactor (bottom)
A summary of the technical characteristics of the ultrasound systems is presented in Table 3-1.

Table 3-1: Characteristics of ultrasound systems

<table>
<thead>
<tr>
<th>Equipment type</th>
<th>Sonix probe</th>
<th>Ultrawaves probe</th>
<th>ASPS reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supplier</strong></td>
<td>Sonico Ltd (UK)</td>
<td>Ultrawaves GmbH (Germany)</td>
<td>Advanced Sonic Processing Systems (USA)</td>
</tr>
<tr>
<td><strong>Transducer type</strong></td>
<td>Piezoelectric</td>
<td>Piezoelectric</td>
<td>Magnetostrictive</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
<td>20 kHz</td>
<td>20 kHz</td>
<td>16 &amp; 20 kHz</td>
</tr>
<tr>
<td><strong>Power input (Max power)</strong></td>
<td>2.2 kW (3 kW)</td>
<td>1 kW (1 kW)</td>
<td>2.4 kW (2.4 kW)</td>
</tr>
<tr>
<td><strong>Max power intensity</strong></td>
<td>NA</td>
<td>50 W cm⁻²</td>
<td>7 W cm⁻²</td>
</tr>
<tr>
<td><strong>Max power density</strong></td>
<td>1.5 kW L⁻¹</td>
<td>0.5 kW L⁻¹</td>
<td>2.5 – 11.7 kW L⁻¹</td>
</tr>
<tr>
<td><strong>Reactor volume</strong></td>
<td>1.5 L</td>
<td>2 L</td>
<td>0.205 – 0.945 L</td>
</tr>
<tr>
<td><strong>Reactor configuration</strong></td>
<td>Beaker</td>
<td>Flow cell</td>
<td>Flow cell</td>
</tr>
<tr>
<td><strong>Temperature control</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**High intensity systems**

The two high intensity probe systems used in this study were:

- **Sonix probe**: ultrasound probe with a radial horn from Sonico Ltd, UK, a joint venture between Atkins and Enpure Ltd (formally Purac Ltd)
- **Ultrawaves probe**: ultrasound probe from Ultrawaves GmbH, in collaboration with Sonotronic GmbH, Germany.

The main difference between the Sonix and Ultrawaves probes is the design of the horn. Both systems are fitted with piezoelectric transducers, were specifically designed/optimised for sludge disintegration and have already been installed at full-scale in different WWTPs around the world, mainly to enhance anaerobic digestion.

**Low intensity system**

The **ASPS reactor** is an ultrasound dual-frequency reactor for continuous flow operation, with adjustable reactor volumes and temperature control from Advanced Sonic Processing Systems, US. This system has a reactor shaped like a rectangular narrow pipe with two irradiating plates separated by a small gap. The gap between the radiating surfaces could be varied using three different spacers of 6.3, 16 and 29 mm,
to get an active volume of 0.205, 0.52 and 0.945 L. Two banks of magnetostrictive transducers are bonded onto each opposing plate and are driven at two different frequencies: 16 and 20 kHz. Temperature was controlled with cooling manifolds underneath the transducer covers where cold water was injected and flowed off by gravity. This system was not specifically designed for sludge disintegration but for the industrial processing of liquids to enhance a variety of physical and chemical reactions (e.g. degassing, dispersion, mixing, emulsification, extraction) (Suslick, 2008). At the present time, no full-scale installation of this system in a WWTP for sludge disintegration is known.

3.2.2 Mechanical system

A Pilao DTD-10” Spider Deflaker (Pilao deflaker) from Pilao International Ltd, UK was used to compare ultrasound with a mechanical, non-cavitation based technology (Figure 3-2).

![Pilao DTD-10” spider deflaker](image)

Figure 3-2: Pilao DTD-10” spider deflaker

The main technical characteristics of the deflaker used in this study are presented in Table 3-2.
### Table 3-2: Characteristics of Pilao deflaker

<table>
<thead>
<tr>
<th>Equipment type</th>
<th>Pilao deflaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment model</td>
<td>Pilao DTD-10” Spider Deflaker</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Pilao International Ltd (UK)</td>
</tr>
<tr>
<td>Disc size</td>
<td>230 mm</td>
</tr>
<tr>
<td>Stator/Rotor gap</td>
<td>0.6-0.9 mm</td>
</tr>
<tr>
<td>Rotation speed</td>
<td>3000-3600 rpm</td>
</tr>
<tr>
<td>Installed power</td>
<td>50-150 HP</td>
</tr>
<tr>
<td>Installed motor power</td>
<td>30 kW</td>
</tr>
<tr>
<td>Actual power consumption</td>
<td>5.7 kW</td>
</tr>
<tr>
<td>Power density</td>
<td>1.14 kW L⁻¹</td>
</tr>
<tr>
<td>Reactor volume</td>
<td>5 L</td>
</tr>
<tr>
<td>Temperature control</td>
<td>No</td>
</tr>
</tbody>
</table>

Deflakers are already an established technology in the pulp and paper industry. Thanks to their simple, robust design and small footprint, they are considered a potential cheaper “off-the-shelf” mechanical system for sludge disintegration. Instead of cavitation, deflakers exploit the high degrees of fluid shear and turbulence caused by the rotation of a central disc against two adjacent static discs to disperse and homogenise sludge flocs and possibly disrupt sludge bacterial cells.

#### 3.3 Material II: Pilot-scale plant

The pilot-scale plant (PSP) used during dynamic studies was constructed by Balmoral Group (Aberdeen, UK), and located indoors in the pilot hall facilities of the WWTP of Cranfield University, UK.

The pilot-scale plant was operated as a nitrifying/denitrifying activated sludge plant and was comprised of two lanes, operated as a test and control (Figure 3-3 and Figure 3-4).
Chapter 3: Material and methods

Figure 3-3: Pilot-scale plant schematics showing the integration of the ultrasound system along the RAS line of the test lane

Figure 3-4: Side view of the pilot-scale plant
Chapter 3: Material and methods

To keep the same flow rates in both lanes, each flow line was fitted with an independent peristaltic pump. All peristaltic pumps and tubing were purchased from Watson-Marlow Bredel Pumps Limited, Cornwall, UK.

The pilot-scale plant was seeded with RAS taken from a municipal wastewater treatment works (Cotton Valley WWTP, Milton Keynes, operated by Anglian Water). Each lane was initially filled half with RAS collected in the same day, and half with Cranfield University WWTP settled wastewater. After seeding, it was left to stabilise for more than a month.

Each lane was comprised of a reactor tank (890 L) and a final clarifier (330 L). The reactor tank was divided in one aerobic (590 L) and two anoxic areas (first area: 100 L; second area: 200 L). The aerobic areas were supplied with air providing agitation and a dissolved oxygen concentration of 2-4 mg L$^{-1}$. The anoxic areas were kept at a dissolved oxygen concentration lower than 0.2 mg L$^{-1}$ and stirred by Lightnin® Industrial Batch Mixers (Cole-Palmer Instrument Co Ltd., London UK). The minimum temperature of the mixed liquor (ML) in the reactor tank was kept around 18-20 ºC by using three fish tank heaters per lane. Due to seasonal temperature variations occurring inside the unheated indoor test facility, the ML temperature varied between 18 and 24 ºC.

Clarifiers were filled by overflow from the aerobic areas of the reactor tank and fitted with slow rotating scrapers (0.4 rpm) (Cole-Palmer Instrument Co Ltd., London UK). RAS was pumped back at 1000 mL min$^{-1}$ from the base of the clarifiers to the first anoxic areas. During dynamic studies, an ultrasound system was integrated along the RAS line of test lane for continuous in-line RAS disintegration, treating from 1.7 to 12.5 % of the RAS flow.

Both lanes were fed with the Cranfield University WWTP settled wastewater (average TSS, COD, NH$_4$ and NO$_3$: 117±44, 291±71, 32±8 and 2±1 mg L$^{-1}$, respectively) at a flow rate of 1500 mL min$^{-1}$. In addition to settled wastewater and RAS, an acetate solution was also fed into the first anoxic areas (flow rate: 3 mL min$^{-1}$, concentration: 8463 mg COD L$^{-1}$) to provide readily biodegradable substrate to enhance denitrification. The total influent COD (i.e. settled wastewater + acetate solution) entering each lane was 650±158 mg TCOD d$^{-1}$, with 5.7 % of it due to the acetate addition. The first anoxic areas acted as a metabolic selector to reduce the number of
filamentous bacteria and improve denitrification, which otherwise would occur at the bottom of the clarifiers causing floating sludge phenomena and loss of accuracy in yield estimation. The total hydraulic retention time (HRT) of the aerobic and anoxic areas was 10 h. The average COD removal in the control lane was 86.2\%.

SAS was removed directly from the aerobic areas via a peristaltic pump (average SAS flow rate: 28±15 mL min\(^{-1}\)) to simplify and improve yield estimation. The amount of SAS removed was dependent on the daily organic load to keep the level of total suspended solids in the mixed liquor of control lane around 2.8±0.35 g L\(^{-1}\), with a resulting average solids retention time (SRT) of 16±5 days. Unless specified differently, the same SRT was kept in both lanes while the total suspended solids in the ML of test lane varied depending on the energy densities and treatment times used during continuous in-line ultrasound disintegration of the RAS in the test lane.

Most of pilot-scale plant operations were designed to be fully automated. Maintenance routines involved:

- Brush-off sludge solids from the inner walls on a daily basis
- Check and ensure, on a daily basis, proper working status of:
  - Pilot-scale plant aeration, pumps, stirrers and heaters
  - Integrated ultrasound system and flow cell
- Check, and if necessary calibrate, all flow rates and dissolved oxygen twice a week.

A summary of the main characteristics and operational parameters for the test and control lanes can be found in Table 3-3.
### Table 3-3: Pilot-scale plant characteristics and operational parameters

<table>
<thead>
<tr>
<th>Volumes [L]</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeration tanks</td>
<td>590</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First anoxic area (metabolic selector)</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second anoxic area</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Settlers</td>
<td>330</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flow rates [mil min⁻¹]</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Settled wastewater</td>
<td>1500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate solution</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS</td>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAS</td>
<td>28±15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TSS [mg L⁻¹]</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Settled wastewater</td>
<td>117±44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effluent</td>
<td>17±8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ML</td>
<td>2800±350</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS</td>
<td>7140±1470</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>COD [mg L⁻¹]</th>
<th>NH₄ [mg L⁻¹]</th>
<th>NO₃ [mg L⁻¹]</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Settled wastewater</td>
<td>291±71</td>
<td>32±8</td>
<td>2±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate solution</td>
<td>8463±1869</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total influent (settled wastewater + acetate solution)</td>
<td>308±71</td>
<td>32±8</td>
<td>2±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effluent</td>
<td>44±18</td>
<td>0.09±0.09</td>
<td>17±4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| COD removal [g d⁻¹] (%)) | 560±155 (86.2 %) |       |       |       |       |

<table>
<thead>
<tr>
<th>Other operational parameters</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RAS recycle ratio [%]</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRT [h]</td>
<td>10 (aerobic: 6.5, anoxic: 3.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRT [d]</td>
<td>16±5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO [mg L⁻¹]</td>
<td>2-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ML/RAS temperature [ºC]</td>
<td>18-24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


3.4 Material III: In-line ultrasound disintegration system set-up

The in-line ultrasound disintegration system set-up was comprised of (Figure 3-5):

- Ultrasound probe
- Flow cell
- Control panel
- Sound abatement enclosure.

![Figure 3-5: Integration of the Ultrawaves probe and flow cell along the RAS flow line in the test lane](image)

3.4.1 Ultrasound probe

The Sonix probe was impossible to integrate for technical reasons. Between the Ultrawaves probe and the ASPS reactor, the former was integrated for its higher performance and its potential to avoid excessive increases of temperature during sludge disintegration.
The Ultrawaves probe could operate at the very low RAS flow rates (250-1000 mL min\(^{-1}\)) used in the pilot-scale plant without overheating due to the higher energy efficiency of its piezoelectric transducers. Furthermore, its electronics made possible the implementation of intermittent operations, i.e. cycles of ultrasound treatment followed by cycles with no treatment. By spreading the ultrasound treatment, using shorter but more frequent sludge disintegration cycles, the cooling effect of the untreated sludge flow on the radiating surfaces of the Ultrawaves probe was enhanced.

### 3.4.2 Flow cell

The decision to integrate a full-scale ultrasound system into the pilot-scale plant was taken to allow a more reliable scale-up of the results. The main problem was to combine the use of an industrial system with the low flow rates characterising the operations in a pilot-scale plant. The only solution was to design a flow cell specifically engineered to be coupled with the Ultrawaves probe for the in-line treatment of the RAS flowing in the pilot-scale plant.

The two main critical design criteria taken into consideration were: (1) the prevention of any form of sludge biomass accumulation within the flow cell itself without compromising (2) the disintegration efficiency of the Ultrawaves probe.

**Prevention of sludge biomass accumulation**

In the Ultrawaves full-scale flow cell, the sludge flows against the probe (Figure 3-6). This type of configuration is implemented in most industrial ultrasound systems because it is considered the most efficient way to disintegrate sludge by cavitation. The configuration of the Ultrawaves full-scale flow cell for sludge disintegration consists of a 25 L reactor divided in five communicating chambers with similar geometry. Each of these chambers is fitted with one Ultrawaves probe.

The initial pilot-scale plant flow cell was therefore designed in collaboration with the Ultrawaves engineers to replicate the configuration of one the five chambers that make up the full-scale unit (Figure 3-6).

The integration of this first ~5 L flow cell in the pilot-scale plant was not successful. The low RAS flow rates and the geometry of the outlet channel were preventing the biomass from flowing out leading to biomass settling and accumulation at the bottom
of the flow cell. This form of accumulation occurred regardless of the way in which the flow cell was positioned, vertically or horizontally.

The accumulation of biomass within the flow cell was totally unacceptable for different reasons. Firstly, it would invalidate the biomass balance necessary to investigated changes in the yield. Secondly, the prolonged permanence of biomass in the anoxic environment present in the flow cell would influence the biomass population dynamics in the overall system in a way that was difficult to predict. Lastly, the increasing amount of biomass in the flow cell was interfering with the ultrasound operations and led to blockage events. Only the presence of the automatic shutdown system triggered by excessive increases in the transducer temperature prevented the ultrasound system from being seriously damaged (the control system is described in the next section).

On the base of this experience, a second (final) ~2 L flow cell was designed (Figure 3-7) to be operated in the vertical position. To facilitate the outflow of the RAS biomass, a funnel-like geometry was adopted together with the downstream flow of the RAS within the flow cell, i.e. the RAS was flowing along the titanium probe instead of against it.
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Flow cell disintegration efficiency

The design of efficient flow cells and their coupling with ultrasound emitters is of critical importance in achieving the necessary levels of disintegration efficiency and, hence, process optimisation.

A completely new and untested design based on the combination of a funnel-like geometry with the downstream flow could have potentially led to reduced disintegration efficiency. The loss of disintegration efficiency would be caused by two main factors: (1) presence of cavitation-free zones and (2) degassing side effects.
The presence of cavitation-free zones would allow the biomass to by-pass the cavitational field generated by the Ultrawaves probe. This problem is generally studied using computational fluid dynamics. Due to time and economical constraints, a more straightforward approach was used. The sonochemical luminescence of ~0.1 g L$^{-1}$ Luminol solutions is able to visualise cavitational fields. The request for providing a picture of the Ultrawaves probe operated within a Luminol-solution was kindly accepted by the Ultrawaves engineers. The shape and dimensions of the flow cell geometry were therefore determined to maximise the exposure of the RAS biomass by implementing a close fit with the cavitational fields highlighted by the Luminol solution.

The degassing of liquids is one of the many effects of cavitational fields that finds useful applications in laboratories and industries. The adoption of a vertical position and a downstream flow would eventually lead to the accumulation of air at the top of the flow cell due to the degassing properties of ultrasound on the influent RAS. Once trapped, the air would not be able to leave the flow cell due to the set-up of the ultrasound disintegration unit. With time, the increasing amount of air in the flow cell would decrease the disintegration efficiency due to the increasing exposure of the probe radiating surfaces to the air instead of to the sludge. Eventually, this situation would also lead to overheating because the Ultrawaves probe should only operate when submerged in liquids for proper cooling. To allow the exit of the trapped air, a purge line was added at the top of the flow cell. The purging was carried out by lifting the purge line above the flow cell and then opening the valve fitted at its end until some RAS was flowing out. The valve would then be closed and the spilled RAS recycled into the oxic tanks. This operation was carried out as part of the daily maintenance routines even if it was really necessary once a week.

Once the design was completed (Figure 3-8), the actual flow cell was manufactured by Nuova Isma s.r.l. (Udine, Italy).
3.4.3 Control panel

The control panel was designed to allow continuous monitoring of the RAS flow rate and transducer temperature by means of an ultrasound flow meter and an infrared temperature sensor, respectively. The purpose was to avoid any damage to the ultrasound equipment due to overheating. If the RAS flow rate was lower than half of the expected one or the transducer temperature was greater than 50 °C, the electrical...
power to the ultrasound generator was automatically shut off by programmable electronic alarm units until a reset button was manually operated.

By using the control panel, it was also possible to configure the ultrasound treatment cycles by manually regulating an analogue electronic timer. The implementation of an intermittent operation mode was necessary for three reasons:

- Best results in enhancing biological activities have been achieved varying time intervals with and without ultrasound treatment (Schläfer et al., 2000)
- It allows a more homogeneous treatment in time of sludge biomass
- It keeps temperature under control because during inactive periods sludge entering the system cools down both the flow cell and the probe.

All electronic components in the control panel were purchased from RS Components Ltd, Corby, UK.

3.4.4 Sound abatement enclosure

The ultrasound probe and flow cell were located inside a soundproof box to comply with all the Health and Safety regulations in place at Cranfield University. The soundproof box was purchased together with the ASPS reactor from Sonic Processing Systems (US).
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3.5 Methods I: Batch studies

Two different groups of batch disintegration trials were completed during the batch studies:

- Equipment comparison trials
- Ultrasound impact trials.

3.5.1 Equipment comparison trials

This group of batch disintegration trials was carried out to

- Compare the performance of the Sonix and Ultrawaves probes, ASPS reactor and Pilao deflaker
- Evaluate the effects on sludge disintegration of the following operational and design factors: power intensity, temperature, solids content and horn design.

Outline of the equipment comparison trials

Table 3-4 provides a summary of the equipment comparison trials in terms of WWTP visited, date of collection, sludge and equipment used, analyses and type of investigation performed.
### Table 3-4: Outline of equipment comparison trials

<table>
<thead>
<tr>
<th>Trial number</th>
<th>WWTP (type)</th>
<th>Date</th>
<th>Sludge type and TSS</th>
<th>Equipment Analyses</th>
<th>Rationale/ Parameters investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Derby (BNR)</td>
<td>05/11/05</td>
<td>RAS: 9.3 g L(^{-1}) SAS: 56.7 g L(^{-1})</td>
<td>Equipment Analyses</td>
<td>Equipment Sonix probe ASPS reactor Pilao deflaker Analyses TSS SCOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Compare the performance of two ultrasound systems and a mechanical one in terms of sludge solubilisation. Effects on sludge disintegration due to:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Power intensity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Temperature</td>
</tr>
<tr>
<td>2</td>
<td>Brampton (OD)</td>
<td>28/09/05</td>
<td>RAS: 1.8 g L(^{-1})</td>
<td>Equipment Analyses</td>
<td>Equipment Sonix probe ASPS reactor Analyses TSS SCOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Further investigation on the effects on sludge disintegration due to:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Power intensity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Temperature</td>
</tr>
<tr>
<td>3</td>
<td>Cranfield pilot-scale plant (CAS)</td>
<td>06/04/07</td>
<td>ML: 3.3 g L(^{-1}) RAS: 7.3 g L(^{-1})</td>
<td>Equipment Analyses</td>
<td>Equipment Ultrawaves probe Analyses TSS SCOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Investigation on the effects on sludge disintegration due to:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Solids content</td>
</tr>
<tr>
<td>4</td>
<td>Cotton Valley (CAS)</td>
<td>19/08/05 – 22/08/07</td>
<td>RAS: 7.3 – 8.3 g L(^{-1})</td>
<td>Equipment Analyses</td>
<td>Equipment Sonix probe Ultrawaves probe Analyses TSS SCOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Investigation on the effects on sludge disintegration due to:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Horn design</td>
</tr>
</tbody>
</table>

The equipment comparison trials were performed using all the disintegration systems available. For each system, the actual power usage varied depending on the experimental conditions and procedures applied. A summary of experimental conditions and procedures used during the equipment comparison trials is presented in Table 3-5.
### Table 3-5: Operational conditions and procedures during equipment comparison trials

<table>
<thead>
<tr>
<th>Trial number</th>
<th>Equipment</th>
<th>Power</th>
<th>Reactor volume</th>
<th>Retention times</th>
<th>Flow rates</th>
<th>Energy densities</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>Sonix probe</td>
<td>2300 W</td>
<td>1.5 L</td>
<td>0, 30, 60, 120 s</td>
<td>NA</td>
<td>0, 46, 92, 184 kJ L⁻¹</td>
<td>A 1.5 L beaker was filled with sludge. Sonix horn was then immersed in the</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>beaker and stirred with no temperature control</td>
</tr>
<tr>
<td>1, 2</td>
<td>ASPS reactor</td>
<td>2400 W</td>
<td>0.205 L</td>
<td>0, 30, 60, 120 s</td>
<td>410, 205, 102.5 mL min⁻¹</td>
<td>0, 351, 702, 1405 kJ L⁻¹</td>
<td>Sludge flew through the 0.205 L ASPS reactor by means of a peristaltic pump. ASPS reactor was operated with temperature control except during a replica at retention time of 60 s (i.e. 102.5 mL min⁻¹, 702 kJ L⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>Pilao deflaker</td>
<td>5700 W</td>
<td>5 L</td>
<td>0,120, 300, 600 s</td>
<td>NA</td>
<td>0, 137, 342, 684 kJ L⁻¹</td>
<td>The reactor of Pilao deflaker was filled with 5 L of sludge. The reactor was then sealed and Pilao deflaker was operated without temperature control</td>
</tr>
<tr>
<td>3</td>
<td>Ultrawaves probe</td>
<td>700 W</td>
<td>6 L</td>
<td>0, 360 s</td>
<td>NA</td>
<td>0, 42 kJ L⁻¹</td>
<td>A 6 L beaker was filled with sludge. Ultrawaves horn was then immersed in the beaker and stirred with no temperature control</td>
</tr>
<tr>
<td>4</td>
<td>Sonix probe</td>
<td>2300 W</td>
<td>1.5 L</td>
<td>0, 30, 46, 60, 120 s</td>
<td>NA</td>
<td>0, 46, 69, 92, 184 kJ L⁻¹</td>
<td>A 1.5 L beaker was filled with sludge. Sonix horn was then immersed in the beaker and stirred with no temperature control</td>
</tr>
<tr>
<td>4</td>
<td>Ultrawaves probe</td>
<td>700 W</td>
<td>2 L</td>
<td>0, 120, 240, 480 s</td>
<td>1, 0.5, 0.25 L min⁻¹</td>
<td>0, 42, 84, 168 kJ L⁻¹</td>
<td>Sludge flew through the 2 L flow cell by means of a peristaltic pump. Ultrawaves probe was operated with no temperature control</td>
</tr>
</tbody>
</table>

#### 3.5.2 Ultrasound impact trials

This group of batch disintegration trials using the Sonix probe was carried out to investigate if the impact of the ultrasound treatment on sludge biomass varied significantly depending on the type of WWTP. The sludge samples were collected from WWTPs with three different configurations: (1) conventional activated sludge (CAS); (2) oxidation ditch (OD) and (3) biological nutrient removal (BNR). The effects of ultrasound disintegration on sludge biomass were investigated in terms of floc breakage and sludge solubilisation.
Outline of the ultrasound impact trials

Table 3-6 provides a summary of the ultrasound impact trials in terms of WWTPs visited, date of collection, sludge and equipment used, analyses and type of investigation performed.

<table>
<thead>
<tr>
<th>WWTP (type)</th>
<th>Date</th>
<th>Sludge: TSS [g L⁻¹]</th>
<th>Equipment</th>
<th>Analysis</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Cambridge (CAS)</td>
<td>12/05/05</td>
<td>RAS: 8.1 g L⁻¹</td>
<td>Sonix probe</td>
<td>TSS</td>
<td>Effects of sludge disintegration on:</td>
</tr>
<tr>
<td>• Huntingdon (CAS)</td>
<td>16/20/05</td>
<td>RAS: 6.0 g L⁻¹</td>
<td></td>
<td>SCOD</td>
<td>• Floc breakage</td>
</tr>
<tr>
<td>• Cotton Valley (CAS)</td>
<td>23/05/05</td>
<td>RAS: 6.9 g L⁻¹</td>
<td></td>
<td>Particle size analysis (*)</td>
<td>• Sludge solubilisation</td>
</tr>
<tr>
<td>• Brampton (OD)</td>
<td>28/09/05</td>
<td>1.8 g L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Derby (BNR)</td>
<td>05/11/05</td>
<td>RAS: 9.3 g L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ultrasound impact trials were performed using the Sonix probe according to the experimental condition and procedures described in Table 3-7.

Table 3-7: Operational conditions and procedures during ultrasound impact trials

<table>
<thead>
<tr>
<th>Equipment Power Reactor volume</th>
<th>Retention times Flow rates Energy densities</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonix probe 2300 W 1.5 L</td>
<td>0, 30, 46, 60 s NA 0, 46, 69, 92, 184 kJ L⁻¹</td>
<td>A 1.5 L beaker was filled with sludge. Sonix horn was then immersed in the beaker and stirred with no temperature control</td>
</tr>
</tbody>
</table>

3.5.3 Sludge samples used during the batch studies

All WWTPs visited were treating mixed municipal and industrial wastewaters. All sludge samples were treated with ultrasound and mechanical equipment within 4 hours of collection.
3.5.4 Parameters used during the batch studies

Different ranges of energy inputs were applied during the equipment comparison and ultrasound impact trials depending on:
- The performance of the system under investigation
- The rationale and parameters investigated during each specific trial.

To allow a comparison between batch disintegration trials performed on sludge samples with different equipment, operational modes and solids contents, it was necessary to normalise the way in which energy and the release of soluble COD were presented.

**Energy input**

Energy inputs were always given in terms of energy densities, in \([\text{kJ L}^{-1}]\)

**Release of soluble COD**

During the equipment comparison trials and the ultrasound impact trials, to compare the COD release from sludge samples with different solids content, the “Degree of SCOD release” or “Degree of disintegration” (DD\(_{\text{COD}}\)) was used, as it is the most common index used in the literature (equation (2.14) in the Literature review chapter).
3.6 Methods II: Dynamic studies

As part of the dynamic studies, a combination of batch and dynamic trials were completed to evaluate the impact of ultrasound treatment on the pilot-plant sludge biomass, sludge production and process performance.

3.6.1 Batch disintegration trials: the ultrasound potential trials

During the ultrasound potential trials, the effects on the pilot-scale plant sludge biomass due to batch ultrasound disintegration were investigated and compared with the similar effects due to continuous in-line ultrasound disintegration during the dynamic trials. The effects on the pilot-scale plant sludge biomass were investigated in terms of floc breakage, sludge solubilisation and biological activity.

These effects due to sludge disintegration are of great interest because they can theoretically trigger the three different mechanisms of sludge reduction: enhanced metabolism by floc breakage, lysis-cryptic growth by sludge solubilisation and increased maintenance metabolism by induced stress. Lysate biodegradability was also assessed in comparison to synthetic sewage and Cranfield University WWTP settled sewage. The reason was that the biodegradability of lysate is a key parameter in the evaluation of the sludge reduction mechanism based on the lysis-cryptic growth.

Outline of the ultrasound potential trials

Table 3-8 provides a summary of the ultrasound impact trials in terms of WWTPs visited, sludge and equipment used, analyses and type of investigation performed.
Table 3-8: Outline of the batch disintegration trials completed as part of the dynamic studies

<table>
<thead>
<tr>
<th>WWTP (type)</th>
<th>Equipment</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton Valley (CAS)</td>
<td></td>
<td>Effects of sludge disintegration on:</td>
</tr>
<tr>
<td>Date/Period</td>
<td></td>
<td>• Floc breakage</td>
</tr>
<tr>
<td>Sludge: TSS [g L$^{-1}$]</td>
<td></td>
<td>• Sludge solubilisation</td>
</tr>
<tr>
<td>22/08/07</td>
<td>Equipment:</td>
<td>• Biological activity</td>
</tr>
<tr>
<td>RAS: 8.3 g L$^{-1}$</td>
<td>Ultrawaves probe</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Analyses:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Particle size analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCOD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Respirometric analyses</td>
<td></td>
</tr>
<tr>
<td>Cranfield pilot-scale plant test and control lanes (CAS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15/01/07-04/09/07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS: 6.7-9.6 g L$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The batch disintegration trials were performed using the Ultrawaves probe according to the procedure described in Table 3-9.

Table 3-9: Operational conditions and procedure during the batch disintegration trials completed as part of the dynamic studies

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Retention times</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>Flow rates</td>
<td>Sludge flew through the 2 L flow cell by means</td>
</tr>
<tr>
<td>Reactor volume</td>
<td>Energy densities</td>
<td>of a peristaltic pump. Ultrawaves probe was</td>
</tr>
<tr>
<td></td>
<td></td>
<td>operated with no temperature control</td>
</tr>
<tr>
<td>Ultrawaves probe 700 W 2 L</td>
<td>0, 120, 240, 480 s, 1, 0.5, 0.25 L min$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0, 42, 84, 168 kJ L$^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>

3.6.2 Dynamic trials: the pilot-scale plant trials

The seven pilot-scale plant trials completed as part of the dynamic studies comprised the baseline and six disintegration trials.

The pilot-scale plant was initially seeded and left to stabilise for one month. After stabilisation, a 2-month baseline was completed. In this period, no ultrasound treatment was applied to the test lane. The sludge production and performance of the control and test lanes were monitored to evaluate differences due to the natural variability present in any biological system. In the following six months, six disintegration trials were completed during which the RAS in the test lane was exposed to continuous in-line ultrasound disintegration. RAS disintegration was performed using different ultrasound operational conditions (i.e. different energy densities and treatment periods) to evaluate the impact of ultrasound treatment on
sludge production and process performance and investigate the role played by different sludge reduction mechanisms.

**Analyses and monitoring regime**

Control and test mixed liquor, RAS, influent and effluent samples were generally collected from the pilot-scale plant every day in the early afternoon and analysed within one hour. The list of the analyses performed during the disintegration trials, their rationale and monitoring regime is detailed in Table 3-10.

**Table 3-10: Analyses, rationale and monitoring regime during the pilot-scale plant trials**

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Rationale</th>
<th>Minimum frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (test and control ML and RAS) TCOD (test and control effluent influent)</td>
<td>Impact on sludge production (effects on the observed yields) Impact on process performance (effects on TCOD and solid removal)</td>
<td>5x week</td>
</tr>
<tr>
<td>VSS (test and control ML and RAS)</td>
<td>Changes in the ratio VSS/TSS</td>
<td>5x week</td>
</tr>
<tr>
<td>Lysate COD (test RAS)</td>
<td>Effects on sludge solubilisation Impact on sludge production due to the lysis cryptic growth by sludge solubilisation</td>
<td>3x week</td>
</tr>
<tr>
<td>Respirometry (test and control RAS)</td>
<td>Effects on biological activity Impact on sludge production due to the increased maintenance metabolism by induced stress</td>
<td>2x month</td>
</tr>
<tr>
<td>Size analysis (test and control ML and RAS)</td>
<td>Effects on floc breakage Impact on sludge production due to the enhanced metabolism by floc breakage</td>
<td>2x month</td>
</tr>
<tr>
<td>Ammonium, nitrate and total phosphorus (test and control effluent and influent)</td>
<td>Impact on process performance (effects on nutrient removal efficiency)</td>
<td>3x week (NH₄, NO₃) 1x week (Total P)</td>
</tr>
<tr>
<td>Turbidity (test and control effluent and influent)</td>
<td>Impact on process performance (effects on effluent quality)</td>
<td>3x week</td>
</tr>
<tr>
<td>SSVI (test and control RAS)</td>
<td>Impact on process performance (effects on sludge settleability)</td>
<td>3x week</td>
</tr>
<tr>
<td>Capillary suction time (test and control RAS)</td>
<td>Impact on process performance (effects on sludge filterability/dewaterability)</td>
<td>3x week</td>
</tr>
<tr>
<td>Specific resistance to filtration (test and control RAS)</td>
<td>Impact on process performance (effects on sludge dewaterability)</td>
<td>1x week</td>
</tr>
</tbody>
</table>
Outline of the pilot-scale plant trials

The six disintegration trials were named after the level of the energy input used, and, when the energy input was the same, after the level of the energy density used (Table 3-11):

- Trial L: disintegration trial completed using a low energy input (1008 kJ d\(^{-1}\))
- Trials M (trial M/L (F/M), trial M/L, trial M/M and trial M/H): disintegration trials completed using the same medium energy input (6048 kJ d\(^{-1}\)) but different energy densities. Trial M/L (F/M) and trial M/L were completed applying an energy density of 42 kJ L\(^{-1}\), trial M/M an energy density of 84 kJ L\(^{-1}\), trial M/H an energy density of 168 kJ L\(^{-1}\)
- Trial H: disintegration trial completed using a high energy input (15120 kJ d\(^{-1}\)).

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Trial description</th>
<th>Energy input [kJ d(^{-1})]</th>
<th>Energy density [kJ L(^{-1})]</th>
<th>Treatment period [min h(^{-1})]</th>
<th>Treated RAS [%]</th>
<th>Stress factor [d(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bas. L</td>
<td>Baseline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Low energy</td>
<td>1008</td>
<td>42</td>
<td>1</td>
<td>~1.7</td>
<td>0.05</td>
</tr>
<tr>
<td>M/L (F/M)</td>
<td>Medium energy input Low energy density (similar F/M for both control and test lane)</td>
<td>6048</td>
<td>42</td>
<td>6</td>
<td>10</td>
<td>0.28</td>
</tr>
<tr>
<td>M/L</td>
<td>Medium energy input Low energy density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/M</td>
<td>Medium energy input Medium energy density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/H</td>
<td>Medium energy input High energy density</td>
<td>168</td>
<td>84</td>
<td>2.5</td>
<td>12.5</td>
<td>0.07</td>
</tr>
<tr>
<td>H</td>
<td>High energy input</td>
<td>15120</td>
<td>84</td>
<td>15</td>
<td>12.5</td>
<td>0.33</td>
</tr>
</tbody>
</table>

During the baseline period, the most stable condition for the pilot-scale plant was reached when the total suspended solids (TSS) in the mixed liquor was at 2.8 g L\(^{-1}\) with a solids retention time (SRT) of 16 days.

Unless specified, during all pilot-scale plant trials (Table 3-11), the control and test lanes were operated using the same SRT for both of them. However, the SRT was not kept constant but it varied (16±5 d) to keep the total suspended solids (TSS) in the
control lane at the same optimal level detected during the baseline (~2.8 g L⁻¹). The TSS in the test lane was free to vary in response to the operational conditions used during the disintegration trials.

The trial M/L (F/M) was the only exception to this rule. During the trial M/L (F/M) the SRT in the control and test lanes was varied independently to keep the same optimal TSS in both lanes, i.e. to keep the same food on microorganism ratio (F/M) in both lanes.

The rationale behind the implementation of pilot-scale plant trials is given below:

- **Bas.**: Baseline to compare sludge production and the performance of control and test lane without ultrasound treatment and assess the suitability of the pilot-scale plant for this research
- **Trial L, trials M, trial H**: Disintegration trials at low, medium and high energy inputs to assess the degree of sludge reduction and overall performance at three different levels of energy input
- **Trials M/L, M/M, M/H**: Disintegration trials performed at the same (medium) energy input but at three different energy densities to assess the effects of different operational conditions on sludge production and the process performance and, possibly, trigger some form of enhanced maintenance metabolism due to stress induced by ultrasound
- **Trial M/L (F/M) and M/L**: Disintegration trials at medium energy input and low energy density to assess variability within results provided by the pilot-scale plant. Trial M/L (F/M) and M/L were performed at the same energy input and density to assess differences in sludge production and the process performance between the control and test lanes when using either the same food to microorganism ratios (trial M/L (F/M)) or the same SRTs (trial M/L).

The pilot-scale plant trials covered a period of 8 months. Their chronological sequence, duration and number of samplings are presented in Table 3-12.
Table 3-12: Pilot-scale plant trials chronological sequence, duration, number of days and samplings

<table>
<thead>
<tr>
<th>Trial name (chronological order)</th>
<th>Duration</th>
<th>Days [#]</th>
<th>Samplings [#]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bas.</td>
<td>09 Jan – 27 Feb</td>
<td>50</td>
<td>43</td>
</tr>
<tr>
<td>M/L (F/M)</td>
<td>01 Mar – 04 Apr</td>
<td>35</td>
<td>29</td>
</tr>
<tr>
<td>M/L</td>
<td>18 Apr – 01 May</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>L</td>
<td>15 May – 08 Jun</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>M/M</td>
<td>15 Jun – 17 Jul</td>
<td>33</td>
<td>29</td>
</tr>
<tr>
<td>M/H</td>
<td>19 Jul – 16 Aug</td>
<td>29</td>
<td>23</td>
</tr>
<tr>
<td>H</td>
<td>17 Aug – 04 Sep</td>
<td>19</td>
<td>15</td>
</tr>
</tbody>
</table>

Sludge biomass undergoes significant reduction in the floc size when exposed to ultrasound treatment and tends to return to the original state when the treatment is stopped. The shortest interval possible between the end of a disintegration trial and the start of a new one was adopted. This was to minimise any form of re-aggregation into bigger flocs, thus preserving the effects of long-term ultrasound treatment on sludge biomass.

3.6.3 Sludge samples used during the dynamic studies

Either RAS samples were collected from the Cranfield University pilot-scale plant or the Cotton Valley WWTP, which treats mixed municipal and industrial wastewaters and is located close the Cranfield University. All RAS sludge samples were treated with ultrasound within 1 hour of collection.

3.6.4 Parameters used during the dynamic studies

**Energy input**

Energy inputs were always given in terms of energy densities, in [kJ L⁻¹]

**Frequency of the ultrasound treatment**

The frequency of the ultrasound treatment was specified either in terms of percentage of RAS treated or in terms of stress factor. The stress factor is the most common index used in the literature and is defined as the ration between the amount of sludge treated per day and the total sludge amount in the system (equation (2.20) in the Literature
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review chapter).

**Release of soluble COD (SCOD)**

During the ultrasound potential trials and the pilot-scale plant trials, to compare the COD release from sludge samples with different solids content, the “Degree of SCOD release” or “Degree of disintegration” ($\text{DD}_{\text{COD}}$) was used, as it is the most common index used in the literature (equation (2.14) in the Literature review chapter).

**Evaluation of the impact on the activated sludge process**

This section describes the most important parameters used during the pilot-scale plant trials to quantify the impact of ultrasound on process performance and sludge production.

**Total COD removal efficiency**

The efficiency in the removal of the total COD ($\text{TCOD}_{\text{Rem}}$) was defined according to equation (3.1):

$$\text{TCOD}_{\text{Rem}} \left[\%\right] = \frac{\text{TCOD}_{\text{Inf}} - \text{TCOD}_{\text{Eff}}}{\text{TCOD}_{\text{Inf}}} \cdot 100 \quad (3.1)$$

Where:

- $\text{TCOD}_{\text{Inf}} = \text{The total COD concentration in the influent, in [mg TCOD L}^{-1}]$
- $\text{TCOD}_{\text{Eff}} = \text{The total COD concentration in the effluent, in [mg TCOD L}^{-1}]$

**Ammonium removal efficiency**

The efficiency in the removal of ammonium ($\text{NH}_4_{\text{Rem}}$) was defined according to equation (3.2):

$$\text{NH}_4_{\text{Rem}} \left[\%\right] = \frac{\text{NH}_4_{\text{Inf}} - \text{NH}_4_{\text{Eff}}}{\text{NH}_4_{\text{Inf}}} \cdot 100 \quad (3.2)$$

Where:

- $\text{NH}_4_{\text{Inf}} = \text{The ammonium concentration in the influent, in [mg NH}_4\text{ L}^{-1}]$
- $\text{NH}_4_{\text{Eff}} = \text{The ammonium concentration in the effluent, in [mg NH}_4\text{ L}^{-1}]$
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**Total phosphorus removal efficiency**

The efficiency in the removal of the total phosphorus (TotP\textsubscript{Rem}) was defined according to equation (3.3):

\[
\text{TotP}\textsubscript{Rem} [\%] = \frac{\text{TotP}\textsubscript{Inf} - \text{TotP}\textsubscript{Eff}}{\text{TotP}\textsubscript{Inf}} \cdot 100 \tag{3.3}
\]

Where:
- \(\text{TotP}\textsubscript{Inf}\) = The total phosphorus concentration in the influent, in [mg total P L\(^{-1}\)]
- \(\text{TotP}\textsubscript{Eff}\) = The total phosphorus concentration in the effluent, in [mg total P L\(^{-1}\)]

**Total suspended solids removal efficiency**

The efficiency in the removal of the total suspended solids (TSS\textsubscript{Rem}) was defined according to equation (3.4):

\[
\text{TSS}\textsubscript{Rem} [\%] = \frac{\text{TSS}\textsubscript{Inf} - \text{TSS}\textsubscript{Eff}}{\text{TSS}\textsubscript{Inf}} \cdot 100 \tag{3.4}
\]

Where:
- \(\text{TSS}\textsubscript{Inf}\) = The total suspended solids concentration in the influent, in [mg TSS L\(^{-1}\)]
- \(\text{TSS}\textsubscript{Eff}\) = The total suspended solids concentration in the effluent, in [mg TSS L\(^{-1}\)]

**Reduction in sludge production**

Every day \(d\) the increment in sludge biomass \(\Delta X(d)\) and TCOD uptake \(\Delta S(d)\) was evaluated for both control and test lane.

The biomass increment at day \(d\), \(\Delta X(d)\), was defined according to equation (3.5):

\[
\Delta X(d) = \Delta X_{\text{ML}} (d) - \Delta X_{\text{ML}} (d-1) - \Delta X_{\text{Wasted, SAS}} (d) - \Delta X_{\text{Lost, Effluent}} (d) \tag{3.5}
\]

Where:
- \(\Delta X(d)\) = Increment of biomass at day \(d\) in [g TSS]
- \(\Delta X_{\text{ML}}(d)\) = ML total biomass at day \(d\)
- \(\Delta X_{\text{ML}}(d-1)\) = ML total biomass at day \(d-1\)
- \(\Delta X_{\text{Wasted, SAS}}\) = Biomass wasted as SAS at day \(d\)
\[ \Delta X_{\text{Lost, Effluent}} = \text{Biomass lost in effluent at day } d \]

The TCOD uptake at day \( d \), \( \Delta S(d) \), was defined according to equation (3.6):

\[ \Delta S(d) = \Delta S_{\text{Sew, Influent}}(d) - \Delta S_{\text{Sew, Effluent}}(d) + \Delta S_{\text{Acetate}}(d) \] (3.6)

Where:
\[ \Delta S(d) = \text{TCOD uptake at day } d, \text{ in [g COD]} \]
\[ \Delta S_{\text{Sew, Influent}}(d) = \text{Total amount of TCOD in the influent at day } d, \text{ in [g COD]} \]
\[ \Delta S_{\text{Sew, Effluent}}(d) = \text{Total amount of TCOD in the effluent at day } d, \text{ in [g COD]} \]

The cumulative TSS production at day \( d \) is the cumulative sum of \( \Delta X(d) \) from the first day of treatment to day \( d \) according to equation (3.7):

\[ \text{TSS Production}(d) = \Delta X(1) + \Delta X(2) + \ldots + \Delta X(d - 1) + \Delta X(d) = \sum_{d=1}^{d} \Delta X(d) \] (3.7)

At the same way, the cumulative TCOD removal is defined as the cumulative sum of \( \Delta S(d) \) from the first day of treatment to day \( d \) according to equation (3.8):

\[ \text{TCOD Removed}(d) = \Delta S(1) + \Delta S(2) + \ldots + \Delta S(d - 1) + \Delta S(d) = \sum_{d=1}^{d} \Delta S(d) \] (3.8)

The slope of the linearisation (not passed through zero) of the correlation between cumulative TSS production and TCOD removal can be defined as the observed sludge production yield (\( Y_{OBS} \)). Based on this, the reduction of sludge production RSP\[%\] can be calculated according to equation (3.9):

\[ \text{RSP \[%\]} = \left( 1 - \frac{Y_{OBS,\text{TEST}}}{Y_{OBS,\text{CTRL}}} \right) \cdot 100 \] (3.9)

RSP \[%\], which is also referred to as the degree of sludge reduction, gives positive values when a reduction in sludge production in test lane occurs while negative values would mean that test lane \( Y_{OBS} \) is actually higher than control lane’s one.
Ratio between the degree of sludge reduction and the daily average release of lysate COD

Ultrasound treatment can theoretically implement three different sludge reduction mechanisms: enhanced metabolism by floc breakage, lysis-cryptic growth by sludge solubilisation and increased maintenance metabolism by induced stress. To gain information on the role played by the lysis-cryptic growth mechanism based on sludge solubilisation, the ratio $RSP/ΔS_{Lys}$ between the degree of sludge reduction achieved and the daily average release of lysate COD was evaluated according to equation (3.10):

$$\text{Ratio } RSP/ΔS_{Lys} = \frac{\text{RSP} [%]}{ΔS_{Lys, AVERAGE}(d)} \quad (3.10)$$

Where:

- $\text{RSP} [%]$ = Reduction in sludge production
- $ΔS_{Lys, AVERAGE}(d)$ = Daily average release of lysate COD, in [g lysate COD d$^{-1}$]

The ratio $RSP/ΔS_{Lys}$ was measured for each of the pilot-scale plant trials completed at the same medium energy density (trials M at 6048 kJ d$^{-1}$) but applying different energy densities: 42 kJ L$^{-1}$ (trial M/L (F/M) and trial M/L), 84 kJ L$^{-1}$ (trial M/M) and 168 kJ L$^{-1}$ (trial M/H). A constant value for the ratio $RSP/ΔS_{Lys}$ during the different trials completed at the same energy input would suggest a strong relationship between the degree of sludge reduction and the release of lysate regardless of the energy density used.

Short-term and long-term specific oxygen uptake increments

Two different groups of respirometric trials were completed as part of the ultrasound potential trials to investigate the impact of ultrasound on the biomass biological activity.

For convenience, the two groups were defined as:

- **Short-term respirometric trials** – Respirometric analyses using untreated and treated RAS samples collected from the control and test lane of the pilot-scale plant and the Cotton Valley WWTP
• **Long-term respirometric trials** – Respirometric analyses using RAS samples collected from the control and test lane of the pilot-scale plant.

The distinction between short-term and long-term does not refer to the length of the respirometric trials. For all the respirometric trials, the length was determined by the time needed by the biomass to reach the endogenous respiration. The distinction refers to the short-term and long-term effects of the ultrasound treatment on the RAS biomass.

During the short-term respirometric trials, using untreated and treated RAS samples collected from the control lane and test lane and the Cotton Valley WWTP, the ultrasound treatment was applied just before the respirometric trials.

During the long-term respirometric trials, using the RAS samples collected from the control lane and test lane, no ultrasound treatment was performed on the RAS samples before the respirometric trials. The RAS samples from the test lane were simply collected before entering the ultrasound disintegration unit. Statistically, the minimum time elapsed since the last pass through the ultrasound system for the test RAS samples was longer than a week, depending on the pilot-scale plant disintegration trial under progress at the time of collection.

For the quantitative analysis of the short-term and long-term respirometric trials, two indexes were defined:

- The short-term specific oxygen uptake
- The long-term specific oxygen uptake.

**Short-term specific oxygen uptake**

The specific oxygen uptake (SOU) was evaluated at the end of each short-term respirometric trial. To highlight differences in the biological activity induced by ultrasound treatment between untreated and treated RAS samples, the increment in the SOU values in the RAS biomass treated at different energy densities against the corresponding one in the untreated RAS biomass was calculated according to equation (3.11):
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Short-term SOU increment [%] = \left( \frac{\text{SOU}_{\text{RAS, treated}} - \text{SOU}_{\text{RAS, untreated}}}{\text{SOU}_{\text{RAS, untreated}}} \right) \cdot 100 \quad (3.11)

Where:

\text{SOU}_{\text{RAS, treated}} = \text{Specific oxygen uptake of the treated RAS samples, in [mg O}_2 \text{ mg}^{-1} \text{TSS]}}

\text{SOU}_{\text{RAS, treated}} = \text{Specific oxygen uptake of the untreated RAS samples, in [mg O}_2 \text{ mg}^{-1} \text{TSS]}}

Long-term specific oxygen uptake increment

The specific oxygen uptake (SOU) was evaluated at the end of each long-term respirometric trial. To highlight the differences in biological activity induced by ultrasound treatment between the RAS samples collected from the test lane and the control lane, the increment in the SOU values in the test lane RAS biomass against the corresponding one in the control lane RAS biomass was calculated according to equation (3.12):

Long-term SOU increment [%] = \left( \frac{\text{SOU}_{\text{TEST RAS}} - \text{SOU}_{\text{CTRL RAS}}}{\text{SOU}_{\text{CTRL RAS}}} \right) \cdot 100 \quad (3.12)

Where:

\text{SOU}_{\text{TEST RAS}} = \text{Specific oxygen uptake of the RAS samples collected from the test lane, in [mg O}_2 \text{ mg}^{-1} \text{TSS]}}

\text{SOU}_{\text{CTRL RAS}} = \text{Specific oxygen uptake of the RAS samples collected from the control lane, in [mg O}_2 \text{ mg}^{-1} \text{TSS]}}

Interpretation of the short-term and long-term specific oxygen uptake increments

The short-term and long-term SOU increments differ only in relation to the RAS samples to which they apply. The short-term SOU increment (equation (3.11)) is applied to the SOU of the RAS samples before and after the ultrasound treatment (\text{SOU}_{\text{RAS, treated}} and \text{SOU}_{\text{RAS, untreated}}), the long-term SOU increment (equation (3.12)) to
the SOU of the RAS samples collected from the control and test lanes (SOU_{TEST\ RAS} and SOU_{CTRL\ RAS}).

For both the short-term and long-term SOU increments, positive values indicate an increase in the SOU of the treated RAS samples or the RAS samples collected from test lane, respectively. An increase in the biological activity could suggest the presence of some form of increased maintenance metabolism by induced stress. Negative values indicate a decrease in the SOU of the treated RAS samples or the RAS samples collected from test lane, respectively. A decrease in the biological activity, therefore, would exclude the presence of increased maintenance metabolism by induced stress.
3.7 Methods III: Analytical methods

This section describes all the analytical methods used during both batch and dynamic studies to evaluate the impact of ultrasound treatment on the activated sludge biomass and the overall process.

3.7.1 Sample preparation

The solid free fraction of sludge samples was required for the determination of soluble COD, ammonium, nitrate and phosphorous. The sludge samples were centrifuged for 20 minutes at 7200 rpm (RCF: 11712 x g) in a Rotanta 96 R centrifuge (Hettich Zentrigugen, Tuttlingen, Germany). The supernatant was filtered through a 0.45 μm pore size glass fibre filter paper to remove any suspended particles.

3.7.2 Total suspended solids

Total suspended solids (TSS) content was determined by Standard Method 2540D (APHA, 1998). Schleicher & Schuell Grade GF 52 glass fibre filter papers (Patterson Scientific, Bedfordshire, UK) were pre-dried in an oven at 105°C for at least 1 hour and then cooled in a desiccator until needed. The pre-dried filter papers were weighed immediately prior to use. Well mixed sludge samples (5-100 mL, depending on solids contents) were filtered under vacuum through the pre-dried and pre-weighed filter papers. The filter papers were subsequently dried in an oven at 105°C overnight, cooled in a desiccator and re-weighed. The suspended solids content was calculated according to equation (3.13):

\[
TSS \ [g \ L^{-1}] = \frac{(A-B) \cdot 1000}{C} \tag{3.13}
\]

Where  
A = Weight of filter and dried residue [g]  
B = Weight of filter [g]  
C = Sample volume filtered [ml]

3.7.3 Volatile suspended solids

Volatile Suspended Solids (VSS) were determined by Standard Method 2540E (APHA, 1998). Filter papers supporting the suspended solids residue were ignited in a
furnace at 550°C for 4 hours. Following cooling in a desiccator, the filter papers were immediately re-weighed and the VSS concentration calculated according to equation (3.14):

\[
\text{VSS [g L}^{-1}] = \frac{(A-B) \cdot 1000}{C \cdot D}
\]

Where:
A = Weight of filter and dried residue before ignition in [g]
B = Weight of filter and dried residue after ignition in [g]
C = Sample volume filtered in [mL]
D = Sample dilution factor

3.7.4 Microscopic examination

Qualitative flocs structural characteristics were investigated with an Olympus BHB phase contrast light microscope (Olympus European, Hamburg, Germany).

3.7.5 Particle size analysis

Sludge particle sizes were measured using a Malvern Mastersizer 2000 particle analyser (Malvern Instruments Ltd, Worcestershire, UK). The Mastersizer uses an optical unit to detect the light scattering pattern of sludge particles dispersed in deionised water. The dilute sludge suspension is circulated through a measurement cell where the particle fields are exposed to an analyzing laser beam. The pattern of light scatter can be used to calculate the particle sizes that created the scatter by using Mie theory, which predicts the way light is absorbed and scattered by spherical particles.

All sludge samples were analysed using the same standard operating procedure. The optical properties of the material (sludge) were set at default (refractive index 1.52, absorption 0.1) appropriate for the majority of naturally occurring samples. The stirrer pump speed was set at 7 rpm. Sludge samples were added to the deionised water (dispersant) tank supplying the particle suspension to the measurement cell until the laser obscuration (fraction of light ‘lost’ by scatter and absorption from the analysing beam) was between 10 % and 20 %. Five measurement cycles were taken with a 5 s delay between cycles and an average measurement calculated. The
measurement time was set at 20 s (at 1000 snaps s⁻¹) in order to ensure that the particle size distributions of the sludge samples were adequately represented by allowing coarser particles time to flow through the measurement beam. The Mastersizer measurement is volume based and, according to Mie theory, assumes that the particles causing light absorption and scatter are perfect spheres. Consequently, the results are both volume based and expressed in terms of equivalent spheres. The percentage volume of particles is plotted against particle size (µm).

3.7.6 Stirred sludge volume index

The stirred sludge volume index (SSVI) is the volume in millilitres occupied by 1 g of a suspension after 30 min settling in a stirred cylinder. The SSVI is typically used to evaluate the settleability of activated sludge and other biological suspensions. This test is based on Standard Method 2710C and D (APHA, 1998). Around 3.5 L of RAS were collected and settled for 30 minutes in a special graduated settling column. The column, 50 cm deep and about 10 cm in diameter, was fitted with a wire stirrer rotating at 1rpm (Triton Electronics Ltd, UK). The volume occupied by the sludge is reported in millilitres. The SSVI is computed by dividing the result of the settling test in mL per litre by the TSS concentration in mg L in the aeration tank times 1000 according to equation (3.15):

\[
\text{SSVI \ [ml \ g^{-1}]} = \frac{\text{settled volume of sludge \ [mL L^{-1}]} \cdot 1000 \ [mg \ g^{-1}]}{\text{TSS \ [mg \ L^{-1}]}} \quad (3.15)
\]

In terms of SSVI, a sludge with good settling characteristics has a value < 120 ml g⁻¹, while one with poor settleability a value > 200 mg g⁻¹. The SSVI is generally preferred over the SVI because the SSVI reproduces better the situation in the sedimentation tanks, minimizes wall effects on the settled sludge volume and is less dependent on solids concentration (Gray, 2004). However, in this study, the SSVI was mainly chosen to minimise the effects of sludge floating during the traditional non-stirred SVI test, caused by an enhanced form of denitrification occurring in the RAS samples treated with ultrasound.
Chapter 3: Material and methods

3.7.7 Capillary suction time

Sludge filterability and conditionability governs the output of nearly all the various types of dewatering equipment and was assessed by the capillary suction time (CST) test based on Standard Method 2710 G (APHA, 1998) and performed using a 200 Type CST tester supplied by Triton Electronics Ltd, Essex, UK (Figure 2.10.). The CST test provides a quantitative measure of the rate of water release from sludge and is used to evaluate sludge conditioning processes and doses. A 6.4 mL sludge sample was placed in the cylindrical reservoir on top of the CST filter paper. The filter paper removes water from the sludge sample by capillary action and the time required for the water to travel between electrodes E1 and E2 was measured by monitoring the conductivity change occurring at the electrodes, which are in contact with the filter paper. The time taken for the water to travel the specified distance is indicative of the water drainage rate. The solids content of the sludge influences the CST. Higher solids contents increase the CST by increasing the flow resistance of the cake formed in the reservoir during drainage. Consequently, the CST’s of different sludge samples were normalised by dividing the sludges CST value by its corresponding solids concentration (g TSS L⁻¹), as detailed in Standard Method 2710 G.

![Diagram of the Triton 2000 capillary suction time (CST) test apparatus. E1 and E2 are electrodes 1 and 2 connecting to the electronic counter. A) Transverse section, B) Plan view](image)

3.7.8 Specific resistance to filtration

Dewaterability was also assessed by measuring the specific resistance to filtration (SRF) based on the methodology of Coakley and Jones (1956) using a KST 47 Model Pressure Filtration Apparatus (Figure 3-10), supplied by M – Tech Diagnostics Ltd,
Warrington, Cheshire, UK. Diluted sludge samples were placed in the sludge reservoir and filtered across a 0.8 µm membrane / filter (M – Tech Diagnostics Ltd) at a pressure of 1 bar (100 kPa). A beaker placed on top of a balance was used to collect the filtrate. Filtrate volume V was recorded at one-second intervals for at least 5000 seconds by a laptop connected to the balance. Filtrate mass (in kg) was assumed to be equivalent to its volume (in m\(^3\)). The filter area was 962×10\(^{-6}\) m\(^2\). Filtrate viscosity was measured at 10 rpm using a Brookfield DV – E Viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, Massachusetts, USA) fitted with UL Adaptor 00. The specific resistance to filtration was calculated by Equation (3.16) according to Coakley and Jones (1956).

\[
SRF = \frac{2 \times P \times A^2 \times G}{U \times C} \tag{3.16}
\]

Where:

- SRF = Specific resistance to filtration in [m kg\(^{-1}\)]
- P = Pressure in [Pa]
- A = Filter area in [m\(^2\)]
- G = Gradient (slope of plot of t/V vs V)
- V = Volume of filtrate in [m\(^3\)]
- U = Filtrate viscosity in [Pa s]
- C = Solids content in [kg m\(^{-3}\)]
3.7.9 Total and soluble chemical oxygen demand

The total chemical oxygen demand (TCOD) was determined using Merck Spectroquant COD cell test 1.14555.0001 (500 – 10,000 mg COD L$^{-1}$) (VWR International Ltd, Dorset, UK), analogous to standard method 5220 D (APHA, 1998). Where necessary samples were diluted to ensure the measured value was within the range of the test kit. The vial was heated at 150°C for 2 hours and following cooling the total COD value (mg L$^{-1}$) was measured in a Spectroquant Nova 60 Spectrophotometer (VWR International Ltd, UK).

The soluble COD is commonly defined as the sum of the truly soluble COD and the colloidal matter that normally passes through 0.45 μm membrane filters (Mamais et al., 1993). The soluble chemical oxygen demand (SCOD) was determined using Merck Spectroquant COD cell test 1.14540.0001 (10 – 150 mg COD L$^{-1}$) or
1.14541.0001 (25 – 1500 mg COD L⁻¹). A 3 mL sample of the filtered supernatant was added to the cell test vial and heated at 148°C for 2 hours. After cooling to room temperature, the SCOD value (mg L⁻¹) was measured in the Spectroquant Nova 60 Spectrophotometer.

3.7.10 **Cell lysate chemical oxygen demand**

Cell lysate chemical oxygen demand was determined using Merck Spectroquant COD cell test 1.14540.0001 (10 – 150 mg COD L⁻¹) or 1.14541.0001 (25 – 1500 mg COD L⁻¹). In this study, the chemical oxygen demand of the cell lysate was defined as the sum of the truly soluble COD and the colloidal COD.

Sludge samples were first treated at different energy densities with ultrasound. The sludge samples were then centrifuged for 20 minutes at 7200 rpm (RCF: 11712 x g) in a Rotanta 96 R centrifuge (Hettich Zentriugugen, Tuttlingen, Germany). The supernatant was not filtered as for the measurement of the soluble COD. A 3 mL sample of non-filtered supernatant was added to the cell test vial and heated at 148°C for 2 hours. After cooling to room temperature, the COD value (mg L⁻¹) was measured in the Spectroquant Nova 60 Spectrophotometer.

3.7.11 **Alkaline hydrolysis**

The alkaline hydrolysis of sludge was carried out in a 0.5 M NaOH solution prepared using NaOH pellets (Fisher chemicals, Fisher Scientific UK Ltd) and kept at 20°C for 22 h before COD measurements were taken. This is a well-established technique to maximise the release of COD by cell lysis (Neis and Tiehm, 1999; Rai et al., 2004; Nickel and Neis, 2007).

3.7.12 **Ammonium**

Ammonium (NH₄⁺) concentrations were determined using Merck Spectroquant ammonium cell test 1.14559.0001 (5.2 – 103.0 mg L⁻¹ NH₄⁺) (VWR International Ltd, UK). A 0.1 mL solid free sample was transferred to the test vial and the contents mixed. One dose of reagent NH₄⁺-1K was added and the vial left to stand at room temperature for 15 minutes. Ammonium concentrations were determined photometrically using a Spectroquant Nova 60 Spectrophotometer (VWR International Ltd, UK). Where necessary, samples were diluted before analysis in
order to ensure ammonium concentrations were within the test range (5.2 – 103.0 mg L\(^{-1}\) NH\(_4^+\)).

### 3.7.13 Nitrate

Nitrate (NO\(_3^-\)) concentrations were determined using by Merck Spectroquant nitrate cell test 1.14563.0001 (VWR International Ltd, UK). A 1.0 mL sample was transferred to the test vial, followed by 1.0 mL of reagent NO\(_3^-\)-1K and the contents mixed. The cell was left to stand for 10 minutes. Nitrate concentrations were determined photometrically using a Spectroquant Nova 60 Spectrophotometer (VWR International Ltd, UK).

### 3.7.14 Soluble total phosphorus

Soluble total phosphorus (Total P) was determined using Merck Spectroquant phosphate cell test 1.14729.0001 (0.5 – 25 mg L\(^{-1}\) P) (VWR/Merck), analogous to standard method 4500-P E (APHA, 1998). The samples of solid free fraction of sludge and wastewater were digested at 120 °C in the thermoreactor for 30 minutes for the determination of total phosphorus. Samples were diluted to produce a measured value of phosphorus in the vial concentration range.

### 3.7.15 Respirometric analysis

A CES multi-Channel Aerobic Respirometer (Coordinated Environmental Services Ltd, Kent, UK) was used for respirometric analysis. This system has eleven respirometric cells. The working principle of each cell is based on the coupling of an electrolytic oxygen generation process to a sensitive manometric system within a closed sample flask kept at constant temperature. The sample solution in the sample flask of each cell is continuously stirred by magnetic bars to ensure uniform mixing during the experimental period. Following sealing of the sample flask from the outside environment, any biodegradation within a sample causes a decrease in oxygen concentration coupled with production of carbon dioxide. The use of an alkali carbon dioxide trap inside the sample flask leads to a net reduction in gas pressure within the cell, which is detected by the manometric system and triggers electrolysis of an aqueous copper sulphate solution (25%). Electrolysis produces oxygen, which replenishes that utilised by the biomass. In this way, magnitude and duration of the
electrolytic current required are together directly proportional to the amount of oxygen consumed by microorganisms within the sample. The electrolytic current is logged automatically by the system and translated into the equivalent oxygen consumption.

Respirometric trials were performed on untreated and treated sludge samples, using different types of substrate. The energy densities investigated varied between 0 and 168 kJ L\textsuperscript{-1}. The different types of substrate considered in this study were:

- Settled sewage from Cranfield WWTP
- Synthetic sewage
- Cell lysate released by sludge biomass during ultrasound treatment.

This set-up for the respirometric trials was used to investigate the effect of different energy inputs and substrate biodegradability on the biological activity in terms of specific oxygen uptake and specific oxygen uptake rates. Initial and final soluble COD was also measured to evaluate the ratio between oxygen uptake and COD removal at the end of the test and get a better insight on ultrasound impact on biological activity. The procedure used during the respirometric trials was the following:

- The initial soluble COD of sludge samples under study was measured
- 50 mL of sludge biomass, treated and/or untreated, was added to each sample flask
- Substrate or simply tap water was added to reach an overall volume of 350 mL for each sample flask
- A minimum of 3 replicas for each set-up were prepared
- The carbon dioxide traps were set up using 10ml of a 2M NaOH solution and a fluted filter paper wick
- Magnetic stirrers were placed in the sample flasks
- Water bath temperature was set at 25 °C and stirrers were started
- Cells were sealed, placed in the water bath, configured to record oxygen consumption every minute and started
- Cells were stopped when biomass had reached endogenous respiration
Chapter 3: Material and methods

- Final soluble COD of samples was measured.

The composition of the synthetic sewage used in this study is given in Table 3-13 and was based on the work of Rai et al. (2004) (all chemicals were purchased from Fisher Scientific UK Ltd).

**Table 3-13: Composition of synthetic sewage**

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity [g L(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>0.79</td>
</tr>
<tr>
<td>Meat extract</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium acetate (CH(_3)COONa)</td>
<td>0.79</td>
</tr>
<tr>
<td>Ammonium sulphate ((NH(_4))(_2)SO(_4))</td>
<td>0.9</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>0.9</td>
</tr>
<tr>
<td>Calcium chloride (CaCl(_2))</td>
<td>0.05</td>
</tr>
<tr>
<td>Magnesium sulphate heptahydrate (MgSO(_4).7H(_2)O)</td>
<td>0.03</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate (K(_2)HPO(_4))</td>
<td>0.35</td>
</tr>
</tbody>
</table>

In most of the trials, 50 mL of synthetic sewage were used as substrate and then deionised water was added to reach the total volume of 350 mL. Average COD content of 50 mL of synthetic sewage was 80 g COD. For this reason when other types of substrate were used, enough volume was added to provide the same amount of COD.

The respirometric procedure detailed above was based on the technique used by Rai et al. (2004). On the other hand, in this study:
- A complete different type of respirometer was used
- No allyl thiourea (ATU) was added to suppress nitrification
- Different types of substrate were investigated.

**3.7.16 Statistical analysis**

MATLAB\(^{\text{®}}\) 7.1.0.246 (R14) Service Pack 3 (The MathWorks, Inc.) was used for assessing averages, standard deviations, modelling. The robust linear least squares fitting method was adopted for curve fitting, unless specified otherwise. Multiple comparison tests were used in conjunction with the one-way ANOVA test, applying the Tukey's honestly significant difference criterion.
Chapter 4: Batch studies

4.1 Introduction

This chapter is divided in two sections that report on the results from two groups of batch disintegration trials:

- **Equipment comparison trials** – Batch disintegration trials completed to compare the performance of the Sonix and Ultrawaves probes, ASPS reactor and Pilao deflaker in terms of sludge solubilisation and evaluate the effects on sludge disintegration of the following operational and design factors: power intensity, temperature, solids content and horn design.

- **Ultrasound impact trials** – Batch disintegration trials completed using the Sonix probe to investigate in terms of floc breakage and sludge solubilisation if the impact of the ultrasound treatment on sludge biomass varied significantly depending on the types of WWTP: (1) conventional (CAS); (2) oxidation ditch (OD) and (3) biological nutrient removal (BNR).

The first section of this chapter examines the results from the equipment comparison trials. The second section of this chapter examines the results from the ultrasound impact trials. The two sections of this chapter address the following project objectives:

- Comparison of different ultrasound equipment and evaluation of the best operational conditions for sludge disintegration
- Evaluate the impact of ultrasound treatment on activated sludge biomass from different types of WWTP
4.2 Comparison of different ultrasound equipment and evaluation of the best operational conditions for sludge disintegration

The equipment comparison trials comprised five different sets of trials completed to:

- Compare the performance of two ultrasound systems (the Sonix probe and the ASPS reactor) and a mechanical one (the Pilao deflaker). The comparison was made in terms of sludge solubilisation in the RAS samples treated at different energy densities during the Derby WWTP trial
- Investigate the role played by the power intensity and temperature in terms of sludge solubilisation in the RAS samples treated with the Sonix probe and the ASPS reactor during the Brampton WWTP trial
- Investigate the role played by the solids content in terms of sludge solubilisation in the RAS samples treated with the Ultrawaves probe during the Cranfield pilot-scale plant trial
- Investigate the role played by the horn design in two high intensity ultrasound systems in terms of sludge solubilisation in the RAS samples treated with the Sonix and Ultrawaves probes during the Cotton Valley WWTP trial.

4.2.1 Comparison of two ultrasound and a mechanical equipment during the Derby WWTP trial

Derby WWTP operates in biological nutrient removal (BNR) mode. RAS and thickened SAS were collected from the RAS line and immediately after the belt thickener, respectively. The total suspended solid (TSS) concentration was 9.3 g L\(^{-1}\) for RAS and 56.7 g L\(^{-1}\) for thickened SAS. RAS and SAS disintegration was carried out after less than 3 hours from collection.

The Sonix probe and Pilao deflaker were operated in batch mode and with no temperature control. The ASPS reactor was operated in flow-through mode and with temperature control for all energy densities. In addition, at 702 kJ L\(^{-1}\), the ASPS reactor was also operated with no temperature control.
Chapter 4: Batch studies

The comparison among the different equipment was made in terms of the ultrasound impact on sludge solubilisation, by measuring the degree of SCOD release (DDCOD). Results were described taking account differences in power intensity and temperature. The role played by these parameters during sludge disintegration by ultrasound was investigated in more depth during the Brampton WWTP trial. A summary of results from the Derby WWTP trial can be found in Table 4-1.

Table 4-1: Summary of operational condition and SCOD release for Derby WWTP trial (NTC: no temperature control)

<table>
<thead>
<tr>
<th></th>
<th>Untr.</th>
<th>Sonix probe</th>
<th>ASPS reactor</th>
<th>Pilao deflaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor volume [L]</td>
<td>-</td>
<td>1.5</td>
<td>0.205</td>
<td>5</td>
</tr>
<tr>
<td>Power [W]</td>
<td>-</td>
<td>2300</td>
<td>2400</td>
<td>5700</td>
</tr>
<tr>
<td>Retention time [s]</td>
<td>-</td>
<td>30 60 120</td>
<td>30 60 60</td>
<td>180 120 600</td>
</tr>
<tr>
<td>Energy density [kJ L⁻¹]</td>
<td>0 46 92 184 351 702 702 2107 137 342 684</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS Temp. [°C]</td>
<td>18 26 33 46</td>
<td>18 20 58 24</td>
<td>42 62 93</td>
<td></td>
</tr>
<tr>
<td>RAS SCOD [mg L⁻¹]</td>
<td>119 315 616 1473 162 222 1515 438 384 1090 1143</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS DDCOD [%]</td>
<td>0 4 11 30</td>
<td>1 2 31 7</td>
<td>6 21 23</td>
<td></td>
</tr>
</tbody>
</table>

The equipment performance of the Sonix probe, the ASPS reactor and the Pilao deflaker in terms of sludge solubilisation were compared at 184 kJ L⁻¹ because it was the maximum energy density considered for the Sonix probe and within the range considered for the other two systems (Table 4-1). There was no reason to consider energy densities higher than 184 kJ L⁻¹, because this energy density is already well above the one used at full-scale, which is generally around 15 kJ L⁻¹ or lower (Wolff et al., 2007).

In the RAS samples treated at 184 kJ L⁻¹, the Sonix probe triggered the highest degree of SCOD release (DDCOD), 30%, followed by the Pilao deflaker, 9.5%, ASPS reactor
operated without temperature control, 8%, and ASPS reactor operated with temperature control, 0.5% (Figure 4-1).

![Graph showing the degree of SCOD release (DDCOD) vs energy density in the RAS (9.3 g L⁻¹) samples collected from Derby BNR WWTP and treated with different equipment: Sonix probe, ASPS reactor (with and without temperature control) and Pilao deflaker.]

Temperature can increase the release of SCOD during sludge disintegration (Chu et al., 2001; Grönroos et al., 2005). Therefore, before comparing the degree of SCOD released by the different disintegration systems, it was necessary to evaluate the increases in temperature reached during the disintegration trials.

For energy densities between 0 and 184 kJ L⁻¹, there was an almost linear relationship between temperature increases and energy densities applied. At 184 kJ L⁻¹, the RAS samples treated with the Sonix probe and the Pilao deflaker reached a temperature of 46 °C. The RAS samples treated with the ASPS reactor without temperature control reached a temperature of 28 °C, no temperature increase was observed in those treated with the ASPS reactor operated with temperature control (Figure 4-2).
Figure 4-2: Temperature increase vs energy density in the RAS (9.3 g L⁻¹) samples collected from Derby BNR WWTP and treated with different equipment: Sonix probe, ASPS reactor (with and without temperature control) and Pilao deflaker.

The ASPS reactor without temperature control caused a SCOD release 16 times higher than with temperature control. The 16-fold increase in the SCOD caused by a 10 ºC increase in the temperature proved the important role played by the temperature. For the same reason, it would not be fair to further compare the performance of the ASPS reactor operated with temperature control with the Sonix probe and the Pilao deflaker.

The Pilao deflaker and the ASPS reactor operated without temperature control released similar amounts of SCOD: 9.5 and 8 %, respectively. On the other hand, the Sonix probe caused a SCOD release 3.2 times higher than the Pilao deflaker and 3.8 higher than the ASPS reactor operated without temperature control. The higher degree of SCOD released by the Sonix probe in comparison to the Pilao deflaker indicated that the cavitation effects generated by a high intensity ultrasound system could disintegrate sludge more efficiently than the effects of high degrees of fluid shear and turbulence generated by the Pilao deflaker.
At least part of the higher degree of SCOD released by the Sonix probe in comparison to the ASPS reactor operated without temperature control could be attributed to the higher temperature reached in the RAS samples treated with the Sonix probe, 46 instead of 28 ºC. However, two other factors could contribute to the higher degree of SCOD released by the Sonix probe: the use of higher power intensity and more efficient transducers.

The Sonix probe is designed to operate with power intensity at least 7-fold higher than the ASPS reactor. Various studies in the literature reported that, using the same energy input, larger SCOD increases were obtained with higher ultrasound intensities and shorter treatment times rather than with lower ultrasound intensities and longer treatment times (Neis et al., 2000; Grönroos et al., 2005; Nickel, 2005; Zhang et al., 2007b). Furthermore, the Sonix probe is fitted with piezoelectric transducers, the ASPS reactor with magnetostrictive ones. Piezoelectric transducers are considered ~75% more efficient than magnetostrictive ones (Blackstone, 2008).

In this trial, in terms of sludge solubilisation, the performance of the ultrasound systems was either better, using a high intensity ultrasound system (the Sonix probe) or similar, using a low intensity system (the ASPS reactor), to the mechanical system under study (the Pilao deflaker). This result is in disagreement with various studies in the literature where, generally, mechanical systems were found more energy efficient than ultrasound systems (Springer et al., 1996; Kopp et al., 1997; Müller et al., 1998; Müller, 2000b; Winter, 2002; Boehler and Siegrist, 2006). A possible reason is that ultrasound systems have reached an advanced stage of development and optimisation, with the Sonix probe being one of the most successful design in terms of number of full-scale applications (Müller, 2001; Panter, 2002; Enpure, 2008).

For a better evaluation, the SCOD released by the Sonix probe in this study and by two high pressure homogenisers (HPH), based on data from the literature, were compared. For convenience, the degrees of SCOD release (DDCOD) were compared at 56 kJ L⁻¹, the maximum energy density used by Müller (2000b). At 56 kJ L⁻¹, the
SCOD released by the two HPHs was 24 % and 18 %, according to the results provided by Müller (2000b) and Strünkmann et al. (2006). At the same energy input, in this trial, the SCOD released from the Sonix probe was 6 %. Therefore, in terms of sludge solubilisation, the HPH used by Strünkmann et al. (2006) and Müller (2000b) were 3 and 4 times more efficient than the Sonix probe, respectively (Figure 4-3).

Figure 4-3: The degree of SCOD release (DDCOD) vs energy density in sludge samples treated with the Sonix probe (this study) and a high pressure homogeniser (Müller, 2000b)

The higher efficiency of the HPHs over the Sonix probe seems to confirm what was found in the literature (Kopp et al., 1997; Müller et al., 1998; Müller, 2000b; Winter et al., 2002; Boehler and Siegrist, 2006). On the other hand, it should also be noted that often technologies such as cavitation prove to be more efficient at laboratory scale (Gogate and Pandit, 2004b). Therefore, it is difficult to compare the performance of a well know industrial system, such as the Sonix probe, and the HPHs used by Müller (2000b) and Strünkmann et al. (2006), whose characteristics are only partially available and their use at full-scale not documented.
4.2.2 The role of power intensity and temperature during the Brampton WWTP trial

The Derby WWTP trial indicated that the Sonix probe at 184 kJ L\(^{-1}\) was able to cause a release of SCOD 3.8 times higher than the ASPS reactor. The higher SCOD release was attributed to three main factors in favour of the Sonix probe: a 7-fold higher power intensity, a higher increase in the temperature in the treated RAS samples, 46 instead of 28 °C, and the use of more efficient transducers.

However, during the Derby WWTP trials it was not possible to evaluate the contribution of those three factors one by one. Therefore, RAS samples were collected from the Brampton WWTP to provide further information. The RAS total suspended solid (TSS) concentration was 1.8 g L\(^{-1}\). The unusual low solids content was the consequence of a series of rainy days. RAS samples were treated within a couple of hours from collection with the Sonix probe and ASPS reactor.

The Sonix probe was operated in batch mode and with no temperature control. The temperature of the RAS samples treated with the Sonix probe increased from 18 °C to 50 °C. The ASPS reactor was operated in flow-through mode during three different runs with temperature control but different initial RAS temperatures: 18 °C, 33 °C and 52 °C. The rationale was to differentiate and quantify the contribution due to power intensity, temperature and the use of more efficient transducers. A summary of the operational conditions and SCOD releases for this trial can be found in Table 4-2.
Table 4-2: Summary of the operational conditions and SCOD release for Brampton trial

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Sonix probe</th>
<th>ASPS reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor volume [L]</td>
<td>-</td>
<td>1.5</td>
<td>0.205</td>
</tr>
<tr>
<td>Power [W]</td>
<td>-</td>
<td>2300</td>
<td>2400</td>
</tr>
<tr>
<td>Retention time [s]</td>
<td>0</td>
<td>30 60 120</td>
<td>30 60 120</td>
</tr>
<tr>
<td>Energy density [kJ L⁻¹]</td>
<td>0</td>
<td>46 92 184</td>
<td>351 702 1405</td>
</tr>
<tr>
<td>Temperature [ºC]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First run</td>
<td>18 28 37 50</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Second run</td>
<td>18 - - -</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Third run</td>
<td>18 - - -</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>SCOD [mg L⁻¹]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First run</td>
<td>84 128 180</td>
<td>384 120 152</td>
<td>240</td>
</tr>
<tr>
<td>Second run</td>
<td>84 - - -</td>
<td>186 238 312</td>
<td></td>
</tr>
<tr>
<td>Third run</td>
<td>84 - - -</td>
<td>390 - -</td>
<td></td>
</tr>
<tr>
<td>DD_{COD} [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First run</td>
<td>0 5 12 37</td>
<td>4 8 19</td>
<td></td>
</tr>
<tr>
<td>Second run</td>
<td>0 - - -</td>
<td>13 19 28</td>
<td></td>
</tr>
<tr>
<td>Third run</td>
<td>0 - - -</td>
<td>38 - -</td>
<td></td>
</tr>
</tbody>
</table>

**Role of power intensity**

During the first run, the temperature in the RAS samples treated with the Sonix probe reached 50 ºC, while the temperature in the RAS samples treated with the ASPS reactor was kept at 18 ºC. At 184 kJ L⁻¹, the degree of SCOD release (DD_{COD}) in the RAS samples treated with the Sonix probe was 37 %, in those treated with the ASPS reactor 2.3 %. However, at 184 kJ L⁻¹, in the second and third runs using the ASPS reactor, the DD_{COD} values in the RAS samples pre-heated at 33 ºC and 52 ºC increased to 6.6 % and 10 %, respectively. (Figure 4-4).
Figure 4-4: Effects of ultrasound intensity and temperature on the degree of SCOD release in RAS (1.8 g L\(^{-1}\)) from Brampton WWTP treated with the Sonix probe (i.e. high intensity system) without temperature control and with the ASPS reactor (i.e. low intensity system). The ASPS reactor was operated with temperature control but using different initial RAS temperatures (18, 33 and 52 °C). The degree of SCOD release that the ASPS reactor could potentially achieve at 52 °C using the more efficient piezoelectric transducers is also shown for comparison (the cyan line).

According to the previous results, in the first run at 184 kJ L\(^{-1}\), the Sonix probe caused a SCOD release 16 times higher than the ASPS reactor operated with temperature control. However, the final temperature in the RAS samples treated with the Sonix probe was 50 °C, while the temperature in the RAS samples treated with the ASPS reactor with temperature control was kept at 18 °C.

From the literature, the combination of cavitation and temperature is known to enhance the SCOD release (Chu et al., 2001; Grönroos et al., 2005). To differentiate the effects due to the power intensity and temperature, the RAS samples were preheated at 33 °C and 52 °C before treatment with the ASPS reactor during the second and third runs. The ASPS reactor was then was operated to keep the initial temperature constant during the disintegration trials.
In this way, in the second run, there was a 5.6-fold increase in the SCOD release caused by the Sonix probe in comparison to the ASPS reactor working at 33 ºC, instead of the initial 16-fold increase.

Finally, in the third run, the ASPS reactor was operated with an initial RAS temperature of 52 ºC. This value was chosen to match the temperature reached by the RAS samples treated with the Sonix probe at the highest energy density considered, i.e. 50 ºC at 184 kJ L⁻¹. In this last run, when the Sonix probe and the ASPS reactor were working at the same temperature, 50-52 ºC, the Sonix probe caused a SCOD release 3.7 times higher the ASPS reactor: a much lower value than the initial 16-fold increase in SCOD release due to the combined action of higher power intensity and temperature.

However, the Sonix probe is fitted with a piezoelectric transducer, ASPS with a magnetostrictive one. Taking into account the different efficiency of the two types of transducer, the result was that at least a 2.2-fold increase in SCOD release could still be attributed to the higher intensity alone.

Other studies have investigated the role of intensity. Neis et al. (Neis et al., 2000) using a 3.8 k/31 kHz system found a linear relationship between SCOD release and intensities within 6 and 8 W cm⁻². In their study, a 2-fold increase in SCOD release was caused by a 1.3 increase in intensity. The explanation given was that, at higher intensities, higher mechanical shear forces are produced which are able to cause more cell lysis. Grönroos et al. (2005), using a 27 kHz system, gave the same explanation when they found a 6.9 fold increase in SCOD release with a 3.5 increase in intensity. In assessing the role of intensity, another important contribution came from the work of Nickel and Neis (2007). In their paper describing the development of a full-scale ultrasound reactor for sludge disintegration (equipped with the same Ultrawaves probe used in this study) they clearly recommended the use of high intensity systems. Their suggestion is even more interesting because their new system evolved from a previous model that can be classified as a low intensity system, with multiple flat transducers mounted on the walls of a tube shaped reactor. The two systems were
compared in an earlier work of Nickel (2005) which gave results in agreement with this study. Working at 38 kJ L$^{-1}$, the SCOD release from the high intensity system was 4.8 higher than from the low intensity system. The high intensity system was operated with estimated power intensity 3 times higher than the previous low intensity system.

Direct comparisons in terms of figures are difficult because too dependent on the equipment and operational conditions. However, the higher efficiency of high intensity systems was also explained by the theoretical work of Moholkar et al., (1999). Based on mathematical simulations, they showed that, by increasing the intensity, the amplitude of ultrasound is increased together with the magnitude of the pressure pulse generated by the imploding cavitation bubbles and hence the magnitude of the hydromechanical forces. The increased magnitude of the hydromechanical forces leads to higher levels of floc and cell disruption.

**Role of the temperature**

Operating the ASPS reactor, the higher the initial temperature of RAS was, the higher the degree of SCOD release was. In this study, on average, there was a 1.4-fold increment in the SCOD release every 10 ºC of temperature increment. Grönroos et al. (2005) found a 0.8 increase every 10 ºC.

According to Chu et al. (2001) bulk temperature is an essential factor that controls transformation of solid-state organic compounds and it seems to be equally important as ultrasound in inducing sludge floc disintegration and cell lysis. Both Chu et al. (2001) and Grönroos et al. (2005) agreed that heating alone already increased SCOD release, but it was the combination of heat and ultrasound which greatly enhanced it. Save (1994) suggested as an explanation that temperature makes bacterial cells more susceptible to cavitation forces, most likely by weakening bacteria cell walls.

**4.2.3 The role of the solids content during the Cranfield pilot-scale plant trial**

The role of solids content in the release of SCOD release was investigated during the Cranfield pilot-scale plant.
Sludge samples were collected from the Cranfield pilot-scale plant and thickened at different total suspended solid (TSS) concentrations from 3.2 to 50.7 g TSS L\(^{-1}\). Sludge samples were placed inside a 6 L beaker and then treated at 42 kJ L\(^{-1}\) with no temperature control by immersing and stirring the Ultrawaves probe inside the beaker. The flow-through mode generally adopted with the Ultrawaves probe was not used during this trial because the peristaltic pump was not able to let the thickened sludge samples flow properly through the flow cell due to their high solids content.

The SCOD released by the sludge samples with a TSS concentration of 3.2, 5.7, 28.4 and 50.7 g TSS L\(^{-1}\) was: 168, 408, 1412 and 2850 g SCOD L\(^{-1}\). When the SCOD release was plotted against the TSS concentration, an almost linear relationship was found with an R-squared value of 0.99 (Figure 4-5).

Figure 4-5: SCOD release vs solids content in sludge from the Cranfield pilot-scale plant. Sludge samples were thickened up to a concentration of ~5 % DS (i.e. 50.7 g TSS L\(^{-1}\)) and treated with the Ultrawaves probe at 42 kJ L\(^{-1}\).
Around 55 mg of COD were released for every gram of total suspended solids present in the sludge samples treated at 42 kJ L$^{-1}$. Other studies in the literature found this form of linearity but generally a lower range of concentrations was investigated (Neis et al., 2000; Lehne et al., 2001; Eder and Günthert, 2002; Gonze et al., 2003). Two explanations were given for this linearity (Neis et al., 2001; Tiehm et al., 2001; Gonze et al., 2003):

- The presence of more solids delivers more cavitation sites
- The statistical probability that a solid is affected by the mechanical jet streams during bubble implosion increases with higher solids concentration until an optimum solids concentration beyond which sludge solids reduce the cavitation effect by absorbing the ultrasound wave

Show et al. (2007) reached this critical concentration at 3 % DS, but they were working using lab-scale equipment. Results from this study showed that, working with industrial equipment designed for sludge disintegration at full-scale, it is possible to treat sludge with concentration at least as high as 5-6 % DS without dampening effects on the cavitation field.

The possibility of treating sludge with solids content of 5-6 % or higher without loss in disintegration performance is important because it confirms the advantages of treating thickened sludge (Lehne et al., 2001). Furthermore, it allows a sounder scale-up of the results obtained in this study during the disintegration trials completed as part of the dynamic studies.

### 4.2.4 The role of the horn design using two high intensity ultrasound probes during the Cotton Valley WWTP trial

The design of ultrasound horns can play a major role in achieving high levels of process optimisation and low energy consumption (Mason, 2003). Both the Sonix and Ultrawaves probes are high intensity systems (~50 W cm$^{-2}$). On the other hand, their horn design is completely different. The horn of the Sonix probe has a distinctive radial shape while the horn of the Ultrawaves probe is an optimised variation of the stepped horn (Figure 3-1 in the Material and method chapter). Both systems were
operated without temperature control and their performance in terms of sludge solubilisation was compared evaluating the SCOD release in RAS samples collected from Cotton Valley WWTP on two occasions. The total suspended solids concentrations of the RAS samples varied between 7.3 and 8.3 g L$^{-1}$.

The RAS samples treated with the Sonix probe were placed inside a 1.5 L beaker. The Sonix probe was then immersed in the beaker and stirred. By varying the retention time between 0 and 120 s, energy densities between 0 and 184 kJ L$^{-1}$ were used to treat the RAS samples with the Sonix probe.

The RAS samples treated with the Ultrawaves probe were pumped through the 2 L flow cell by means of a peristaltic pump. By varying the flow rate between 0.25 and 1 L min$^{-1}$, energy densities between 0 and 168 kJ L$^{-1}$ were used to treat the RAS samples with the Ultrawaves probe.

The maximum degree of SCOD release (DD$_{\text{COD}}$) was 27.5 % at 184 kJ L$^{-1}$ in the RAS samples treated with the Sonix probe, and 22.7 % in the RAS samples treated with the Ultrawaves probe at 168 kJ L$^{-1}$. The differences in the degree of SCOD release (DD$_{\text{COD}}$) in the RAS samples treated with the Sonix and Ultrawaves probes were below 5% (Figure 4-6).
Figure 4-6: Comparison between the SCOD released in the RAS samples (7.3 and 8.3 g TSS L\(^{-1}\)) collected from the Cotton Valley WWTP. RAS samples were treated with the Sonix and Ultrawaves probes at energy densities between 0 and 184 kJ L\(^{-1}\) and 0 and 168 kJ L\(^{-1}\), respectively. Both systems were operated without temperature control.

During the dynamic studies, the standard deviation in the DD\(_{\text{COD}}\) values in the RAS samples collected from the control lane in the pilot-scale plant and treated with the Ultrawaves probe was ±6 % (Figure 5-16 in the Dynamic studies chapter). Therefore, the 5 % difference between the DD\(_{\text{COD}}\) values in the RAS samples treated with the Sonix and Ultrawaves probe could be considered not significant.

These results suggested that both horn designs were equally efficient in disintegrating sludge. However, the significance of this trial might be limited because the real performance of both ultrasound systems should be evaluated using their specific full-scale flow-through reactors. These reactors are specifically engineered to maximise the overall disintegration performance but could not be used during this trial, mainly for financial reasons. According to Mason (2003), high level of efficiency can be reached by optimising the design of the reactors. No other studies comparing two high intensity ultrasound systems were found in the literature.
4.3 Evaluation of the impact of ultrasound treatment on activated sludge biomass from different types of WWTP

During the ultrasound impact trials, the effects of sludge disintegration by ultrasound on floc breakage and sludge solubilisation were investigated to assess if these effects varied significantly depending on the type of WWTP.

The three different types of WWTP considered in this study were: (1) conventional activated sludge (CAS); (2) oxidation ditch (OD) and (3) biological nutrient removal (BNR).

All the RAS samples were treated with the Sonix probe, without temperature control, at energy densities between 0 and 184 kJ L\(^{-1}\). A 1.5 L beaker was filled with sludge, the Sonix probe was then immersed in the beaker and stirred for retention times between 0 and 120 s.

4.3.1 Floc breakage

The effects of sludge disintegration on floc breakage were investigated in the RAS samples collected from two CAS WWTPs (Cotton Valley and Huntingdon) and from a BNR WWTP (Derby).

The initial sizes of the flocs in the RAS samples collected from the Cotton Valley, Huntingdon and Derby WWTPs were 135, 86 and 129 μm, respectively.

At 184 kJ L\(^{-1}\), the floc sizes in the RAS samples collected from the Cotton Valley, Huntingdon and Derby WWTPs, were reduced by 84, 76 and 28 % respectively.

In the RAS samples collected from the Cotton Valley and Huntingdon WWTPs the floc sizes were reduced by 80 and 59 % at 46 kJ L\(^{-1}\) (Figure 4-7).
Chapter 4: Batch studies

The reductions in the floc size of the RAS samples collected from the Cotton Valley and Huntingdon WWTPs were in accordance with those reported in the literature. In most studies in the literature, at 184 kJ L⁻¹, floc size reductions varied between 70 and 90 %, with reductions between 25 and 78 % occurring at 46 kJ L⁻¹ (Figure 2-12 in the Literature review chapter) (Biggs and Lant, 2000; Chu et al., 2001; Lehne et al., 2001; Neis and Blume, 2002). On the contrary, only a limited floc reduction could be achieved in the RAS samples collected from the Derby WWTP, 28 % at 184 kJ L⁻¹.

Due to the limited amount of WWTPs considered in relation to floc breakage, it was not possible to evaluate if the effects of ultrasound treatment on floc breakage varied significantly depending on the type of WWTP. It would be a difficult task to accomplish anyway because, according to Wilén et al. (2003), it is always difficult to compare results from particle size analysis using sludge samples from different WWTPs. Floc stability and strength are influenced by many factors (e.g. WWTP design and operating conditions, wastewater characteristics) which are not fully understood.
4.3.2 Sludge solubilisation: the degree of SCOD release (DD\textsubscript{COD})

To evaluate if there were significant differences in sludge solubilisation depending on the types of WWTP, the degrees of SCOD release (DD\textsubscript{COD}) in RAS and ML samples collected from 10 different WWTPs were compared. The RAS and ML samples were collected and treated during batch disintegration trials completed as part of this study and the one undertaken by Deans (2003) (Table 4-3). In both studies the same ultrasound equipment (the Sonix probe), similar operational conditions and procedures were used. The only difference was that in this study the RAS samples were treated at energy densities between 0 and 184 kJ L\textsuperscript{-1}, while Deans (2003) treated the ML samples with energy densities between 0 to 48 kJ L\textsuperscript{-1}.

Table 4-3: Type and list of WWTPs visited during this study and in the one undertaken by Deans (2003)

<table>
<thead>
<tr>
<th>Type</th>
<th>WWTP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional activated sludge (CAS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton Valley</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>Cambridge</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>Huntingdon</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td><strong>Oxidation Ditches (OD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brampton</td>
<td>This study</td>
<td>(Deans, 2003)</td>
</tr>
<tr>
<td>Brackley</td>
<td>(Deans, 2003)</td>
<td></td>
</tr>
<tr>
<td>Bourne</td>
<td>(Deans, 2003)</td>
<td></td>
</tr>
<tr>
<td>Bungay</td>
<td>(Deans, 2003)</td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td>(Deans, 2003)</td>
<td></td>
</tr>
<tr>
<td>Uppingham</td>
<td>(Deans, 2003)</td>
<td></td>
</tr>
<tr>
<td><strong>Biological nutrient removal (BNR)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Derby</td>
<td>This study</td>
<td></td>
</tr>
</tbody>
</table>

The outline of the ultrasound impact trials completed in this study is provided in the Table 3-6 and Table 3-7 in the Material and methods chapter. The outline of the batch disintegration trials completed by Deans (2003) is provided in Table 4-4.
Table 4-4 Outline of disintegration trials completed by Deans (2003)

<table>
<thead>
<tr>
<th>WWTP (type)</th>
<th>Date</th>
<th>Sludge: TSS [g L⁻¹]</th>
<th>Analysis (WWTPs)</th>
<th>Experimental conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bourne (OD)</td>
<td>19/03/02</td>
<td>ML: 6.1 g L⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brackley (OD)</td>
<td>16/01/02</td>
<td>ML: 3.8 g L⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brampton (OD)</td>
<td>13/02/02</td>
<td>ML: 3.3 g L⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bungay (OD)</td>
<td>06/02/02</td>
<td>ML: 2.5 g L⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye (OD)</td>
<td>06/02/02</td>
<td>ML: 4.8 g L⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uppingham (OD)</td>
<td>10/03/02</td>
<td>ML: 5.7 g L⁻¹</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Analysis:**
- TSS
- SCOD

**Equipment:** Sonix probe
**Power:** 2000 W
**Reactor volume:** 5 L
**Retention times:** 0, 2, 5, 10, 20, 60, 120 s
**Energy densities:** 0, 0.8, 2, 4, 8, 24, 48 kJ L⁻¹
**Procedure:** A 5 L beaker was filled with sludge. Sonix horn was then immersed in the beaker and stirred with no temperature control.

Only the range of energy densities between 0 and 48 kJ L⁻¹ was considered for the comparison of the degrees of SCOD release (DDₐₗₖ) from different types of WWTPs and for their statistical analysis. This decision was based on three main reasons. Firstly, 0 to 48 kJ L⁻¹ was the maximum range considered by Deans (2003). Secondly, this range of energy densities can already provide significant information because is wider than the range generally used at full-scale. For sludge disintegration at full-scale, high intensity probes such as the Sonix and Ultrawaves probes are generally operated at energy densities between 4 and 15 kJ L⁻¹ (Enpure, 2005; Wolff et al., 2007). Lastly, within this range of energy densities, the temperature effects on the SCOD release were limited. For technical reasons it was not possible to operate the Sonix probe with temperature control. At around 48 kJ L⁻¹, there was already an increase of 10 °C in the temperature of the treated sludge. At 184 kJ L⁻¹, the temperature increase was around 30 °C. This temperature increase would have influenced the statistical analysis considering that in this study, on average, there was a 1.4-fold increment in the SCOD release every 10 °C.
At 48 kJ L\(^{-1}\), the range of DD\(_{\text{COD}}\) values varied between 3 and 7% in the RAS samples collected from CAS WWTPs, between 1.4 and 7.7% from the OD WWTPs and was around 5% from the BNR WWTP. The overall range of DD\(_{\text{COD}}\) from all the WWTPs considered in this study, between 1.4 and 7.7%, was within the average range found in the literature (0.5 – 27%) (Figure 4-8).

![Figure 4-8: Summary of the degrees of SCOD release in the RAS and ML samples collected from different WWTPs and treated at different energy densities with the Sonix probe. The maximum and minimum degrees of SCOD release reported in the literature review were also reported for comparison (black lines).](image)

A statistical analysis was performed even if the number of DD\(_{\text{COD}}\) values available for each energy density and type of WWTP was not the same. As previously discussed, the range of energy densities considered in this analysis was between 0 and 48 kJ L\(^{-1}\). The rationale was to see if there was any significant difference in the degree of SCOD released in RAS and ML samples collected from CAS, OD and BNR WWTPs. A robust linear least square fitting method was adopted for fitting the DD\(_{\text{COD}}\) values to the energy densities. The 95% confidence bounds for the linear fitting to the OD
DD\textsubscript{COD} values and the only BNR DD\textsubscript{COD} values available lay within the 95% confidence bounds for the CAS DD\textsubscript{COD} values (Figure 4-9). This means that, at least within the DD\textsubscript{COD} measurements available, there was no significant difference in the release of SCOD from RAS and ML samples collected from different type of WWTPs.

![Graph showing linear fitting and 95% confidence bounds for DD\textsubscript{COD} values](image)

**Figure 4-9:** Linear fitting and 95% confidence bounds for the DD\textsubscript{COD} values available from the RAS and ML samples collected from different CAS, OD and BNR WWTPs and treated with the Sonix probe using from 0 to 48 kJ L\textsuperscript{-1}. 


Chapter 5: Dynamic studies

5.1 Introduction

According to the WIRES report (Ginestet, 2006), it was not possible to predict the degree of sludge reduction only on the base of the effects of the selected disintegration treatment on the activated sludge biomass. Therefore, in this study, a combination of both batch and dynamic trials were completed to investigate the potential of the ultrasound treatment for sludge reduction based on its effects on the activated sludge biomass and its actual impact on sludge production.

This chapter is divided in three sections that report on the results from the batch and dynamic trials completed as part of the dynamic studies:

- **Ultrasound potential trials** – Batch trials completed to investigate the effects on the pilot-scale plant biomass that can theoretically trigger sludge reduction mechanisms

- **Pilot-scale plant trials** – Dynamic trials completed to investigate: (1) the effects on the pilot-scale plant biomass that can theoretically trigger sludge reduction mechanisms; (2) the actual impact on sludge production and (3) the performance of the activated sludge process.

The first section of this chapter examines the impact of ultrasound treatment on the performance of the activated sludge process during the pilot-scale plant trials. The second section of this chapter investigates the potential of ultrasound for sludge reduction by evaluating the effects of ultrasound treatment on floc breakage, sludge solubilisation and biological activity during the ultrasound potential trials and the pilot-scale plant trials. The third section of this chapter investigates the potential of ultrasound for sludge reduction by evaluating the actual reduction in the sludge production (RSP) during the pilot-scale plant trials.

The three sections of this chapter address the following project objectives:

- Evaluation of the ultrasound impact on the activated sludge process
- Investigation on the mechanisms of sludge reduction based on the effects of
ultrasound treatment on the activated sludge biomass

- Evaluation of the degree of sludge reduction.

### 5.1.1 The pilot-scale plant trials

The seven pilot-scale plant trials completed as part of the dynamic studies comprised the baseline and six disintegration trials. The baseline was completed to compare sludge production and overall performance of the control and test lane and assess the suitability of the pilot-scale plant for this research. The six disintegration trials were completed using different ultrasound operational conditions to trigger different degrees of reduction. The six disintegration trials were named after the level of the energy input used, and, when the energy input was the same, after the level of the energy density used (Table 5-1):

- Trial L: disintegration trial completed using a low energy input (1008 kJ d⁻¹)
- Trials M (trial M/L (F/M), trial M/L, trial M/M and trial M/H): disintegration trials completed using the same medium energy input (6048 kJ d⁻¹) but different energy densities. Trial M/L (F/M) and trial M/L were completed applying an energy density of 42 kJ L⁻¹, trial M/M an energy density of 84 kJ L⁻¹, trial M/H an energy density of 168 kJ L⁻¹
- Trial H: disintegration trial completed using a high energy input (15120 kJ d⁻¹).

**Table 5-1: Summary of the operational conditions used during the disintegration trials in this study.**

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M</th>
<th>M</th>
<th>M</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy input [kJ d⁻¹]</td>
<td>-</td>
<td>1008</td>
<td>6048</td>
<td></td>
<td></td>
<td>15120</td>
</tr>
<tr>
<td>Energy density [kJ L⁻¹]</td>
<td>0</td>
<td>42</td>
<td>42</td>
<td>84</td>
<td>168</td>
<td>84</td>
</tr>
<tr>
<td>Stress factor [d⁻¹]</td>
<td>-</td>
<td>0.05</td>
<td>0.28</td>
<td>0.14</td>
<td>0.07</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Unless specified, during all pilot-scale plant trials (Table 3-11), the control and test lanes were operated using the same SRT for both of them. However, the SRT was not kept constant but it varied (the second and third columns in Table 5-2) to keep the total suspended solids (TSS) in the control lane at the same optimal level detected during the baseline (~2.8 g L⁻¹). The TSS in the test lane was free to vary in response to the operational conditions used during the disintegration trials.
The trial M/L (F/M) was the only exception to this rule. During the trial M/L (F/M) the SRT in the control and test lanes was varied independently to keep the same optimal TSS in both lanes, i.e. to keep the same food on microorganism ratio (F/M) in both lanes.

In practice, due to the different concentrations in the total suspended solids in the test and control mixed liquors and effluents, even if the same volume of mixed liquor was wasted from the aeration tanks, it was not possible to keep the same SRT in both lanes (the second and third columns in Table 5-2). On average, the differences in the SRT between the test and control lanes varied from 0 (during the baseline) to 2.6 days (during the trial M/M).

The observed yield is a function of the SRT, and the theoretical relationship between the observed yield, the SRT and other wastewater characteristics and microbial kinetics parameters is given by the equation (2.7) in the Literature review chapter. Equation (2.7) was used to evaluate theoretically the test and control observed yields (the fourth and fifth column in Table 5-2) as a function of the corresponding SRT values in the control and test lanes, default microbial growth kinetics parameters and the Cranfield settled wastewater characteristics. The variation $\Delta Y_{OBS}$ in the test and control observed yields due to the different SRT values was then evaluated using equation (5.1):

$$
\Delta Y_{OBS}[\%] = \frac{(Y_{OBS,CTRL} - Y_{OBS,TEST})}{Y_{OBS,CTRL}} \cdot 100
$$

Using this approach the differences in the SRT values between the test and control lanes were able to cause only limited variations in the test and control observed yield: between -0.1 % in the baseline and 2.9 % in the trial M/L (F/M) (the sixth column in Table 5-2).
Table 5-2: SRT values in the control and test lanes during the pilot-scale plant trials and the variation $\Delta Y_{OBS}$ caused by their difference on the corresponding observed yields evaluated using equation (2.7).

<table>
<thead>
<tr>
<th>Trials</th>
<th>SRT [d$^{-1}$]</th>
<th>(Theoretical) $Y_{OBS}$ [g TSS g$^{-1}$ COD]</th>
<th>$\Delta Y_{OBS}$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control lane</td>
<td>Test lane</td>
<td>Control lane</td>
</tr>
<tr>
<td>Baseline</td>
<td>14±4</td>
<td>14±4</td>
<td>0.351</td>
</tr>
<tr>
<td>Trial L</td>
<td>17±5</td>
<td>18±5</td>
<td>0.325</td>
</tr>
<tr>
<td>Trial M/L (F/M)</td>
<td>13±1</td>
<td>16±4</td>
<td>0.324</td>
</tr>
<tr>
<td>Trial M/L</td>
<td>11±3</td>
<td>11±2</td>
<td>0.337</td>
</tr>
<tr>
<td>Trial M/M</td>
<td>21±3</td>
<td>23±2</td>
<td>0.343</td>
</tr>
<tr>
<td>Trial M/H</td>
<td>20±4</td>
<td>23±5</td>
<td>0.347</td>
</tr>
<tr>
<td>Trial H</td>
<td>20±2</td>
<td>20±3</td>
<td>0.341</td>
</tr>
</tbody>
</table>

During the trial M/L (F/M), the difference in the SRT between the control and test lane was on average 2.5 days, which led to a difference of only 2.9 % between the control and test observed yields evaluated using equation (2.7) (the sixth column in Table 5-2). Therefore, the trial M/L (F/M) and trial M/L could be considered as two replicas completed using the same ultrasound operational conditions, i.e. medium energy input (6048 kJ d$^{-1}$) and low energy density (48 kJ L$^{-1}$).

In all the graphs of this chapter showing the performance and the degrees of sludge reduction for the pilot-scale plant test and control lane, the pilot-scale plant trials were ordered for increasing energy inputs and, when the same energy input was used, for increasing energy densities. Therefore, results are presented in the graphs according to the following order: baseline, trial L, trial M/L (F/M), trial M/L, trial M/M, trial M/H, trial H. However, chronologically, the pilot-scale plant trials were completed according to the following order: baseline, trial M/L (F/M), trial M/L, trial L, trial M/M, trial M/H, trial H (Table 3-12 in the Materials and methods).

Whenever possible the results from the six disintegration trials were compared with those from the most significant disintegration trials found in the literature and completed as part of the other dynamic studies investigating the use of cavitation-based technologies for sludge reduction (Table 5-3).
Table 5-3: Summary of the operational conditions used during the disintegration trials in the other studies investigating the use of cavitation-based technology for sludge reduction (UH: ultrasound; HPH: high pressure homogeniser).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Equipment</th>
<th>Energy density [kJ L(^{-1})]</th>
<th>Stress factor [d(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Strünkmann et al., 2006)</td>
<td>UH</td>
<td>103</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>117</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>(Camacho et al., 2002b)</td>
<td>HPH</td>
<td>~45</td>
<td>0.2</td>
</tr>
<tr>
<td>(Zhang et al., 2007a)</td>
<td>UH</td>
<td>216</td>
<td>~0.2</td>
</tr>
<tr>
<td>(Cao et al., 2006)</td>
<td>UH</td>
<td>150</td>
<td>NA</td>
</tr>
</tbody>
</table>

The volume of the lab-/pilot-scale plants used in the different dynamic studies varied significantly: from 7 L (Zhang et al., 2007a) to 1220 L per lane (this study) (Table 5-4).

Table 5-4: Comparison among the volumes of the lab-/pilot-scale plant used in this and in the other studies investigating the use of cavitation-based technology for sludge reduction.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Lab-/pilot-scale plant volume [L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>1220</td>
</tr>
<tr>
<td>(Strünkmann et al., 2006)</td>
<td>36</td>
</tr>
<tr>
<td>(Camacho et al., 2002b)</td>
<td>200</td>
</tr>
<tr>
<td>(Zhang et al., 2007a)</td>
<td>7</td>
</tr>
<tr>
<td>(Cao et al., 2006)</td>
<td>13.6 (aerobic reactor only)</td>
</tr>
</tbody>
</table>

The energy input is greatly influenced by the volume of the lab-/pilot-scale plant used. Therefore, without applying specific conversions, the ultrasound operational conditions of the disintegration trials completed in different studies could only be compared in terms of the energy density and stress factor and not in terms of the energy input.

5.1.2 Multiple comparison tests
Whenever possible, the results from the different trials completed during the dynamic studies were assessed to see if they were significantly different or not. For this purpose, multiple comparison tests were used in conjunction with the one-way ANOVA test, applying the Tukey's honestly significant difference criterion. This criterion, using the statistical information from the one-way ANOVA test, provided comparison intervals. Two averages were significantly different if their comparison
intervals were disjoint, and were not significantly different if their intervals overlap. All tests were implemented using MATLAB®. The results from the multiple comparison tests can be found in the Appendix I.
5.2 Evaluation of the impact on the performance of the activated sludge process

During the dynamic studies, seven pilot-scale plant trials were completed examining the impact of continuous in-line disintegration on: the TCOD removal, the NH₄ removal and the effluent NO₃ concentrations, the total P removal, the effluent turbidity and the suspended solids removal, the settleability and the dewaterability.

5.2.1 TCOD Removal

In this study, the total COD removal during the baseline and the disintegration trials at low energy input (trial L) and medium energy input (trials M) varied from 79.3±7.2 % (baseline, test lane) to 89.8±5 % (trial M/L (F/M), test lane). However, during the trial at low energy input (trials L) and medium energy input (trials M), the removal differences between the test and control lane were within –1 and +2.2 %. Only during the last trial at high energy input (trial H), there was a 9.2 % decrease in the removal: from, on average, 81.3 % in the control lane down to 72.1 % in the test lane (Figure 5-1).

![Figure 5-1: TCOD removal performance in the test and control lanes during the pilot-scale plant trials.](image-url)
The differences in the soluble COD present in the test and control effluent were, on average, lower than 6 mg L\(^{-1}\) in the trial L and trials M, and up to 24 mg L\(^{-1}\) in the trial H. The multiple comparison tests indicated that only the difference in trial H was significantly higher than the other trials (Figure 11-1 in the Appendix I).

The results from the pilot-scale plant trials performed at low (trial L) and medium energy inputs (trials M) were in agreement with the other studies investigating the use of cavitation-based technology for sludge reduction (Table 5-3). During those studies, differences lower that 10 mg L\(^{-1}\) were reported.

The significant decrease in the TCOD removal during the trial H at high energy input could be a result of the following:

- Ultrasound treatment might negatively affected the microbial metabolism by reducing the ability of microorganisms to remove soluble and particulate COD
- It could be the consequence of extensive cell lysis, which reduced the number of metabolically active microorganisms
- In general, by solubilising part of the sludge biomass, ultrasound treatment leads to an increase in the COD load of the test systems. Part of the COD released is not biodegradable within the system hydraulic retention time (Strünkmann et al., 2006), and part of it is not biodegradable at all (Rocher et al., 1999; Andreottola et al., 2007). Therefore, losses of COD in the effluent could appear because at higher energy inputs ultrasound treatment solubilised organic inert compounds that could not be biodegraded within the 10-hour hydraulic retention time for the pilot-scale plant.
5.2.2 Ammonium removal and effluent nitrate

Ammonium removal was consistently over 97.5% in the control and test lanes during the baseline, the disintegration trial at low energy input (trial L), the disintegration trials at medium energy input and low (trial M/L (F/M) and trial M/L) and medium (trial M/M) energy density. It then decreased in the test lane to 92% during the trial at medium energy input and high energy density (trial M/H) and to 29.5% during the trial at high energy density (trial H) (Figure 5-2).

The difference in the ammonium concentration between the test and control lanes were, on average, 0.12 mg L\(^{-1}\) in the baseline, trial L, trial M/L (F/M), trial M/L and trial M/M, 2.3 mg L\(^{-1}\) in the trial M/H and 25.6 mg in the trial H. The multiple comparison tests indicated that only the differences in the trial M/H and trial H were significantly higher than in the baseline (Figure 11-2 in the Appendix I).
The trend in the differences in the effluent nitrate concentrations between the test and control lanes also highlighted the increasing loss of nitrification in the trial M/H and the trial H. The differences in the effluent nitrate concentration between the test and control lanes were between –4.4 and +3.2 mg L\(^{-1}\) in the baseline, trial L, trial M/L (F/M), trial M/L and trial M/M, –7 mg L\(^{-1}\) in the trial M/H and –14 mg L\(^{-1}\) in the trial H (Figure 5-3).

Figure 5-3: Differences in the effluent nitrate concentrations between the test and control lanes during the pilot-scale plant trials.

Strünkmann et al. (2006), Camacho et al. (2002b) and Zhang et al. (2007a), applied a stress factor around 0.2 d\(^{-1}\), and reported that nitrogen removal was only slightly deteriorated. Cao et al. (2006) did not specify the stress factor they applied. In their study, they reported little/no variation in the ammonium removal and a limited increase in the nitrate concentrations in the test system (+10 mg) which was mainly attributed to the release of organic nitrogen during the lysis process. The significant decrease in NH\(_4\) removal at high energy inputs observed in this study could be related to the stress factor applied during the trial H, which was 0.33 d\(^{-1}\) (Table 5-1). A loss of
nitrification using such a high stress factor was predicted by the model developed by Yoon and Lee (2005). According to their model, the ammonium removal efficiencies should not be significantly affected by the sludge disintegration until the stress factor reaches a value around 0.4 d\(^{-1}\). After that level, the decrease of the autotrophic biomass concentration and the increase in the release of organic nitrogen due to the solubilisation process, would lead to low nitrification rates and, when an anoxic stage is present, reduced nitrogen removal.

### 5.2.3 Total phosphorus removal

The pilot-scale plant was not configured for biological phosphorus removal. The total phosphorus removal in the control lane varied between 27±9.7 % (baseline) and 42.2±5.2 % (trial M/M) and was, on average, 10.6±14.4 % higher than in the test lane (Figure 5-4).

![Figure 5-4: Total phosphorus removal performance in the test and control lanes during the pilot-scale plant trials.](image)

The disintegration of sludge generally leads to the release of the phosphorus compounds incorporated into the sludge biomass. Therefore, an increase in the
concentration of the soluble total phosphorus in the effluent of systems exposed to some form of sludge disintegration is generally expected (Müller et al., 2004). However, in this study, the multiple comparison tests indicated that there were no significant differences between the test and control lanes among the pilot-scale plant trials (Figure 11-3 in the Appendix I).

The average difference between the concentrations of total phosphorus in the test and control effluent was 0.7±1.2 mg L\(^{-1}\). A similar result was found by Cao et al. (2006), who reported a difference of 2 mg L\(^{-1}\) between the test and control systems. Strünkmann et al. (2006), Camacho et al. (2002b) and Zhang et al. (2007a) did not provide information on the total phosphorus removal.

### 5.2.4 Turbidity and total suspended solids removal

In this study, the effluent turbidity in the test and control lanes varied from 3.8±0.9 NTU (trial M/L (F/M), control lane) to 13.8±3.7 NTU (trial H, test lane). The difference in the effluent turbidity between the test and control lanes was almost zero (0.0±0.9 NTU) during the baseline and -1.5±2.6 NTU during the six disintegration trials (Figure 5-5).
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Figure 5-5: Effluent turbidity in the test and control lanes during the pilot-scale plant trials.

The trial at medium energy input and low energy density (trial M/L (F/M)) and the trial at high energy input (trial H) were the only disintegration trials were the effluent turbidity in the test lane was higher than in the control lane: +3.2±3.3 NTU and +1.6±2.7 NTU, respectively. However, the multiple comparison tests applied to the difference in the effluent turbidity between the test and control lanes indicated that only the difference in trial M/L (F/M) was significantly higher than in the baseline (Figure 11-4 in the Appendix I).

A possible explanation is that the trial M/L (F/M) was, chronologically, the first disintegration trial completed (Table 3-12 in the Materials and Methods). The 3.2±3.3 NTU increase in turbidity in the trial M/L (F/M) might be the consequence of two factors related to the initial disintegration process that led to a 61 % reduction in the floc size of the test lane:

- The release of extracellular polymeric substances (EPS) due to floc breakage
- A 7% decrease of the total suspended solid (TSS) removal, on average, during the trial M/L (F/M) (Figure 5-6), which was also the only disintegration trial with a
TSS concentration in the test lane effluent significantly higher than in the baseline (Figure 11-5 in the Appendix I).

The hypothesis that the increase in turbidity was due to the disintegration process triggering the initial floc breakage was supported by the fact that during trial M/L, completed using exactly the same ultrasound operational conditions, no increase in turbidity was detected. During the trial M/L, the turbidity in the test lane was $1.6 \pm 2.2$ NTU lower than in the control lane (Figure 5-5).

Camacho et al. (2002b) and Zhang et al. (2007a) reported an increase in the effluent turbidity but they did not provide enough information for a numerical comparison. However, Camacho et al. (2002b) did correlate the increase in turbidity with a slight decrease in the TSS removal. Strünkmann et al. (2006) and Cao et al. (2006) did not provide any information on changes in the effluent turbidity.
5.2.5 Settleability

The RAS stirred sludge volume index (SSVI) in the control lane varied between 38.8±3 (trial H) and 75.1±6.4 (trial M/L (F/M)) mL g⁻¹. During the baseline, the RAS SSVI was similar in both the control and test lanes: 66.4±9.2 mL g⁻¹ and 66.7±8.9 mL g⁻¹, respectively. During all the disintegration trials, the RAS SSVI in the test lane was, on average, 35.2±7.3 mL g⁻¹ higher than in the control lane (Figure 5-7).

![Figure 5-7: Settleability in the test and control lanes during the pilot-scale plant trials.](Image)

According to the multiple comparison tests, applied to the differences in the RAS SSVI between the test and control lanes, in all the disintegration trials the differences were significantly higher than in the baseline (Figure 11-6 in the Appendix I). This suggests that the ultrasound treatment caused an overall decrease in the settleability in the test lane during all the six disintegration trials. However, the RAS SSVI values in the test lane always stayed between 64.6 and 99.2 mL g⁻¹, a range that corresponds to sludge of good settling characteristics (Tchobanoglous et al., 2003; Dewil et al., 2006a).
Quantitative comparisons of the sludge settling characteristics among different studies are always difficult because a variety of settling indexes are used in practice (e.g. SVI, DSVI, SQI, SSVI, SSVI$_{3.5}$ ...). In particular, measurements based on the traditional non-stirred SVI method can be significantly affected by the initial concentration of suspended solids (Gray, 2004). However, an increase in the RAS SSVI values after ultrasound treatment was in apparent disagreement with other studies in the literature (Müller et al., 1998; Wünsch et al., 2002). Camacho et al. (2002b) reported a decrease in the SVI from 151 to 104 mL g$^{-1}$, Cao et al. (2006) from 140 to 90 mL g$^{-1}$. On the other hand, Zhang et al. (2007a) found a slight increase in all the systems treated with ultrasound. Strünkmann et al. (2006) did not provide any information on the impact of ultrasound on the settleability.

An explanation for the decrease in the settleability detected in this study is provided by Low and Chase (1999a) and Zhang et al. (2007a). According to those authors, ultrasound treatment can increase the settleability of bulking sludge (i.e. sludge with SVI values above 120 mL g$^{-1}$) due to the breakage of filamentous organisms. However, in the absence of bulking sludge, by disintegrating the activated sludge flocs with already good settling properties, ultrasound can also lead to deterioration in the settleability, due to changes in the surface chemistry and/or the characteristics and quantity of the extracellular polymeric substances (EPS).
5.2.6 Dewaterability

Dewaterability was investigated by monitoring the RAS capillary suction time (CST) and the RAS specific resistance to filtration (SRF) in the control and test lanes of the pilot-scale plant.

The RAS CST values in the control lane varied from 10.8±0.7 (trial H) to 15.1±1.7 (trial M/L) s. During the baseline, the RAS CST values were similar in both the control and test lanes: 12.8.4±1.5 s and 13±1.4 s, respectively. During all the disintegration trials, the RAS CST values in the test lane were, on average, 58.9±14 s higher than in the control lane (Figure 5-8).

![Figure 5-8: CST values in the test and control lanes during the pilot-scale plant trials.](image)

According to the multiple comparison tests, in all the disintegration trials, the differences in the CST values between the test and control lane RAS were significantly higher than in the baseline (Figure 11-7 in the Appendix I). However, no particular trend could be highlighted in relation to the energy input, energy density or stress factor used.
The RAS SRF values in the control lane varied from $1.6 \pm 0.8 \times 10^{10}$ m kg$^{-1}$ (trial M/M) to $3.5 \pm 1.4 \times 10^{10}$ m kg$^{-1}$ (trial M/L). During all the disintegration trials, the RAS SRF values in the test lane were, on average, $7.5 \pm 3.8 \times 10^{10}$ m kg$^{-1}$ higher than in the control lane (Figure 5-9). The highest increases in the SRF values were observed in the trials with a stress factor greater than 0.14 d$^{-1}$ (trial M/L, trial M/M and trial H (Figure 5-25).

![Figure 5-9: SRF values in the test and control lanes during the pilot-scale plant trials.](image)

The RAS SRF values for the test and control lanes during the baseline were not available. Therefore, for the multiple comparison tests, the differences in the RAS SRF values in the control lane among the other pilot-scale trials were used to get a reference value. Using this approach, the difference in the RAS SRF values between the test and control lane RAS were significantly higher during all the disintegration trials investigated (Figure 5-25).

The significant increase in the RAS CST and SRF values, during all the disintegration trials, indicated that ultrasound treatment caused an overall decrease in the dewaterability, in particular when a stress factor greater than 0.14 d$^{-1}$ was applied.
None of the other studies investigating the use of cavitation-based technology for sludge reduction provided information on dewaterability. Furthermore, there is not common agreement on the effects of ultrasound treatment on sludge dewaterability. Some studies reported that, using the appropriate dose, ultrasound treatment could increase sludge dewaterability when applied before anaerobic digestion (Kopp et al., 1997; Friedrich, 2002; Yin et al., 2004). However, the results from this study were in agreement with the research undertaken by other authors (Müller, 2000a; Chu et al., 2001; Winter et al., 2002; Boehler and Siegrist, 2006; Dewil et al., 2006a; Wang et al., 2006). According to these authors, ultrasound treatment leads to a decrease in the dewaterability of the disintegrated sludge. This decrease was mainly attributed to the increased amount of water retained by the biopolymers released by floc breakage and to the increased amount of free water adsorbed on the floc surface, which is greatly extended due to the reduction in the floc size.


5.3 Investigation on the mechanisms of sludge reduction based on the effects of ultrasound treatment on the activated sludge biomass

The potential of the ultrasound treatment for sludge reduction based on the effects on floc breakage, sludge solubilisation and biological activity was investigated during the ultrasound potential trials and pilot-scale plant trials. These effects due to sludge disintegration were investigated because they can theoretically trigger three different sludge reduction mechanisms: enhanced metabolism by floc breakage, lysis-cryptic growth by sludge solubilisation and increased maintenance metabolism by induced stress.

The cell lysate biodegradability was also evaluated in comparison to synthetic sewage and Cranfield University WWTP settled sewage. The reason was that the biodegradability of lysate is a key parameter in the evaluation of the sludge reduction mechanism based on the lysis-cryptic growth by sludge solubilisation.

5.3.1 Floc breakage

The floc size was monitored because its decrease might lead to some form of sludge reduction by enhanced metabolism based on sludge self-consumption (Abbassi et al., 2000; Cao et al., 2006).

Floc breakage due to ultrasound treatment had an impact on the floc size, the strength and shape, the floc size distribution and the volatile fraction (i.e. the ratio VSS/TSS). Similar changes to the ones described in the following paragraphs for the RAS from the test lane occurred in the ML from the test lane.

None of the four studies investigating the use of cavitation-based technologies for sludge reduction (Table 5-3) provided information on the floc size reduction caused by the sludge treatment.
Floc size

The control RAS floc size varied between 247 and 475 μm. During the first disintegration trial at 42 kJ L⁻¹, the test RAS floc size decreased to 39% of the initial value: from 247±15 μm during the baseline down to 96±13 μm. After this initial decrease, the test RAS floc size remained almost constant at 77±15 μm during all the disintegration trials, which were completed using energy densities between 42 and 168 kJ L⁻¹ (Figure 5-10).

Regardless of the energy density used, from 42 to 168 kJ L⁻¹, there were limited changes in the floc sizes detected during the pilot-scale plant, from 65 to 96 μm. This limited change was in agreement with the results from the ultrasound potential trials in which 88% of the total decrease in the floc size of the control RAS treated with ultrasound was already achieved at 42 kJ L⁻¹ (Figure 5-11).

Figure 5-10: Comparison of test and control RAS and ML median floc size during the pilot-scale plant trials.
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Figure 5-11: Floc size reduction in the pilot-scale plant control RAS (~7 g L\(^{-1}\)) treated at different energy densities with the Ultrawaves probe.

The test RAS floc size did not show any sign of increase during the disintegration trial at low energy input (trial L), in which ultrasound treatment was on for only 1 minute per hour for 24 days. This suggests that, once flocs have been broken, little periods of ultrasound treatment are necessary to keep their reduced size. The same result was found by Skelton (Skelton, 2007) who, based on the work of Ding et al. (2006), suggested that the lack of re-aggregation might also be the consequence of the shear provided by the aeration.

**Structure and shape**

According to Eikelboom (2000), the microscopic image can give information on a number of visually observable characteristics of the sludge under study, such as the size, structure and shape of the flocs. Two microscopy images of the pilot-scale plant test RAS (Figure 5-12 (a)) and control RAS (Figure 5-12 (b)) were taken at the end of the trial at high energy input (trial H).
Floc size analysis indicated that the size of the flocs in the control RAS was on average 4.7 times bigger than in the test RAS during the trial H (Figure 5-10). A proper microscopy examination would require the use of digital processing techniques. Nevertheless, even with a less rigorous approach, the microscopy images of the test RAS (Figure 5-12 (a)) and control RAS (Figure 5-12 (b)) confirmed the net decrease in the floc size of the test RAS. Furthermore, flocs in the control RAS (Figure 5-12 (b)) appeared to have a denser and firmer structure with a rather rounded shape while the flocs in the test RAS (Figure 5-12 (a)) appeared characterised by a weaker structure and a more irregular shape.
Figure 5-12: Microscopic photographs (100x) showing the impact of ultrasound treatment on test RAS floc structure (a) compared to the control RAS floc (b) at the end of the last disintegration trial (trial H, September 2007).
**Floc size distribution**

Gonze *et al.* (2003) proposed a model of the floc structure based on the contribution of five main particle size populations with an average diameter of: 0.8 (primary particles: isolated small microorganisms, cellular fragments), 14 (micro-flocs, isolated microorganisms), 55 (flocs), 80 (flocs) and 400 (macro-flocs, large protozoans) μm. Taking that model as a reference to analyse changes in floc size distribution of the pilot-scale plant biomass, the ultrasound treatment caused the almost complete disappearance of the macroflocs (300-500 μm) and a shift towards smaller floc with sizes around 100 μm (Figure 5-13).

![Floc size distribution graph](image)

*Figure 5-13: Example of test and control RAS and ML size distribution at the end of the last disintegration trial (trial H, September 2007).*
Volatile fraction

The RAS volatile fraction varied from 83.2±3.9 % (trial L, control lane) to 88.9±1.5 % (trials M/L, test lane). There was a consistent increase in the RAS volatile fraction during all the pilot-scale plant trials. The increase in the RAS volatile fraction was, on average, 3.4±1.5 % (Figure 5-14).

![Figure 5-14: Percentage of the volatile fraction in test and control RAS during the pilot-scale plant trials.](image)

The multiple comparison tests indicated that in all the disintegration trials, the differences in the RAS volatile fraction between the test and control lanes were significantly higher than in the baseline (Figure 11-9 in the Appendix I). However, it was not possible to highlight any relationship between the increase in the RAS volatile fraction in the test lane and the energy input, energy density or stress factor applied during the pilot-scale plant trials.
Ultrasound potential to implement the enhanced metabolism by floc breakage

The results presented in the previous paragraphs confirmed the ability of ultrasound treatment to effectively reduce the floc size and theoretically trigger the sludge reduction mechanism based on the enhanced metabolism by floc breakage.
5.3.2 Sludge solubilisation

The amount of sludge solubilisation was evaluated in terms of the release of soluble COD or cell lysate COD. The soluble COD is commonly defined as the sum of the truly soluble COD and the colloidal matter that normally passes through 0.45 μm membrane filters (Mamais et al., 1993). In this study, the cell lysate COD was defined as the sum of the truly soluble COD and the colloidal COD. In this study, the ratio between soluble COD and cell lysate COD for energy densities between 42 kJ and 168 kJ L⁻¹ was 0.69 g soluble COD g⁻¹ cell lysate COD. The ratio between the standard deviation and the average was 12.7 %, which indicated that the ratio between soluble COD and cell lysate COD was reasonably constant within the range of energy densities investigated.

During the dynamic studies, the release of cell lysate COD was investigated because of its potential to trigger the sludge reduction mechanism based on the lysis-cryptic growth by sludge solubilisation. Increasing the amount of cell lysate released should lead to a higher degree of sludge reduction. To establish the relationship between the amount of cell lysate and the degree of sludge reduction achievable, different energy inputs and energy densities were used during the six disintegration trials (trial L, trials M and trial H).

Energy inputs were increased by increasing the treatment period from 1 min h⁻¹ in the trial at low energy input (trial L), to 6 min h⁻¹ in the trials at medium energy input (trials M) and 15 min h⁻¹ in the trial at high energy input (trial H) (Table 5-5). In the trials at medium energy input (trials M), the energy densities were increased by decreasing the RAS flow rate through the disintegration unit from 1 L min⁻¹ in the trials at low energy density (trial M/L (F/M) and trial M/L), to 0.5 L min⁻¹ in the trial at medium energy density (trial M/M), to 0.25 L min⁻¹ in the trial at high energy density (trial M/H) (Table 5-5).
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Table 5-5: Summary of the ultrasound operational conditions and the average daily lysate release during the six disintegration trials

<table>
<thead>
<tr>
<th>Trial name</th>
<th>L</th>
<th>M</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy input [kJ d⁻¹]</td>
<td>1008</td>
<td>6048</td>
<td>15120</td>
</tr>
<tr>
<td>Energy density [kJ L⁻¹]</td>
<td>42</td>
<td>42</td>
<td>84</td>
</tr>
<tr>
<td>Treatment period [min h⁻¹]</td>
<td>1</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>RAS flow rate [L min⁻¹]</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Daily average lysate release [g lysate COD d⁻¹]</td>
<td>8.5±0.6</td>
<td>46.2±8.6</td>
<td>55.1±7.3</td>
</tr>
</tbody>
</table>

The effects of increasing energy inputs on the release of cell lysate were investigated in terms of the daily average lysate release during the six disintegration trials (the sixth row in Table 5-5 and Figure 5-15).

The daily average lysate release in the trial H was 2.4 higher than in the trials M and 12.9 higher than in the trial L (Figure 5-15). The reason of these differences among the trial L, trials M and trial H is that the daily average release of lysate is mainly a function of the energy input.

![Figure 5-15: Daily average release of cell lysate during the six disintegration trials.](image-url)
Among the trials at the same energy input (trials M), the highest release occurred during the trial M/L (F/M) and was 1.5 higher than the lower one occurred during the trial M/H (Table 5-5 and Figure 5-15). This smaller difference observed among the trials M was the consequence of the almost linear increase in the release of soluble COD, for increasing energy densities (Figure 5-16).

![Figure 5-16: The degree of SCOD release (DD COD) vs energy density in the control and test RAS. The RAS samples were collected at different times during the pilot-scale plant trials from the control and test lane. The control RAS samples were never exposed to the ultrasound treatment before the disintegration trial. On the opposite, the test RAS samples were already exposed to the continuous in-line treatment of the RAS in the test lane.]

Among the trials M, the highest release of lysate occurred during the trial M/L (F/M) (Table 5-5). The most likely reason is that, chronologically, the trial M/L (F/M) was the first disintegration trial to be completed and therefore the ultrasound treatment was applied to biomass that had never been previously exposed to it. Batch disintegration trials proved that the release of soluble COD from untreated RAS biomass (i.e. the control RAS) was on average 1.7 times higher than from biomass
already exposed to ultrasound treatment (i.e. the test RAS) (Figure 5-16). This decreased release of soluble COD in the biomass already exposed to ultrasound treatment suggests some form of biomass adaptation to it.

In this study, the degrees of SCOD release (DD\textsubscript{COD}) achieved with ultrasound treatment were between 6 and 19\% for energy densities between 42 and 168 kJ L\textsuperscript{-1}. Strünkmann \textit{et al.} (2006) achieved similar results with the ultrasound treatment: DD\textsubscript{COD} values between 11.4 and 20 \% were achieved for energy densities between 103 and 117 kJ L\textsuperscript{-1}. Camacho \textit{et al.} (2002b) achieved higher degrees of COD release using a high pressure homogeniser (i.e. hydrodynamic cavitation): up to 50\% at 45 kJ L\textsuperscript{-1}. The higher disintegration efficiency of hydrodynamic cavitation was confirmed by other studies in the literature (Müller, 2000b; Boehler and Siegrist, 2006).

Not enough information to estimate the degree of SCOD release was provided by the other dynamic studies (Cao \textit{et al.}, 2006; Ginestet, 2006; Zhang \textit{et al.}, 2007a).

**Lysate biodegradability**

The biodegradability of cell lysate is necessary for the viability of the lysis-cryptic growth as a sludge reduction mechanism. Respirometry was used not only to evaluate the biodegradability of cell lysate but also to compare it with the biodegradability of the synthetic sewage and Cranfield University settled sewage. RAS samples from the pilot-scale plant control lane were fed with the three different substrates under study. Enough volume of each substrate was added to have an initial average COD content of 80 g COD. The synthetic sewage used in this study (refer to the Respirometric analysis paragraph for its composition) was the most biodegradable substrate with 87 \% removal after 100 h, followed by cell lysate with 78 \% and settled sewage with 74 \% (Figure 5-17). The result for the cell lysate is in agreement with the 75\% removal rate found by Rocher \textit{et al.} (1999).
Figure 5-17: Percentage of COD removal of different substrates by pilot-scale plant untreated RAS (9.6 g L⁻¹) at the end of the respirometric analysis to assess lysate biodegradability in comparison to synthetic sewage and Cranfield University WWT settled sewage.

The good quality of the lysate as a substrate was confirmed by triggering the highest levels of specific oxygen uptake and specific oxygen uptake rates (Figure 5-18), in agreement with the fact that the use of cell-free extracts has even been investigated for the enhancement of the biological wastewater treatment (Jones and Schroeder, 1989).
Chapter 5: Dynamic studies

Figure 5-18: Specific oxygen uptake (a) and specific oxygen uptake rates vs time of pilot-scale plant untreated RAS (9.6 g L\(^{-1}\)) fed with 80 g of COD from different substrates: cell lysate, synthetic sewage and Cranfield University WWTP settled sewage.
Ultrasound potential to implement the lysis-cryptic growth by sludge solubilisation

The results related to the release of cell lysate by ultrasound treatment (Figure 5-15) and to the biodegradability of the cell lysate (Figure 5-17) presented in the previous paragraphs confirmed the potential of ultrasound treatment to implement the sludge reduction mechanism based on the lysis-cryptic growth by sludge solubilisation.
5.3.3 Biological activity

Introduction
Changes in the biological activity, such as an initial decrease in the oxygen uptake (biological inactivation) followed by recovery of the initial activity or an overall increase in the oxygen uptake, might be a sign of increased maintenance metabolism that could eventually lead to some form of growth reduction (Camacho et al., 2002b; Rai et al., 2004).

To detect the presence of increased maintenance metabolism, the changes in the biological activity of the RAS biomass were monitored in terms of the specific oxygen uptake (SOU) and specific oxygen uptake rate (SOUR) using respirometry. Each SOU value or SOUR curve showed in the graphs of this section is the average of at least three replicas.

Two different groups of respirometric trials were completed. For convenience, the two groups were defined as:

- **Short-term respirometric trials**
- **Long-term respirometric trials.**

The distinction between short-term and long-term trials does not refer to the length of the respirometric trials. For all the respirometric trials, the length was determined by the time needed by the biomass to reach the endogenous respiration. The distinction refers to the short-term and long-term effects of the ultrasound treatment on the RAS biomass.

**Short-term respirometric trials**
Three different respirometric trials were completed as part of the short-term respirometric trials. In these trials, the biological activity in untreated RAS samples was compared to the one present in RAS samples that were treated with ultrasound immediately before the respirometric trial, as part of the ultrasound potential trials. The rational was to investigate the “short-term” effects of ultrasound treatment on the
RAS biomass, as it is usually done in other studies in the literature (Camacho et al., 2002b; Rai et al., 2004).

The RAS samples for the three short-term respirometric trials were collected from: (1) the pilot-scale plant control lane, (2) the Cotton Valley WWTP and (3) the pilot-scale plant test lane, respectively. Some of the RAS samples were treated with the Ultrawaves probe at energy densities between 42 kJ and 168 kJ L\(^{-1}\). The treated and untreated samples were then used for the short-term respirometric trials.

The first trial was completed using the RAS samples collected from the pilot-scale plant control lane. In accordance with the respirometric procedure described in the Material and methods chapter, 50 ml of synthetic sewage were added as substrate and then the oxygen uptake was monitored until the biomass reached the endogenous respiration.

The profile analysis of the SOUR curves indicate that only the RAS samples treated at 168 kJ L\(^{-1}\) exhibited a different behaviour than the one followed by the control samples (Figure 5-19).

![Figure 5-19: Specific oxygen uptake rates vs time in the RAS samples (TSS: 9.6 g L\(^{-1}\)) collected from the pilot-scale plant control lane. The RAS samples were treated at different energy densities with the Ultrawaves probe. Synthetic sewage was added at the beginning of respirometric trial.](image)
The SOUR of the samples treated at 168 kJ L\(^{-1}\) samples took more than 15 h before rising to the level that all the other samples had reached within 1 h. Even after the peak at \(~23\) h, the SOUR profile was different until the end of the trial when the endogenous respiration phase was reached. This kind of profile might suggest some form of biological inactivation (Camacho et al., 2002b).

The second trial was completed using the RAS samples collected from the Cotton Valley WWTP to see if similar profiles in the SOUR were also present in the biomass from a full-scale WWTP. To further investigate the changes in the biological activity found in samples treated at 168 kJ L\(^{-1}\), 50 ml of synthetic sewage were added both at the beginning and after the first endogenous phase. The rationale was to see if the changes were just temporarily or more persistent.

The SOUR of the samples treated at 168 kJ L\(^{-1}\) showed changes in their profile (Figure 5-20) in comparison to the other samples. The profile of the SOUR curves was similar to the one detected in the first trial (Figure 5-19). A different profile in the SOUR curve from all the other samples was still present after the first endogenous phase, suggesting persistence in the effects of ultrasound treatment on biomass treated at high energy densities (168 kJ L\(^{-1}\)) even after 150 h.
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Figure 5-20: Specific oxygen uptake rates vs time in the RAS samples (8.3 g L\(^{-1}\)) collected from the Cotton Valley WWTP. The RAS samples were treated at different energy densities with the Ultrawaves probe. Synthetic sewage was added both at the beginning of respirometric trial and after biomass entered endogenous respiration after ~150 h.

The last trial was completed using the RAS samples collected from the pilot-scale plant test lane. The biomass in the test lane was continuously exposed to ultrasound treatment during the pilot-scale plant disintegration trials completed as part of the dynamic studies. The rationale was to see if treating biomass that had already been exposed to ultrasound treatment would cause changes in the SOUR profiles similar to those underwent by biomass that has never been exposed before. As previously done, 50 ml of synthetic sewage were added both at the beginning and after the endogenous phase.

The SOUR of the RAS samples treated at 168 kJ L\(^{-1}\) showed less significant changes in their profile in comparison to the other RAS samples, which were either untreated or treated at 42 kJ L\(^{-1}\) (Figure 5-21). Furthermore, at the end of the first endogenous phase, after 110 h, their SOUR profile was similar to the other samples. This
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suggested that the overall effects of ultrasound treatment on biological activity became less significant in biomass previously exposed to it.

![Graph showing specific oxygen uptake rates vs time in the RAS samples (6.4 g L⁻¹) collected from the pilot-scale plant test lane.](image)

Figure 5-21: Specific oxygen uptake rates vs time in the RAS samples (6.4 g L⁻¹) collected from the pilot-scale plant test lane. The RAS samples were treated at different energy densities with the Ultrawaves probe. Synthetic sewage was added both at the beginning of this respirometric trial and after biomass entered endogenous respiration after ~110 h.

A quantitative analysis of the three respirometric trials was also undertaken to detect if there was an increase of the overall specific oxygen uptake in the RAS samples treated with ultrasound, which could be a sign of increased maintenance metabolism (Rai et al., 2004).

The specific oxygen uptake (SOU) was evaluated at the end of each short-term respirometric trial. To highlight differences in the biological activity induced by ultrasound treatment between untreated and treated RAS samples, the increment in the SOU values in the RAS biomass treated at different energy densities against the corresponding one in the untreated RAS biomass was calculated using the short-term SOU increment (equation (3.11) in the Materials and methods chapter).
Positive values for the short-term SOU increment indicate an increase in the SOU of the treated RAS samples. The increase in the biological activity could suggest the presence of some form of increased maintenance metabolism by induced stress. Negative values indicate a decrease in the SOU of the treated RAS samples. The decrease in the biological activity would exclude the presence of increased maintenance metabolism by induced stress.

The short-term SOU increment was greater than zero only for the RAS samples treated at 168 kJ L\(^{-1}\) and varied between 0.2 and 8.5 % (Figure 5-22).

![Figure 5-22: Short-term SOU increments in the RAS samples treated with ultrasound in comparison to the untreated ones. The error bars indicate the standard deviations in the measurements.](image)

The multiple comparison tests indicated that the only significant increase in the SOU in the treated RAS samples in comparison to the untreated ones was present in the RAS samples collected from the control lane and treated at 168 kJ L\(^{-1}\) (Figure 11-10 in the Appendix I).
The overall results from the short-term respirometric trials suggested that, at least with the respirometric equipment and procedure adopted, there were no signs of increased maintenance metabolism in RAS samples treated at 42 and 84 kJ L\(^{-1}\). In the RAS samples treated at 168 kJ L\(^{-1}\), the profile of the SOUR curves showed signs of biological inactivation and a significant increase in the specific oxygen uptake only in the RAS samples previously untreated.

The results related to the RAS samples treated with energy densities as high as 84 kJ L\(^{-1}\) are in agreement with those found by Camacho et al. (2002b). Among the four studies investigating the use of cavitation-based technology for sludge reduction (Table 5-3), Camacho et al. (2002b) were the only ones using energy densities similar to those used during the long-term respirometric trials. In their study, Camacho et al. (2002b) investigated the presence of increased maintenance metabolism during batch and dynamic studies treating sludge with a high pressure homogeniser (HPH). During batch studies at energy densities lower than 90 kJ L\(^{-1}\) and during dynamic studies at 45 kJ L\(^{-1}\), Camacho et al. (2002b) did not detect any sign of temporary biological inactivation linked to increase maintenance requirements. They could find only permanent forms of biological activation linked to cell lysis during their batch studies at high energy densities (\ensuremath{\geq} 90 kJ L\(^{-1}\)).

Only Rai et al. (2004) reported increases in the oxygen uptake in RAS samples treated with ultrasound. Rai et al. (2004) did not provide their actual values, but by reverse engineering the data available, the increase in the oxygen uptake should be around 24% for samples treated at 42 kJ L\(^{-1}\). No other information was available in the literature about the potential of cavitation-based equipment for increasing maintenance metabolism.

**Long-term respirometric trials**

The long-term respirometric trials were performed on the RAS samples collected from the control lane (the control lane RAS samples) and the test lane (the test lane RAS samples) of the pilot-scale plant during the pilot-scale plant trials. No ultrasound treatment was performed on the test RAS samples before the long-term respirometric trial. The test lane RAS samples were simply collected from the test lane before
entering the ultrasound disintegration unit. Statistically, the minimum time elapsed since the last pass through the ultrasound system for the test lane RAS samples was longer than a week, depending on the RAS flow rate and, hence, the energy density used (Table 5-6). This procedure was used to evaluate the presence of “long-term” effects of ultrasound treatment in RAS samples that had been previously exposed to ultrasound treatment but not “just before” the respirometric trial (i.e. the test lane RAS). The rationale was to investigate if ultrasound treatment was able to change the biological activity of RAS biomass in a persistent way during dynamic trials.

Due to time constraints, the RAS samples for the long-term respirometric trials were collected only during the baseline and four out of the six disintegration trials completed. The disintegration trials investigated were the trial at low energy input (trial L) and three trials at medium energy input (trial M/L (F/M) and M/L at 42 kJ L⁻¹ and trial M/M at 84 kJ L⁻¹). The SOU values for the test and control RAS are provided in Table 5-6.

### Table 5-6: Summary of the ultrasound operational conditions and the specific oxygen uptake (SOU) in the test and control lane RAS samples, during four disintegration trials (NA: not available)

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M/L</td>
<td>M/L</td>
<td>M/M</td>
</tr>
<tr>
<td>Energy input [kJ d⁻¹]</td>
<td>0</td>
<td>1008</td>
<td>6048</td>
<td>15120</td>
</tr>
<tr>
<td>Energy density [kJ L⁻¹]</td>
<td>0</td>
<td>42</td>
<td>42</td>
<td>84</td>
</tr>
<tr>
<td>Treatment period [min h⁻¹]</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Treated RAS [%]</td>
<td>0</td>
<td>~1.7</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>RAS flow rate [L min⁻¹]</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Time from the last pass [d]</td>
<td>-</td>
<td>50.8</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Test RAS SOU [mg O₂ mg⁻¹ TSS]</td>
<td>0.46</td>
<td>0.42</td>
<td>0.59</td>
<td>0.47</td>
</tr>
<tr>
<td>Control RAS SOU [mg O₂ mg⁻¹ TSS]</td>
<td>0.45</td>
<td>0.41</td>
<td>0.65</td>
<td>0.53</td>
</tr>
<tr>
<td>Short-term SOU increment [%]</td>
<td>1.4±4.8</td>
<td>2.4±3.7</td>
<td>-8.9±2.9</td>
<td>-12.1±3.9</td>
</tr>
</tbody>
</table>

The energy density applied during the four disintegration trials investigated varied between 42 kJ L⁻¹ and 84 kJ L⁻¹. The maximum energy, 84 kJ L⁻¹, was applied during
the disintegration trial at medium energy density and medium energy density (trial M/M) (Table 5-6).
The profile and quantitative analysis for the long-term respirometric trials was undertaken in the same way as for the short-term respirometric trials.
The profile analysis of the SOUR curves from the long-term respirometric trials indicated that there was no sign of biological inactivation in the RAS samples collected from the control and test lanes (data not shown). This result was in accordance with what found during the short-term respirometric trials in the RAS samples treated at energy densities as high as 84 kJ L\(^{-1}\) (Figure 5-19, Figure 5-20 and Figure 5-21).

As part of the quantitative analysis, the specific oxygen uptake (SOU) was evaluated at the end of each long-term respirometric trial. To highlight the differences in biological activity induced by ultrasound treatment between the RAS samples collected from the test lane and the control lane, the increment in the SOU values in the test lane RAS biomass against the corresponding one in the control lane RAS biomass was calculated using the long-term SOU increment (equation (3.12) in the Materials and methods chapter).

In the same way as for the short-term SOU increment, positive values for the long-term SOU increment indicate an increase in the SOU of the RAS samples collected from the test lane. The increase in the biological activity could suggest the presence of some form of increased maintenance metabolism by induced stress. Negative values indicate a decrease in the SOU of the RAS samples collected from the test lane. The decrease in the biological activity would exclude the presence of increased maintenance metabolism by induced stress.

During the baseline and the disintegration trial at low energy input (trial L), the long-term SOU increments were on average +1.4±4.8 % and +2.4±3.7 % respectively (Table 5-6 and Figure 5-23). During the trials M/L (F/M), M/L and M/M, the long-term SOU increments were all negatives (Table 5-6 and Figure 5-23): on average, their long-term SOU increment was -9.7±3.4 %.
Figure 5-23: Long-term specific oxygen uptake (SOU) increments in the RAS samples from the pilot-scale plant. The long-term SOU increments of four pilot-scale plant trials were evaluated: the baseline, the disintegration trial at low (trial L) and medium (trial M/L (F/M) and trial M/L at 42 kJ L$^{-1}$ and trial M/M at 84 kJ L$^{-1}$) energy input. The test RAS samples were collected just before entering the ultrasound systems and, hence, on average, they underwent ultrasound treatment from 8.5 (trial M/L (F/M)) to 50.8 (trial L) days before the start of the respirometric trials (Table 5-6).

The negative value in the long-term SOU increments indicated that the SOU values of the test lane RAS samples were actually lower than the SOU values of the control lane RAS samples. This implied that there was no sign of increased maintenance metabolism by induced stress. The multiple comparison tests confirmed that none of the differences investigated was significantly higher than in the baseline. They also indicated that only the differences in the disintegration trials at low energy input and low energy density (trial M/L (F/M) and trial M/L) were significantly lower than the baseline (Figure 11-13 in the Appendix I).

The SOU decrease in the trials at low energy input and low energy density (trial M/L (F/M) and trial M/L) could be linked to the cell lysis occurred during the previous exposure of RAS biomass in the test lane to ultrasound treatment during the pilot-
scale plant trials. Cell lysis could lead to an accumulation of organic inert material in the RAS samples that was measured as biomass but was not viable and therefore did not contribute to the respiration process. This would suggest the ability of ultrasound to disrupt cells but not to enhance the biodegradability of the cell residual.

The results from the long-term respirometric trials were in agreement with those from the short-term respirometric trials completed using energy densities between 0 and 84 kJ L^{-1}. No sign of temporary biological inactivation linked or increased oxygen consumption could be detected in the RAS samples collected from the control and test lane of the pilot-scale plant.

**Ultrasound potential to implement the increased maintenance metabolism by induced stress**

During the short-term and long-term respirometric trials, no increased maintenance metabolism was found in the RAS samples treated at 42 and 84 kJ L^{-1} (Figure 5-19, Figure 5-22 and Figure 5-23) and in the RAS samples treated at 168 kJ L^{-1} that were previously exposed to ultrasound treatment (Figure 5-21 and Figure 5-22).

A profile in the SOUR curves indicating the occurrence of temporary biological inactivation (Figure 5-19) and a significant 8.5 % increase in the SOU was found only during the short-term respirometric trials in the RAS samples collected from the control lane and treated at 168 kJ L^{-1} for the first time (Figure 5 19 and Figure 5-22). These results suggested that increased maintenance metabolism occurred only at high energy densities and that RAS samples already exposed to ultrasound treatment are less affected by it.

Therefore, at least on the basis of the result from the respirometric trials, the ultrasound treatment was theoretically able to implement the sludge reduction mechanism based on the increased maintenance metabolism by induced stress only treating RAS samples at energy densities as high as 168 kJ L^{-1}.
5.4 Evaluation of the degree of sludge reduction

In the previous section, the potential of ultrasound treatment was investigated in terms of its effects on floc breakage, sludge solubilisation and biological activity. In this section, the ultrasound potential for sludge reduction is investigated by evaluating the actual impact of the ultrasound treatment on the sludge production during the pilot-scale plant trials.

In the graphs of this section, the observed yields are presented as the slope (i.e. the gradient) of the linearisation (not forced through zero) of the correlation between cumulative sludge production rate and cumulative removed TCOD (Strünkmann et al., 2006). The reduction in the sludge production (RSP, equation (3.9) in the Materials and methods chapter) was used as an index to provide the degree of sludge reduction achieved during the different pilot-scale plant trials. The number of sampling days out of the total number of days of duration of the pilot-scale plant trial is also reported at the top of the graphs of this section.

5.4.1 Baseline

The observed yield of the activated sludge process is influenced by many operational and environmental factors and its assessment is not straightforward due to the intrinsic variability of biological systems. For example, Paul and Salhi (2003) found a 30% variation in sludge production when running for two years a lab-scale pilot plant fed by real municipal wastewater.

In this study, the comparison with a control system was considered essential to provide significant information. Therefore, before starting the disintegration trials, a 2-month baseline was completed to assess the variability between the test and control lanes of the pilot-scale plant.

During the 2-month baseline, there was a wide variation in the influent wastewater strength (from 190 to 490 mg TCOD L\(^{-1}\)), food to microorganism ratio (from 0.19 to 0.54 g TCOD g\(^{-1}\) TSS d\(^{-1}\)) and SRT (from 10 to 33 d\(^{-1}\), with 18 days of complete sludge retention) (Table 5-7).
In spite of these variations, there was only a 5.2\% difference between the test and control lane observed yields (Figure 5-24). This limited difference proved that the pilot-scale plant was suitable to investigate changes in the sludge production by comparing the observed yield in the control lane with the one in the test lane, which was exposed to continuous in-line ultrasound treatment.

![Figure 5-24: The test and control observed yields during the baseline. The reduction in the sludge reduction (RSP) between the two lanes provided the degree of variability between the two lanes in the absence of ultrasound treatment.](image)

**Table 5-7: Summary of the pilot-scale plant operational conditions for both the control and test lanes during the 2-month baseline.**

<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>Max</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Settled wastewater strength [mg TCOD]</td>
<td>190</td>
<td>490</td>
<td>312±72</td>
</tr>
<tr>
<td>Food to microorganism ratio (F/M) [g TCOD g⁻¹ TSS d⁻¹]</td>
<td>0.19</td>
<td>0.54</td>
<td>0.38±0.08</td>
</tr>
<tr>
<td>Sludge retention time (SRT) [d⁻¹]</td>
<td>10</td>
<td>33(*)</td>
<td>14±4</td>
</tr>
</tbody>
</table>

(*) Excluding 18 d of complete sludge retention
None of the other studies investigating the use of cavitation-based technology for sludge reduction provided accurate information about their baseline and, hence, the reliability of their lab-/pilot-scale set-up.

5.4.2 Disintegration trial at low energy input: trial L
The trial L was designed to assess whether it was possible to achieve sludge reduction by simply reducing the floc size, exploiting the reduction mechanism based on the enhanced metabolism by floc breakage, in accordance with the hypothesis proposed by Abbassi et al. (2000).

For this purpose, it was essential for all the biomass to be reduced in size before the start of the trial L. Therefore, the trial L was postponed after trial M/L (F/M) and trial M/L to ensure that floc breakage had properly occurred in the test lane.

The 9.4 % increase in sludge production in the test lane during the trial L (Figure 5-25) indicated that floc breakage alone did not lead to sludge reduction.

![Figure 5-25: The test and control observed yields during the trial L, the disintegration trial at low energy input (1008 kJ d⁻¹), and the related reduction in the sludge production (RSP) between the two lanes.](image-url)
The 9.4 % increase in sludge production in the test lane when ultrasound treatment was operated at 42 kJ L\(^{-1}\) and stress frequency of 0.05 d\(^{-1}\) was in agreement with the results provided by Strünkmann et al. (2006). In their study, they detected an 8 % increase in sludge production in their lab-scale plant, when the ultrasound treatment was operated at 117 kJ L\(^{-1}\) with a stress factor of 0.05 d\(^{-1}\). An even higher increase, 28 %, was detected when ultrasound treatment was operated at 5 kJ L\(^{-1}\) with a stress factor of 0.4 d\(^{-1}\). Camacho et al. (2002b) found a 3 % decrease in sludge production in their pilot-scale plant when they operated a high pressure homogeniser at 2 kJ L\(^{-1}\) with a stress factor of 0.2 d\(^{-1}\). A 3 % decrease is not significant considering the variation in the observed yields of pilot-scale plants (~5 % in this study).

Neither Strünkmann et al. (2006) nor Camacho et al. (2002b) provided information about the floc size reduction achieved in their test systems. Among the four studies investigating the use of cavitation-based technology for sludge reduction (Table 2-6), only Cao et al. (2006) explicitly attributed part of the sludge reduction detected to the improved self-oxidation of activated sludge following the reduction in the floc size. Nevertheless, in their study, they did not either quantify the role played by the enhanced metabolism by floc breakage or give specific information on the actual reduction in the floc size caused by the ultrasound treatment. Therefore in this and in the other dynamic studies, floc breakage caused by ultrasound treatment at low energy input caused either an increase in the sludge production (this study; Strünkmann et al., 2006)) or a not significant decrease (Camacho et al., 2002b) or was simply referred to as one of the mechanisms involved in the sludge reduction observed (Cao et al., 2006).

5.4.3 Disintegration trials at medium energy input: trials M
The trials M, i.e. trial M/L (F/M), trial M/L, trial M/M and trial M/H, were all completed using the same energy input, 6048 kJ L\(^{-1}\), but with different energy densities (Table 5-1).

The reductions in sludge production for the two disintegration trials at low energy density (42 kJ L\(^{-1}\)) were 16.6 % for the trial M/L (F/M) (Figure 5-26 (a)) and 13.4 % for the trial M/L (Figure 5-26 (b)).
Figure 5-26: The test and control observed yields during the trial M/L (F/M) (a) and the trial M/L (b), the two disintegration trials at medium energy input (6048 kJ d⁻¹) and low energy density (42 kJ L⁻¹) and the related reductions in sludge reduction (RSP) between the two lanes.
The reduction in sludge production for the disintegration trial at medium energy density (84 kJ L$^{-1}$), trial M/M, was 17.2 % (Figure 5-27)

\[ \text{RSP[\%]} \approx +17.2 \]

![Graph showing test and control observed yields during the trial M/M at medium energy input (6048 kJ d$^{-1}$) and medium energy density (84 kJ L$^{-1}$) and the related reductions in sludge reduction (RSP) between the two lanes.]

Figure 5-27: The test and control observed yields during the trial M/M at medium energy input (6048 kJ d$^{-1}$) and medium energy density (84 kJ L$^{-1}$) and the related reductions in sludge reduction (RSP) between the two lanes.

The reduction in sludge production for the disintegration trial at high energy density (168 kJ L$^{-1}$), trial M/H, was 14.5 % (Figure 5-28)
Figure 5-28: The test and control observed yields in the trial M/H, the disintegration trial at medium energy input (6048 kJ d⁻¹) and medium energy density (84 kJ L⁻¹), and the related reduction in sludge reduction (RSP) between the two lanes.

Overall, the reductions in sludge production (RSP) for the trials M varied between 13.4 and 17.2 %. Considering the trial M/L (F/M) and the trial M/L as two replicas, the gradient of the linear fit of the degrees of sludge reduction versus the energy densities was almost zero for the trials M (Figure 5-29). The low R-squared value (0.13) indicates that the relationship between the degrees of sludge reduction and the energy densities was not linear. However, a value for the gradient so close to zero suggests that no particular trend could be highlighted in relation to the energy density used.
A much better linear fit (R-squared value of 0.88) could be achieved considering the ratio RSP/ΔS_{Lys} between the degree of sludge reduction achieved and the daily average release of lysate COD (equation (3.10) in the Materials and Methods chapter) versus the energy density. The gradient of the linear fit of the ratio RSP/ΔS_{Lys} versus the energy densities was almost zero for the trials M (Figure 5-30). The good linear fit and a value for the gradient so close to zero indicated a strong relationship between the degrees of reduction achieved and the amounts of lysate released regardless of the energy density used.
Figure 5-30: Linear fit, and related gradient and R-squared value, for the ratio RSP/$\Delta$S$_{Lys}$ between the degree of sludge reduction (RSP) and the daily average lysate release versus the energy density for the disintegration trials at medium energy input. Trial M/L (F/M) and trial M/L were considered as two replicas at medium energy input and low energy density and their average value was used to evaluate the linear fit.

Similar degrees of reduction to the ones observed in this study were found by Camacho et al. (2002b) and Strünkmann et al. (2006). Camacho et al. (2002b) applied a similar energy density (~45 kJ L$^{-1}$) and stress factor (0.2 d$^{-1}$) to those used in trial M/L (F/M) and trial M/L. In their study, they found a 24 % reduction using a high pressure homogeniser. The same reduction (24 %) was reported by Strünkmann et al. (2006) using ultrasound at an energy density of 105 kJ L$^{-1}$ and applying the same stress factor (0.2 d$^{-1}$).

A higher degree of reduction was reported by Cao et al. (2006). In their study, they detected a 40 % reduction using ultrasound at an energy density of 150 kJ L$^{-1}$, but they did not specify the stress factor. This lack of information made their results impossible to compare.
5.4.4 Disintegration trial at high energy input: trial H

A very high energy input (15120 kJ d\(^{-1}\)) was used during the trial H. The energy density used was 84 kJ L\(^{-1}\), with a stress factor of 0.33 d\(^{-1}\). In spite of this intensive ultrasound treatment, the degree of reduction achieved was only 16.2 % (Figure 5-31).

![Figure 5-31: The test and control observed yields in the trial H, the disintegration trial at high energy input (15120 kJ d\(^{-1}\)), and the related reduction in sludge reduction (RSP) between the two lanes.](image)

The 16.2 % degree of reduction observed in the trial H was similar to the 15.4 % reduction observed, on average, during the trials M. However, during the trial H, a 2.5 higher energy input was used, i.e. 15120 instead of 6048 kJ d\(^{-1}\). This fact suggests that high energy inputs might trigger some form of reduction-limiting mechanisms.

The only other study that used a similar high energy input was undertaken by Zhang et al. (2007a). In their study, by using ultrasound at an energy density of 216 kJ L\(^{-1}\) and a stress factor of ~0.2 d\(^{-1}\), they found a 91 % reduction. Such a high degree of reduction is difficult to understand. It must be noted that the experimental set-up used in their research, based on SBR units with a volume of 7 L fed with synthetic sewage, was the less realistic among the dynamic studies investigated.
5.4.5 Comparison with the results reported by Strünkmann et al. (2006)

The degrees of sludge reduction from the disintegration trials completed in this study during the pilot-scale plant trials were compared with those from the study undertaken by Strünkmann et al. (2006). Strünkmann et al. (2006) were the only authors to complete more than one disintegration trial and provide enough information for a comparison (Table 2-7 in the Literature review chapter).

In their study, Strünkmann et al. (2006) used a lab-scale plant of 36 L, in which the solids were separated from the control and test final effluent using submerged membrane plates instead of clarifiers. During their disintegration trials, the lab-scale plant was operated as a conventional activated sludge system but, due to the absence of clarifiers, the ultrasound treatment was applied to the mixed liquor instead of to the RAS.

To compare the energy inputs used in the two studies, the difference in the volumes of the plants and the total suspended solids concentrations (TSS) of the treated sludge had to be accounted for.

The difference in volume could be taken into account for by multiplying the stress factor applied by Strünkmann et al. (2006) by the volume of one lane of the pilot-scale plant (1220 L). This compensated for the higher energy inputs needed if the plant volume, and hence the biomass amount in the plant, is higher.

Treating sludge with a lower TSS concentration is less efficient, but the relationship between the degree of soluble COD release and the energy input is almost linear (Figure 4-5 in the Batch studies chapter). Therefore, the difference in TSS concentrations was taken into account for by dividing the energy input used by Strünkmann et al. (2006) by the ratio between the RAS and ML TSS in the pilot-scale plant. This compensated for the lower energy inputs needed if the RAS is treated instead of the ML.

As a result of the considerations described above, the energy inputs used by Strünkmann et al. (2006) were normalised to the operational conditions used in this study according to equation (5.2):
Normalised energy input(St.) \([kJ \, d^{-1}]\) =
\[ \frac{\text{Energy density(St.)} \,[kJ \, L^{-1}] \cdot \text{Stress factor(St.)} \,[d^{-1}] \cdot \text{Plant volume(this)} \,[L]}{\text{RAS/ML TSS ratio(this)}} \]  \hspace{1cm} (5.2)

Where:

- Normalised energy input(St.) = Energy input used by Strünkmann et al. (2006), normalised to be compared with the ones used in this study
- Energy density(St.) = Energy density used by Strünkmann et al. (2006)
- Stress factor(St.) = Stress factors applied by Strünkmann et al. (2006)
- Plant volume(this) = Volume of the pilot-scale plant used in this study (1220 L): necessary to normalise the differences in the plant volume between the two studies
- RAS/ML TSS ratio(this) = Ratio between the RAS and ML total suspended solids (TSS) in this study (~2.5 g g\(^{-1}\)): necessary to normalise the differences in the TSS concentration of the treated sludge in the two studies

Using this approach, it was possible to visualise the trend followed in the two studies by the degrees of sludge reduction (RSP) versus the energy inputs (Figure 5-32). A form of saturation was present in the degrees of sludge reduction observed in this study. A logarithmic fit, based on the RSP values from both studies, was therefore applied to highlight a possible levelling behaviour in the degree of sludge reduction achievable for increasing energy inputs (black dashed line in Figure 5-32).
Figure 5-32: Reductions in the sludge production vs the energy inputs used during different disintegration trials. Results from this study were compared with those from the study undertaken by Strünkmann et al (2006). The logarithmic fit based on the results from both studies is also shown (black, dashed line).
5.5 **Overall impact on the activated sludge process**

The effects on the sludge production and process performance during the dynamic studies were summarised in a single table to highlight the overall impact of the ultrasound treatment on the activated sludge process (Table 5-8).

Table 5-8: Summary of the overall impact of ultrasound treatment on the activated sludge process (CST: capillary suction time; SRF: specific resistance to filtration).

<table>
<thead>
<tr>
<th>Trials</th>
<th>Bas.</th>
<th>L</th>
<th>M</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy input [kJ d⁻¹]</td>
<td>0</td>
<td>1008</td>
<td>6048</td>
<td>15120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy density [kJ L⁻¹]</td>
<td>0</td>
<td>42</td>
<td>42</td>
<td>84</td>
<td>168</td>
<td>84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress factor [d⁻¹]</td>
<td>-</td>
<td>0.05</td>
<td>0.28</td>
<td>0.14</td>
<td>0.07</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sludge reduction [%]</td>
<td>-5.2</td>
<td>-9.4</td>
<td>16.6</td>
<td>13.4</td>
<td>17.2</td>
<td>14.5</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>TCOD removal (*) [mg L⁻¹]</td>
<td>1.1</td>
<td>-3.1</td>
<td>-1.7</td>
<td>-2.6</td>
<td>1.9</td>
<td>5.6</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>NH₄ removal (*) [mg L⁻¹]</td>
<td>-0.2</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0.8</td>
<td>2.3</td>
<td>25.6</td>
<td></td>
</tr>
<tr>
<td>Total P removal (*) [mg L⁻¹]</td>
<td>0.1</td>
<td>0.3</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
<td>1</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Turbidity (*) [NTU]</td>
<td>0</td>
<td>-4.2</td>
<td>3.2</td>
<td>-1.6</td>
<td>-4.7</td>
<td>-3.1</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Settleability (*) [mL g⁻¹]</td>
<td>0.2</td>
<td>37.2</td>
<td>24.2</td>
<td>52.1</td>
<td>37.7</td>
<td>34.4</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>Dewaterability (CST) (*) [s]</td>
<td>0.2</td>
<td>46.4</td>
<td>67</td>
<td>74.8</td>
<td>67</td>
<td>45.9</td>
<td>52.2</td>
<td></td>
</tr>
<tr>
<td>Dewaterability (SRT) (*) [x10¹⁰ m kg⁻¹]</td>
<td>0</td>
<td>3.6</td>
<td>NA</td>
<td>9.2</td>
<td>10</td>
<td>3.2</td>
<td>11.5</td>
<td></td>
</tr>
</tbody>
</table>

Legend

<table>
<thead>
<tr>
<th></th>
<th>No impact</th>
<th>Positive impact</th>
<th>Negative impact</th>
</tr>
</thead>
</table>

(*) The ultrasound effects on the process performance during the pilot-scale plant trials are defined with the variables used for the multiple comparison tests (Table 5-9). The type of impact (i.e. none, positive or negative) is based on the significance levels provided by the multiple comparison tests (Appendix I).

In the Table 5-8, the average values of the variables used for the multiple comparison tests (Appendix I) were used to describe the impact of ultrasound treatment on the
TCOD, NH₄ and Total P removals, turbidity and dewaterability (in terms of capillary suction time and specific resistance to filtration). A description of these variables is provided in the Table 5-9.

Table 5-9: Variable used to describe the impact of ultrasound treatment on the performance and downstream processes

<table>
<thead>
<tr>
<th>Performance/Process</th>
<th>Variable used</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD removal</td>
<td>Differences in the effluent SCOD concentration between the test and control lanes, in [mg L⁻¹]</td>
</tr>
<tr>
<td>NH₄ removal</td>
<td>Differences in the effluent NH₄ concentration between the test and control lanes, in [mg L⁻¹]</td>
</tr>
<tr>
<td>Total P removal</td>
<td>Differences in the effluent soluble total phosphorus concentration between the test and control lanes, in [mg L⁻¹]</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Differences in the effluent turbidity between the test and control lanes in the pilot-scale plant, in [NTU]</td>
</tr>
<tr>
<td>Settleability</td>
<td>Differences in the RAS sludge volume index between the test and control lanes, in [mL g⁻¹]</td>
</tr>
<tr>
<td>Dewaterability (CST)</td>
<td>Differences in the RAS sludge capillary suction time between the test and control lanes, in [s]</td>
</tr>
<tr>
<td>Dewaterability (SRT)</td>
<td>Differences in the RAS specific resistance to filtration between the test and control lanes, in [x10⁻¹⁰ m kg⁻¹]</td>
</tr>
</tbody>
</table>

Table 5-8 highlights that ultrasound treatment was not able to reduce sludge production at lower energy input (1008 kJ d⁻¹). Sludge production was reduced between 13.4 and 17.2 % at medium energy input (6048 kJ d⁻¹). At high energy input (15120 kJ d⁻¹), the sludge reduction achieved, 16.2 %, was similar to the one achieved, on average, at medium energy input, 15.4 %, using a 2.5 lower energy input.

TCOD and NH₄ removal was significantly decreased only at high energy input (15120 kJ d⁻¹). Total P removal was not significantly affected. Turbidity was only slightly affected, with differences in the effluent turbidity between the test and control lanes varying from -4.2 to 3.2 NTU. In this study, the sludge volume index of the RAS treated with ultrasound was higher than the control RAS but still within the range that corresponds to sludge of good settling characteristics. In terms of capillary suction times and specific resistances to filtration, the dewaterability was significantly decreased during all the disintegration trials.
Chapter 6: Process modelling

6.1 Introduction

A basic model of the pilot-scale plant operations was implemented in MATLAB® (The MathWorks, Inc.). The objective of the modelling was to further investigate the sludge reduction mechanisms implemented by the ultrasound treatment. The model main assumption was that the lysis-cryptic growth was the only sludge reduction mechanism responsible for the degree of sludge reduction experimentally observed in the test lane. In accordance with this assumption, the model was implemented to predict the changes in the observed yield of the pilot-scale plant test lane due to the lysis-cryptic growth. As a result, it was possible to investigate the presence of other mechanisms capable of increasing or limiting the degree of sludge reduction theoretically achievable by the lysis-cryptic growth. Some of the parameters necessary for this modelling were theoretically initialised based on a “reasonable guess”. The results from this modelling can be significantly influenced by the values chosen to initialise those parameters. The results from this modelling cannot therefore be considered definitive until the actual values for those parameters is experimentally measured.

6.2 Model implementation

6.2.1 Modelling the pilot-scale plant test lane

Only heterotrophic organic matter oxidation was modelled. Before the integration of the ultrasound disintegration unit, the pilot-scale plant test lane could be modelled according to the schematics in Figure 6-1 in terms of:

\[
\Delta S_{\text{Sew,TEST}} = \text{TCOD uptake at day } d, \text{ in } [\text{g COD}]
\]

\[
\Delta X_{\text{Sew,TEST}} = \text{Increment of biomass at day } d, \text{ in } [\text{g TSS}]
\]

\[
Y_{\text{OBS}} = \text{Observed yield of the test lane, in } [\text{g TSS g}^{-1} \text{COD}]
\]
Different substrates provide different yields. Before ultrasound treatment, the only substrate available was from the incoming sewage, i.e. $\Delta S_{\text{Sew}}$ (the substrate provided by acetate addition was considered as part of the incoming sewage). Therefore, the $Y_{\text{OBS}}$ of the overall process coincided with the observed yield due to the consumption of the TCOD in the incoming sewage, i.e. $Y_{\text{Sew}}$, under the operational condition of the pilot-scale plant test lane. Furthermore, the 2-month baseline confirmed that, before ultrasound treatment, the behaviour of the control lane and test lane was similar. More specifically, the difference in the observed yields ($Y_{\text{OBS}}$), the TCOD uptake ($\Delta S$) and biomass increment ($\Delta X$) between the control and test lane were as low as -5.2, -0.5 and 3.8%. Therefore, it follows that:

$$
Y_{\text{Sew, TEST}} \approx Y_{\text{OBS,TEST}} \approx Y_{\text{OBS,CTRL}} \\
\Delta S_{\text{Sew,TEST}} \approx \Delta S_{\text{CTRL}} \\
\Delta X_{\text{Sew,TEST}} = Y_{\text{Sew,TEST}} \cdot \Delta S_{\text{Sew,TEST}} \approx Y_{\text{OBS,CTRL}} \cdot \Delta S_{\text{CTRL}} = \Delta X_{\text{CTRL}} \quad (6.1)
$$

**6.2.2 Modelling the integration of the ultrasound disintegration unit**

The pilot-scale plant test lane after the integration of the ultrasound disintegration unit could be modelled according to the schematics in Figure 6-2 in terms of:

- $\Delta S_{\text{MOD}} = \text{The overall model TCOD uptake at day d, in [g COD]}$
- $\Delta X_{\text{MOD}} = \text{The overall model biomass increment at day d, in [g TSS]}$
- $Y_{\text{OBS,MOD}} = \text{The overall model observed yield, in [g TSS g}^{-1} \text{TCOD]}$
\[ \Delta S_{\text{Sew,TEST}} = \] The TCOD uptake at day d in test lane due to the incoming sewage, in [g COD]

\[ \Delta S_{\text{Lys}} = \] The amount of lysate released at day d in the test lane by ultrasound disintegration, in [g COD]

\[ \Delta X_{\text{Sew,TEST}} = \] The increment of biomass at day d due to the consumption of the COD in the incoming sewage, in [g TSS]

\[ \Delta X_{\text{Solub}} = \] The loss of biomass at day d due to solubilisation occurring inside the ultrasound disintegration unit, in [g TSS]

\[ \Delta X_{\text{Lys}} = \] The increment of biomass at day d due to the consumption of cell lysate COD released by ultrasound disintegration, in [g TSS]

\[ \Delta X_{\text{US}} = \] The amount of biomass leaving the ultrasound disintegration unit at day d, in [g TSS]

\[ Y_{\text{Sew}} = \] The observed yield of test lane related to the consumption of TCOD in the incoming sewage, in [g TSS g\(^{-1}\) COD]

\[ Y_{\text{Lys}} = \] The observed yield of test lane related to the consumption of cell lysate, in [g TSS g\(^{-1}\) COD]

\[ K_{\text{Solub}} = \] The conversion factor between biomass solubilised and lysate released, in [g COD g\(^{-1}\) TSS]

---

Figure 6-2: Schematics for modelling the pilot-scale plant test lane in the presence of the ultrasound system for sludge disintegration (system boundary in red)
Chapter 6: Process modelling

Considering equation (6.1), the increment of biomass at day d due to the consumption of the TCOD in the influent sewage ($\Delta X_{\text{Sew,TEST}}$) is given by equation (6.2) and could be considered similar to the corresponding increment in the control lane:

$$\Delta X_{\text{Sew,TEST}} = Y_{\text{Sew,TEST}} \cdot \Delta S_{\text{Sew,TEST}} \approx \Delta X_{\text{CTRL}}$$  \hspace{1cm} (6.2)

The ultrasound disintegration system was modelled as a unit that transforms/converts part of biomass ($\Delta X_{\text{Solub}}$) into the autochthonous substrate ($\Delta S_{\text{Lys}}$), i.e. cell lysate, and cell debris according to the equation (6.3):

$$\frac{\Delta X_{\text{Solub}}}{K_{\text{Solub}}} = \frac{\Delta S_{\text{Lys}}}{K_{\text{Solub}}} - f_d \frac{\Delta S_{\text{Lys}}}{K_{\text{Solub}}} = \frac{\Delta S_{\text{Lys}}}{K_{\text{Solub}}} \cdot (1 - f_d)$$  \hspace{1cm} (6.3)

Where:

A = Amount of biomass lysed, in [g TSS]

B = Amount of biomass left as cell debris, in [g TSS]

$f_d$ = The fraction of biomass that remains as cell debris, about 10 to 15% of the original cell weight (Tchobanoglous et al., 2003), in [g TSS g$^{-1}$ TSS]

Even if the exact value of the conversion factor $K_{\text{Solub}}$ between biomass solubilised and lysate released is not known and must be properly initialised, for the validity of the model, it should not vary significantly during the different disintegration trials. The $K_{\text{Solub}}$ is strictly related to the amount of energy available per unit mass in an organic compound in the form of cell material (Tchobanoglous et al., 2003). This amount of energy per unit mass is related to the oxygen equivalent (OE) of microbial cell, i.e. the amount of oxygen required to oxidise a unit mass of cells to carbon dioxide and water (Gray, 2004). Grady et al. (1975) found that the variance of OE was very low (< 8 %) even under different growth conditions. Therefore, in this study, the $K_{\text{Solub}}$ was assumed constant.

The amount of biomass leaving the ultrasound systems ($\Delta X_{\text{US}}$) is given by the equation (6.4):

\[ \text{\hspace{1cm}} \]
\[ \Delta X_{\text{US}} = \Delta X_{\text{Sew, TEST}} - \Delta X_{\text{Solub}} \quad (6.4) \]

The increment of biomass at day \( d \) due to the consumption of cell lysate COD released by ultrasound disintegration (\( \Delta X_{\text{Lys}} \)) is given by equation (6.5):

\[ \Delta X_{\text{Lys}} = Y_{\text{Lys}} \cdot \Delta S_{\text{Lys}} \quad (6.5) \]

Considering equation (6.4), the overall model biomass increment \( \Delta X_{\text{MOD}} \) at day \( d \) for the test lane is given by equation (6.6):

\[ \Delta X_{\text{MOD}} = \Delta X_{\text{US}} + \Delta X_{\text{Lys}} = \Delta X_{\text{Sew, TEST}} - \Delta X_{\text{Solub}} + \Delta X_{\text{Lys}} \quad (6.6) \]

To take into account the integration of the ultrasound system in the test lane, equation (6.6) shows that \( \Delta X_{\text{MOD}} \) must include two new terms in addition to the biomass increase due to the consumption of the TCOD in the influent sewage (\( \Delta X_{\text{CTRL}} \)):

1. The loss of biomass (\( \Delta X_{\text{Solub}} \)) due to solubilisation by ultrasound treatment
2. The increment of biomass (\( \Delta X_{\text{Lys}} \)) due to bacterial re-growth on the autochthonous substrate, i.e. lysate, released by ultrasound treatment and recycled within the RAS line back into the activated sludge process

Considering equations (6.2), (6.3) and (6.5), \( \Delta X_{\text{MOD}} \) is given by equation (6.7):

\[ \Delta X_{\text{MOD}} = \Delta X_{\text{CTRL}} - \frac{\Delta S_{\text{Lys}}}{K_{\text{Solub}}} \cdot (1 - f_d) + Y_{\text{Lys}} \cdot \Delta S_{\text{Lys}} \quad (6.7) \]

Considering equation (6.1), the overall model TCOD uptake at day \( d \) (\( \Delta S_{\text{MOD}} \)) for test lane is given by the equation (6.8):

\[ \Delta S_{\text{MOD}} = \Delta S_{\text{Sew,TEST}} \approx \Delta S_{\text{CTRL}} \quad (6.8) \]

In equations (6.7) and (6.8), there are three variables (\( \Delta S_{\text{CTRL}} \), \( \Delta S_{\text{Lys}} \) and \( \Delta X_{\text{CTRL}} \)) and three parameters (\( f_d \), \( Y_{\text{Lys}} \) and \( K_{\text{Solub}} \)):

- \( \Delta S_{\text{CTRL}} \) and \( \Delta X_{\text{CTRL}} \) are the same variables used to evaluate the observed yield in the control lane during the six disintegration trials and were measured experimentally during the dynamic studies.
• $\Delta S_{\text{Lys}}$ is the daily average amount of lysate released in the test lane by ultrasound treatment. It was measured experimentally during the dynamic studies and gives a quantitative measurement of the impact of ultrasound treatment on the activated sludge biomass in terms of lysate release by sludge solubilisation.

• $f_d$ gives the fraction of lysed biomass that remains as cell debris. Its value is known from the literature and, in this study, was set to 0.10 g TSS g$^{-1}$ TSS.

• The lysate yield $Y_{\text{Lys}}$ and the conversion factor $K_{\text{Solub}}$ were the only two unknown parameters.

Having defined with equations (6.7) and (6.8) the model daily average biomass increment $\Delta X_{\text{MOD}}$ and TCOD uptake $\Delta S_{\text{MOD}}$, the observed yield for the model is given by the slope of the linearisation (not passed through zero) of the correlation between the model cumulative TSS production (equation (6.9)) and TCOD removal (equation (6.10)):

\[
\text{TCOD Removed}_{\text{MOD}}(d) = \sum_{d=1}^{\text{d}} \Delta S_{\text{MOD}}(d) \tag{6.9}
\]

\[
\text{TSS Production}_{\text{MOD}}(d) = \sum_{d=1}^{\text{d}} \Delta X_{\text{MOD}}(d) \tag{6.10}
\]

The observed yield for the model is therefore a function of the two unknown parameters $Y_{\text{Lys}}$ and $K_{\text{Solub}}$:

\[
Y_{\text{OBS,MOD}} = f(Y_{\text{Lys}}, K_{\text{Solub}}) \tag{6.11}
\]
6.2.3 Model initialisation and definition of the prediction indexes

The exact values of $Y_{\text{Lys}}$ and $K_{\text{Solub}}$ are not known. Their value can significantly influence the results or this modelling. In this study, an attempt was made to initialise $Y_{\text{Lys}}$ and $K_{\text{Solub}}$ in the most reasonable way.

Initialisation of $Y_{\text{Lys}}$

The biodegradability of cell lysate has been investigated in various studies (Rocher et al., 1999; Dewil et al., 2006b; Andreottola et al., 2007) but there is little information on the actual values for the lysate yield. The only study providing some figures for the lysate yield was carried out by Mason and Hamer (1987). In their study on the cryptic growth of Klebsiella pneumoniae based on a series of batch growth experiments, they found that, depending on the dilution rate, between 0.42 and 0.52 mg of carbon were assimilated into new biomass per mg of lysed cellular carbon. In accordance with these results, in the present study, the average value of 0.36 g TSS g$^{-1}$ COD was used to initialise $Y_{\text{Lys}}$. This value for the $Y_{\text{Lys}}$ was calculated using the following equation (6.12):

\[
Y_{\text{Lys}} = \frac{(0.42+0.52)}{2} \frac{[\text{g COD g}^{-1} \text{ lysate COD}]}{\text{COD}_{\text{CELL}}} = 0.36 \frac{[\text{g TSS g}^{-1} \text{ lysate COD}]}{}
\]

COD$_{\text{CELL}}$ is the COD equivalent of cell tissue, i.e. the (total) COD of the pilot-scale plant RAS biomass. Experimentally measured, it was 1.3±0.07 g COD g$^{-1}$ TSS, which is very close to the theoretical value of 1.22 g COD g$^{-1}$ TSS for RAS with a volatile fraction of 0.86±0.02 g VSS g$^{-1}$ TSS (Tchobanoglous et al., 2003).

Initialisation of $K_{\text{Solub}}$

After setting the value of $Y_{\text{Lys}}$ to 0.36 g TSS g$^{-1}$ COD, the predicted $Y_{\text{OBS,MOD}}$ was then a function of the conversion factor $K_{\text{Solub}}$ only. Using equation (6.3), the value of $K_{\text{Solub}}$ was needed to estimate the amount of biomass solubilised ($\Delta X_{\text{Solub}}$) based on the amount of lysate COD released. It would be very difficult to evaluate the amount of lysate COD released by treating 1 due to the ultrasound treatment. To initialise $K_{\text{Solub}}$, it is necessary to know the amount of cell lysate released when 1 gram of
biomass is lysed. It is very difficult to evaluate this relationship for the ultrasound treatment because the amount of cell lysis that cavitation can trigger is not known. A better estimation could be made using a more established technique for cell lysis: alkaline hydrolysis.

It is commonly accepted that the alkaline treatment is able to trigger some of the highest levels of cell solubilisation (Nickel and Neis, 2007). In this study, around 60% of the total COD of the initial treated biomass was converted into cell lysate (i.e. 0.6 g lysate COD g\(^{-1}\) total COD) using the alkaline hydrolysis of RAS biomass from the pilot-scale plant (Figure 6-3). A similar value (50%) was reported by Boehler and Siegrist (2006).

Based on the results from this study, when 1 gram of RAS biomass was lysed with the alkaline hydrolysis, 1.3 gram of total COD was released (i.e. the COD\(_{\text{CELL}}\)). However, only 60 % of the total COD was in the soluble and colloidal form, i.e. was cell lysate.
Therefore, regardless of the treatment used, the lysis of 1 gram of biomass is unlikely to release more than 0.78 gram of cell lysate COD. According to this scenario, \( K_{\text{Solub}} \) is therefore equal to 0.78 g lysate COD g\(^{-1}\) TSS, in accordance with equation (6.13):

\[
K_{\text{Solub}} = 0.6 \text{ g lysate COD g}^{-1} \text{ total COD} \cdot 1.3 \text{ g total COD g}^{-1} \text{ TSS} \\
\quad \quad \quad \quad \quad \quad \quad (60\% \text{ lysate COD}) \quad \quad \quad \quad (\text{COD}_{\text{CELL}}) \\
\quad \quad \quad \quad \quad \quad \quad = 0.78 \text{ g lysate COD g}^{-1} \text{ TSS}
\]

However, cell lysis does not lead to complete solubilisation, thus the parameter \( f_d \) in equation (6.3) and (6.7) is necessary to account for the biomass generated from cell debris.

Even if some parameters in this scenario were experimentally measured, the weight partition implied by this approach and evaluated using equation (6.3) could not be verified. On the other hand, in the only study found in the literature involving the modelling of sludge disintegration, Yoon and Lee (2005) adopted a similar approach. Even if they used a more sophisticated modelling based on the activated sludge model (ASM) no. 1, sludge disintegration was also modelled as a process converting biomass into cell lysate and cell debris. Therefore, in the absence of more specific information in the literature about the loss of biomass occurring during the solubilisation by ultrasound treatment, the value of 0.78 g lysate COD g\(^{-1}\) TSS, i.e. 0.6 * COD\(_{\text{CELL}}\), was used to initialise \( K_{\text{Solub}} \) in the model.

**Definition of the prediction error (PrE)**

The prediction error (PrE) provides the error between the experimental and predicted observed yields for the test lane according to equation (6.14):

\[
\text{PrE} [\%] = \frac{(Y_{\text{OBS,MOD}} - Y_{\text{OBS,TEST}})}{Y_{\text{OBS,TEST}}} \cdot 100 
\]

The prediction error is zero when there is a perfect match between the observed yield experimentally measured during the dynamic studies (\( Y_{\text{OBS,TEST}} \)) and the predicted one (\( Y_{\text{OBS,MOD}} \)), i.e. \( Y_{\text{OBS,MOD}} = Y_{\text{OBS,TEST}} \). This condition implies that the lysis-cryptic...
growth is the only mechanism responsible for the degree of yield reduction experimentally observed. Positive values indicate that other viable mechanisms of sludge reduction have contributed to increase the degree of reduction theoretically achievable by the lysis-cryptic growth. Negative values of PrE indicate that other mechanisms have somehow limited the degree of reduction theoretically achievable by the lysis-cryptic growth.

**Definition of the prediction ratio for the lysis-cryptic growth (PrRatio)**

The prediction ratio is defined as the ratio between the predicted degree of sludge reduction based on modelling and the degree of sludge reduction observed during the pilot-scale plant trials completed as part of the dynamic studies. The prediction ratio (PrRatio) is evaluated according to equation (6.15):

\[
\text{PrRatio} [%] = \frac{(Y_{OBS,CTRL} - Y_{OBS,MOD})}{(Y_{OBS,CTRL} - Y_{OBS,TEST})} \times 100
\]  

(6.15)

The prediction ratio provides quantitative information on the role played by the lysis-cryptic growth when the prediction error is positive, i.e. in the presence of other viable mechanisms of sludge reduction in addition to lysis-cryptic growth. In these cases, the prediction ratio directly provides the percentage of sludge reduction observed during the pilot-scale plant trials that can be attributed to the lysis-cryptic growth mechanism in accordance with the model assumptions and initialisation.

For positive values of the prediction error, the higher the prediction error, the lower is the prediction ratio. Therefore, based on modelling, to investigate the minimum percentage of reduction that can be attributed to lysis-cryptic growth, the prediction ratio must be evaluated for the pilot-scale plant trial associated with the highest prediction error.
Chapter 6: Process modelling

6.3 Comparison of the predictions errors (PrE) and degrees of sludge reduction (RSP)

A baseline and six different disintegration trials were completed during the dynamic studies. During each of the disintegration trials, the operational conditions of the test and control lanes were kept constant but different ultrasound operational conditions (i.e. different energy densities and treatment periods) were used to evaluate the ultrasound impact on sludge production. The model was fed with the data collected during the six disintegration trials in accordance with equations (6.7) and (6.8), keeping the values of $Y_{\text{Lys}}$ and $K_{\text{Solub}}$ constant at 0.36 g TSS g$^{-1}$ COD and 0.78 g COD g$^{-1}$ TSS respectively. As an output, the model provided the predicted observed yields for the test lane due to the lysis-cryptic growth for each of the disintegration trials (Table 6-1).

Table 6-1: Comparison among the experimental and predicted values for the observed yields in the control and test lanes

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Control lane</th>
<th>Test lane</th>
<th>Model output for the test lane</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>0.30</td>
<td>0.32</td>
<td>0.28</td>
</tr>
<tr>
<td>M/L (F/M)</td>
<td>0.36</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>M/L</td>
<td>0.35</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>M/M</td>
<td>0.28</td>
<td>0.23</td>
<td>0.22</td>
</tr>
<tr>
<td>M/H</td>
<td>0.34</td>
<td>0.29</td>
<td>0.27</td>
</tr>
<tr>
<td>H</td>
<td>0.37</td>
<td>0.31</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Using the experimental and predicted observed yields, it was then possible to evaluate and compare the corresponding prediction errors (PrE) and degrees of sludge reductions (RSP). Table 6-2 provides a summary of the results from the process modelling.
Table 6-2: Summary of operational conditions, degrees of sludge reduction (RSP), prediction errors (PrE) and prediction ratios (PrRatio) during the six disintegration trials.

<table>
<thead>
<tr>
<th>Trial name</th>
<th>L</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy input [kJ d^-1]</td>
<td>1008</td>
<td>6048</td>
<td>6048</td>
<td>15120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy density [kJ L^-1]</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>84</td>
<td>168</td>
<td>84</td>
</tr>
<tr>
<td>RSP [%]</td>
<td>-9.4</td>
<td>16.6</td>
<td>13.4</td>
<td>17.2</td>
<td>14.5</td>
<td>16.5</td>
</tr>
<tr>
<td>PrE [%]</td>
<td>-12.5</td>
<td>-0.8</td>
<td>0.4</td>
<td>-5.4</td>
<td>-5.1</td>
<td>-36.7</td>
</tr>
<tr>
<td>PrRatio [%]</td>
<td>-46</td>
<td>&gt; 100 (104)</td>
<td>98</td>
<td>&gt; 100 (126)</td>
<td>&gt; 100 (130)</td>
<td>&gt; 100 (290)</td>
</tr>
</tbody>
</table>

Even if $K_{Solub}$ was assumed constant for the validity of the model, during the simulations a variation in its value was included to show its influence on the prediction error and the robustness of the modelling itself. An 8% variation was used, in accordance to the maximum variation reported by Grady et al. (1975). As indicated by the corresponding error bars (Figure 6-4), this variation did not influence significantly the prediction errors or prevent their comparison. The light green and blue areas in Figure 6-4 refer to the definition of prediction error and indicate the presence of other viable sludge reduction mechanisms (light green), the presence of other reduction-limiting mechanisms (light blue) or the presence of the lysis-cryptic growth only (grey).

The prediction errors showed relative changes between trial L, trials M (i.e. M/L (F/M), M/L, M/M, M/H) and trial H (Figure 6-4). More specifically, there was a decrease in the prediction error in both trial L (PrE: -12.5 %) and trial H (PrE -36.7 %) in comparison to the trials M, whose average prediction error was between 0.4 and -5.4 % (Table 6-2). This suggests that some unknown mechanisms limited the degree of reduction during trial L and trial H more than during trial M/M and M/H, and were almost absent during trial M/L (F/M) and M/L (Figure 6-4). The results provided by the simulations for each trial are described in more details in the following paragraphs.
Chapter 6: Process modelling

Trials

<table>
<thead>
<tr>
<th></th>
<th>Lysis-cryptic growth + other viable reduction mechanisms</th>
<th>Lysis-cryptic growth ONLY</th>
<th>Lysis-cryptic growth + other reduction-limiting mechanisms</th>
<th>PrE[%]</th>
<th>RSP[%] (for comparison with the PrE[%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Green</td>
<td>Grey</td>
<td>Light blue</td>
<td>-40</td>
<td>-40</td>
</tr>
<tr>
<td>M/L</td>
<td>Light green</td>
<td>Grey</td>
<td>Light blue</td>
<td>-30</td>
<td>-30</td>
</tr>
<tr>
<td>M/L (F/M)</td>
<td>Light green</td>
<td>Grey</td>
<td>Light blue</td>
<td>-20</td>
<td>-20</td>
</tr>
<tr>
<td>M/L</td>
<td>Light green</td>
<td>Grey</td>
<td>Light blue</td>
<td>-10</td>
<td>-10</td>
</tr>
<tr>
<td>M/M</td>
<td>Light green</td>
<td>Grey</td>
<td>Light blue</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M/H</td>
<td>Light green</td>
<td>Grey</td>
<td>Light blue</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>H</td>
<td>Light green</td>
<td>Grey</td>
<td>Light blue</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Light green</td>
<td>Grey</td>
<td>Light blue</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Light green</td>
<td>Grey</td>
<td>Light blue</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

Figure 6-4: Prediction errors (PrE) of the six disintegration trials (trials L, M/L (F/M), M/L, M/M, M/H, H) completed as part of the dynamic studies. The error bars indicate the variation in the prediction errors considering an 8% variation in the value of K_{solub}. The light green, grey and light blue areas refer to the definition of prediction error, while the degrees of sludge reduction (RSP, red dotted line) is shown on this graph just for comparison with the prediction errors.

6.3.1 Trial L

The decrease in the value for the prediction error (PrE: -12.5 %) in comparison to trials M indicated the presence of a reduction-limiting mechanism that was confirmed by the 9.4% increase in sludge production detected during this trial. The presence of a reduction-limiting mechanism detected by the modelling could be considered a further proof that enhanced metabolism by floc breakage is not a viable sludge reduction mechanism.

6.3.2 Trial H

During trial H, the degree of sludge production was positive, +16.5 % (Table 6-2), which means that a decrease in sludge production did occur. However, the net decrease (PrE ~ -36.7 %) in the value for the prediction error in comparison to trials M highlighted the fact that some mechanisms must have limited the degree of sludge reduction theoretically achievable as a consequence of the high amount of lysate
released by the high energy input used during the ultrasound treatment in this trial (Table 6-2). The model can only detect the presence of limiting mechanisms not their cause. Nevertheless, it is worth noting that the only way the model could deal with such a low degree of sludge reduction in spite of the high amount of lysate released is to increase the value of the lysate yield $Y_{\text{Lys}}$ in equation (6.5). An increase in the value of $Y_{\text{Lys}}$ could in fact justify the relatively low degree of reduction achieved as a consequence of the increase in the biomass re-growth on its own cell lysate ($\Delta X_{\text{Lys}}$ in equation (6.5)).

6.3.3 Trials M

The prediction errors for the trials M were all similar, with an average value of -2.7±2.9 %. Their consistency suggested the presence of the same mechanisms of reduction when the same energy input was used, regardless of the energy density applied. The presence of the same mechanisms of reduction is reinforced by the similar degrees of reduction detected during trials M (Table 6-2). The higher values for the PrE for the trials M in comparison to the trial L and H suggested that the use of a medium energy input might be the optimal way to achieve some degrees of sludge reduction. Lower prediction errors in the trial L and trial H indicated the presence of reduction-limiting mechanisms at both lower and higher energy inputs.

6.4 Role of the lysis-cryptic growth mechanism

In accordance with the definition of the prediction error, values lower than zero imply that the reductions experimentally observed were lower than those theoretically achievable by the lysis-cryptic mechanism. The highest and the only positive prediction error was 0.4 % for the trial M/L. The corresponding prediction ratio for the trial M/L was 98 %. Therefore, if the parameters used to initialise the model were reasonably accurate, at least 98 % of the sludge reduction observed during the pilot-scale plant trials could be attributed to the lysis-cryptic growth only. The results from this modelling excluded the presence of any other sludge reduction mechanisms other than the lysis-cryptic growth. On the contrary, they highlighted the presence of mechanisms limiting the degree of reduction theoretically achievable.
Chapter 7: Cost analysis

7.1 Introduction

The purpose of this chapter is to provide information on the economics of the sludge reduction process by ultrasound to address the following project objective:

- Outline possible strategies for ultrasound application in Anglian Water activated sludge processes.

Three scenarios based on different assumptions were evaluated to provide an insight on capital and operative costs and potential benefits of integrating an ultrasound disintegration system in the activated sludge process.

The first two scenarios, i.e. scenarios A and B, provide the pilot-scale plant direct cost analysis based on the pilot-scale plant operations during the trials M/L (F/M) and M/L. The assumption made in the second scenario, i.e. scenario B, was the inclusion of a 9-fold increase in the RAS thickening before ultrasound treatment.

The third scenario, i.e. scenario C, provides the scale-up of the cost analysis applied to a full-scale WWTP. The scale-up is based on eleven different assumptions. Some of these assumptions were formulated taking into account the pilot-scale plant operations, some others were based on the available literature.

7.2 Review of main cost data

The currency used to present costs and benefit is the UK pound, the following exchange rate was used whenever there was the need of a conversion from euros and US dollars:

- Euro/Pound: €/£ = 0.9
- US Dollar/Pound: $/£ = 0.72.

Cost of treatment for sludge treatment and reuse

The assessment of the actual overall cost for sludge treatment and reuse in a conventional way is one of the most important steps because it is critical in evaluating the economical feasibility of an alternative route (Table 7-1). The value from the
Chapter 7: Cost analysis

WIRES report (Ginestet, 2006) was finally adopted because it included haulage and is up to date.

Table 7-1: Review of treatment costs for sludge treatment and reuse

<table>
<thead>
<tr>
<th>Cost [£ Mg(^{-1}) DS]</th>
<th>Comments</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>227</td>
<td>Collection, thickening, digestion, dewatering, reuse (excluding hauling)</td>
<td>WERF report (Stensel and Strand, 2004)</td>
</tr>
<tr>
<td>423±252</td>
<td>Collection, thickening, digestion and dewatering plus average value among different disposal/reuse routes including hauling</td>
<td>WIRES report, (Ginestet, 2006)</td>
</tr>
</tbody>
</table>

Equipment cost

Indicative values for Ultrawaves capital and operative expenditures were presented in the study of Wolff et al. (2007) (Table 7-2). The configuration of a full-scale Ultrawaves unit for sludge disintegration consists of a 5kW, 25 L reactor. The reactor is fitted with five probes each powered by a 1kW separate generator. The initial investment includes a 5kW ultrasound unit, control unit, pipe connection and sludge pump.

Table 7-2: Ultrawaves capital and operative expenditures

<table>
<thead>
<tr>
<th>Cost [£ per unit]</th>
<th>Cost [£ per KW installed]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial investment</td>
<td>99000</td>
</tr>
<tr>
<td>Depreciation (10 years)</td>
<td>12821</td>
</tr>
<tr>
<td>Maintenance (per year)</td>
<td>2835</td>
</tr>
</tbody>
</table>

The equipment depreciation (ED) was evaluated using equation (7.1), which was suggested and adopted in the WIRES report (Ginestet, 2006):

\[
ED = I \cdot \frac{IR}{1-(1+IR)^{-DP}}
\]

(7.1)

Where:

I = Investment, i.e. the initial cost of the equipment
IR = Interest rate, 5 %
DP = Depreciation period, 10 years.
Electricity costs

The most updated estimation on average industrial electricity prices for the period between January and June 2008 in the UK were made available by The Department for Business, Enterprise and Regulatory Reform (BERR, 2008) divided by consumer size (Table 7-3). This cost analysis adopted the figure for large size consumers, including not refundable taxes.

Table 7-3: Industrial electricity cost in UK per consumer size

<table>
<thead>
<tr>
<th>Consumer size</th>
<th>Cost excluding taxes [pence per kWh]</th>
<th>Cost including taxes [pence per kWh]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>8.27</td>
<td>8.64</td>
</tr>
<tr>
<td>Medium</td>
<td>6.54</td>
<td>6.82</td>
</tr>
<tr>
<td>Large</td>
<td>6.43</td>
<td>6.61</td>
</tr>
<tr>
<td>Very large</td>
<td>6.64</td>
<td>6.82</td>
</tr>
</tbody>
</table>

Additional aeration cost

Sludge reduction occurs due to an overall higher degree of mineralisation of the organic carbon in the system. Consequently, the increase in the oxygen consumption must be taken into account. This cost analysis adopted the approach proposed in the WERF report (Stensel and Strand, 2004), which is in agreement with the one provided by the WIRES report (Ginestet, 2006) and is based on the following assumptions:

- 0.8 gram of oxygen is needed to oxidise each gram of COD released by sludge solubilisation
- 1.3 gram of COD is released per each gram of VSS solubilised
- 1 kWh is needed to transfer 1500 grams of oxygen in the system.

7.3 Process economical evaluation

7.3.1 Pilot-scale plant direct cost analysis

First step was to evaluate cost and benefits per year for the pilot-scale plant based on two scenarios (Table 7-4):

- **Scenario A** – Direct cost analysis based on the actual pilot plant configuration and operations (i.e. in-line disintegration without RAS thickening) using experimentally measured values during dynamic studies for power consumption, influent strength, observed yield, degree of sludge reduction, equipment usage
• **Scenario B** – Cost analysis for the theoretical application of a 9-fold increase in the RAS thickening before ultrasound treatment (excluding cost involved by thickening process). The immediate consequence of the RAS thickening is a decrease in the equipment usage from 10 down to ~1%.

The scenario parameters provide the information necessary to estimate the CAPEX and OPEX per year for the two scenarios:

- **Equipment**: type of equipment installed in the pilot-scale plant, i.e. the Ultrawaves probe
- **Installed power/Power usage**: the maximum power available and the actual power drawn by the Ultrawaves probe during ultrasound treatment
- **Equipment usage**: percentage of time during which the ultrasound treatment was active, which was 10% (6 minutes per hour) during the trials M/L (F/M) and F/M. In the scenario A, this percentage was equivalent to the actual percentage of the RAS volume treated by ultrasound during the trials M/L (F/M) and F/M, i.e. 10%. In the scenario B, the usage was decreased due the 9-fold increase in the RAS thickening before ultrasound treatment
- **Electricity cost**: value made available by The Department for Business, Enterprise and Regulatory Reform (2008)
- **Sludge treatment and reuse**: average cost found in the WIRES report (Ginestet, 2006)
- **Influent strength**: average value of the TCOD in the influent wastewater measured during the dynamic studies
- **RAS thickening**: none during the pilot-scale plant operations (scenario A), up to concentration of 6-7% DS (scenario B) depending on the type of thickening process used (Tchobanoglous *et al.*, 2003)
- **Observed yield**: average value detected during the dynamic studies
- **Max yield reduction**: average value observed during the trial M/L (F/M) and M/L including the 5.2% offset found during the baseline
- **Process retrofit costs (CAPEX and OPEX)**: these costs were partially unknown and they were omitted in this cost analysis
• **Equipment cost (CAPEX) and Equipment maintenance (OPEX):** values reported by Wolff *et al.* (2007). Due to the low equipment usage (10% in the scenario A, ~1% in the scenario B), the maintenance cost was based on the actual usage of the system in terms of treatment time.

• **Equipment depreciation (OPEX):** based on an annual interest rate of 0.05% and 10-year depreciation period and evaluated using the algorithm (equation (7.1)) suggested in the WIRES report (Ginestet, 2006).

• **Energy consumption (OPEX):** based on the actual power drawn during the ultrasound treatment and the cost of electricity.

• **Additional aeration (OPEX):** cost based on the approach proposed in the WERF report (Stensel and Strand, 2004).

• **Savings from not produced sludge:** calculated on the base of the average influent strength, pilot-scale plant performance and sludge treatment and reuse cost suggested in the WIRES report (Ginestet, 2006).

• **RATIO Total OPEX Costs/Savings:** this index was used to assess the economical viability of the overall process.
### Table 7-4: Pilot plant concise cost analysis

<table>
<thead>
<tr>
<th>Scenario parameters</th>
<th>Units</th>
<th>Scenarios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td><strong>Equipment</strong></td>
<td>Ultrawaves</td>
<td></td>
</tr>
<tr>
<td>Installed power/Power usage</td>
<td>kW</td>
<td>1/0.7</td>
</tr>
<tr>
<td>Equipment usage (time %)</td>
<td>%</td>
<td>10</td>
</tr>
<tr>
<td>Electricity cost</td>
<td>pence kWh(^{-1})</td>
<td>6.61</td>
</tr>
<tr>
<td>Sludge treatment and reuse</td>
<td>£ Mg(^{-1}) DS</td>
<td>423</td>
</tr>
<tr>
<td>Influent strength</td>
<td>mg TCOD L(^{-1})</td>
<td>308</td>
</tr>
<tr>
<td>RAS thickening</td>
<td>% DS</td>
<td>0.7 (None)</td>
</tr>
<tr>
<td>Observed yield</td>
<td>G TSS g(^{-1}) COD</td>
<td>0.31</td>
</tr>
<tr>
<td>Max yield reduction</td>
<td>%</td>
<td>20</td>
</tr>
</tbody>
</table>

#### Costs per year

**CAPEX**
- Process retrofit costs: UNKNOWN
- Equipment cost (per kW installed): £ kW\(^{-1}\) 19800

**OPEX**
- Process retrofit costs (depreciation and OPEX): UNKNOWN
- Equipment Depreciation: £ per year 256 28
- Equipment Maintenance: £ per year 57 6
- Energy Consumption: £ per year 40.5 4.5
- Additional Aeration: £ per year 0.6 0.6
- Total Energy Cost: £ per year 41.1 5.1

**Total OPEX**: £ per year 354 40

**Benefits per year**
- Savings from not produced sludge: £ per year 5.4 5.4

**RATIO Total OPEX Costs/Savings**: # 66 7.4

**Legend**
Change in parameter value from reference scenario (A)
7.3.2 Scale-up of cost analysis applied to full-scale WWTP

The scale-up of the results from the pilot-scale plant to a full-scale WWTP, i.e. the scenario C, is described in Table 7-5. The scenario C was based on the same parameters and costs described for the two previous scenarios. However, to simplify the calculations, the “Equivalent wastewater flow” and, hence, the “Population equivalent” of the reference full-scale WWTP, were determined by the maximum “Equipment RAS treatment capacity” of the Ultrawaves disintegration full-scale unit. The “Equipment RAS treatment capacity” was based on the pilot-scale plant operations and then scaled up taking into account:

- A 2-fold increase in the treatment capacity in comparison to the one used to achieve a 20 % sludge reduction during the trial M/L (F/M) and F/M. The 2-fold increase was applied due to the potential benefits than can be achieved from using an optimised full-scale reactor
- The full-scale configuration of the Ultrawaves equipment, which consists of a 25 L reactor fitted with 5 Ultrawaves probes providing an overall power of 5kW, i.e. 1kW for each probe

In the following list, which provides further details on the scale-up assumptions at the base of the scenario C, each assumption is numbered and the corresponding number refers to “Assumption number” column in Table 7-5:

1. **Installed Power**: a single 5kW Ultrawaves full-scale disintegration unit was considered. In scenario C, the assumption was made that all the installed power was used during the ultrasound treatment
2. **Wastewater strength**: a figure of 200 mg BOD L⁻¹ was considered typical of a medium strength municipal wastewater
3. **RAS thickening**: assumption based (as in scenario B) on the experimental evidence that the Ultrawaves probe could treat sludge thickened up to 9 times the original RAS solids content without losing efficiency in the disintegration process (Figure 4-5)
4. **Equipment RAS treatment capacity**: a 2-fold higher treatment capacity than the one used in the pilot-scale plant operations (as in scenario A and B) was considered due to potential benefits from using an optimised full-scale reactor.

5. **Equivalent wastewater flow**: calculation based on the following WWTP configuration:
   a. RAS recycle ratio: 67% (as in scenarios A and B)
   b. Percentage of RAS treated: 10% (as in scenarios A and B)
   c. RAS thickening ratio: 9-fold (as in scenario B)

6. **Population equivalent**: 300 L of wastewater per day per capita generates 60 g BOD per day per capita considering a strength of 200 mg BOD L⁻¹ for the influent wastewater (assumption 2)

7. **Expected secondary sludge production**: calculation based on sludge production of 30 g DS per day per capita considering an observed yield of 0.5 g TSS g⁻¹ BOD

8. **Expected avoided secondary sludge production**: based on 20 % degree of reduction according to results from dynamic studies (Chapter 5)

9. **CAPEX**: costs to retrofit the ultrasound disintegration process to an existing WWTP including pipework and pumps to allow RAS thickening before ultrasound treatment are unknown. As a consequence, related depreciation and operative costs were not included in this analysis.

10. **Ratio Total OPEX costs/savings**: This index was used to assess the economical viability of the overall process

11. **Break-even point**: This determined the minimum sludge treatment and reuse cost necessary to offset the overall costs involved by the implementation ultrasound treatment

It must be noted that the detrimental effect of ultrasound treatment on the dewaterability of the treated sludge should not be underestimated. However, it was not taken into account in this cost analysis because its economical impact would require a thorough and deeper investigation that goes beyond the limits of this study.
Chapter 7: Cost analysis

Table 7-5: Scale-up of cost analysis for a full-scale WWTP (scenario C)

<table>
<thead>
<tr>
<th>Full-scale scenario parameter</th>
<th>Units</th>
<th>Full-scale estimation</th>
<th>Assumption number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment</td>
<td>Ultrawaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Installed Power</td>
<td>kW</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Electricity cost</td>
<td>pence kWh⁻¹</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>Sludge treatment and reuse</td>
<td>£ Mg⁻¹ DS</td>
<td>423</td>
<td>2</td>
</tr>
<tr>
<td>Wastewater strength</td>
<td>mg BOD L⁻¹</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>RAS thickening</td>
<td>% DS</td>
<td>6.4 (9-fold)</td>
<td>3</td>
</tr>
<tr>
<td>Observed yield</td>
<td>g TSS g⁻¹ BOD</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Max yield reduction</td>
<td>%</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

**Full-scale secondary treatment operations**

| Equipment RAS treatment capacity                                 | m³ d⁻¹      | 21                    | 4                 |
| Equivalent wastewater flow                                       | m³ d⁻¹      | 2777                  | 5                 |
| Population equivalent                                            | PE          | 9257                  | 6                 |

| Expected secondary Sludge production                             | Mg per year | 101                   | 7                 |
| Expected avoided secondary sludge production                     | Mg per year | 20                    | 8                 |

**COSTS PER YEAR**

**CAPEX**

- Process retrofit costs: UNKNOWN
- Equipment cost: 99000

**OPEX**

- Process retrofit costs (depreciation and OPEX): UNKNOWN
- Equipment depreciation: £ per year 12821
- Equipment maintenance: £ per year 2835
- Energy consumption: £ per year 2895
- Additional aeration: £ per year 929

**Total Energy Cost**

- £ per year 3824

**Total OPEX**

- £ per year 19480

**BENEFITS PER YEAR**

- Savings from not produced sludge: £ per year 8576

**RATIO Total OPEX Costs/Savings**

- # fold(s) 2.3 10

**Break-even point for sludge treatment and reuse**

- £ Mg⁻¹ DS ~961 11
7.3.3 Sensitivity of the scaled-up cost analysis

The assumptions made on the costs of sludge treatment and reuse, the implementation of ultrasound disintegration treatment and the full-scale WWTP operational conditions can lead to very different results in the scaled-up cost analysis described in Table 7-5. For this reason, a sensitivity analysis was carried out by varying each one of the following parameters separately. The variations in the parameters were chosen to investigate their potential to reduce the break-even point for sludge treatment and reuse:

- Electricity cost: varied from 6.61 to 2 p kWh⁻¹
- Treatment capacity: varied from 2-fold to 3-fold increase
- Equipment initial cost: varied from full cost to 50%
- Degree of yield reduction: varied from 20 to 40 %.

The outcome of this sensitivity analysis highlighted the parameters that most influenced the result of the scaled-up cost analysis by causing a variation in the break-even point.

In percentage, the impact of the variations in the electricity cost on the variation of the break-even point for the sludge treatment and reuse was less significant than the costs associated with the equipment depreciation (Figure 7-1). Therefore, the initial cost of the equipment plays a significant role in the cost-effectiveness of the overall process.

Results from the dynamic studies (Chapter 5) showed it might be difficult to achieve degrees of yield reduction greater than 20 %. Focusing on the equipment depreciation and the treatment capacity, even considering the most optimistic between the two variations, i.e. an unlikely 50 % reduction in the actual cost of the ultrasound equipment, the minimum break-even point for final sludge treatment and reuse was still as high as £645 per tonne DS (Figure 7-2).
Chapter 7: Cost analysis

Figure 7-1: Percentage variation of the break-even point for the final sludge treatment and reuse when cost analysis parameters are varied.

Figure 7-2: Decrease in the break-even points for the final sludge treatment and reuse when cost analysis parameters are varied.
Chapter 8: Discussion

8.1 Introduction

According to the conclusions reached by the WIRES project, the European funded research on innovative ways to achieve sludge reduction (Ginestet, 2006), it is not possible to predict the degree of sludge reduction only by evaluating the effects of the selected disintegration treatment on the activated sludge biomass in terms of sludge solubilisation and biological activity. The reason given was that the reduction in sludge production is the result of more complex and synergistic biological phenomena triggered by those effects. In this study, the effects of ultrasound treatment on floc breakage, sludge solubilisation, biological activity and sludge production were investigated using a combination of batch disintegration trials (i.e. the ultrasound potential trials), dynamic trials (i.e. the pilot-scale plant trials) and modelling. In this section, the information provided by the ultrasound impact trials, the pilot-scale plant trials and modelling are integrated to discuss the potential of ultrasound treatment for sludge reduction and the possible sludge reduction mechanisms employed.

The main hypothesis of this project was that sludge production could be reduced because, by disintegrating sludge with ultrasound, the following mechanisms might occur:

- Lysis-cryptic growth by sludge solubilisation
- Increased maintenance metabolism by induced stress
- Enhanced metabolism by floc breakage.

The importance of these three mechanisms is discussed in relation to their potential to result in sludge reduction.

8.2 Sludge reduction due to enhanced metabolism by floc breakage

The viability of this mechanism is mainly based on the studies undertaken by Friedrich (2002) and Abbassi et al. (2000).
According to Friedrich (2002), by breaking up sludge flocs, ultrasound treatment is supposed to enhance the transfer and diffusion mechanism of oxygen, substrate and nutrients within the floc. According to Abbassi et al. (2000), the increased diffusion of oxygen within the floc would enhance the hydrolysis of the dead cells and lead to sludge reduction. By increasing the dissolved oxygen concentration from 2 to 6 mg L\(^{-1}\), Abbassi et al. (2000) achieved an overall degree of sludge reduction of 22%. However, to prove their hypothesis, they increased the oxygen diffusion within the floc by increasing the aeration, i.e. the dissolved oxygen concentration, not by reducing the floc size. Therefore, there is no direct evidence that a reduction in floc size alone, achieved by ultrasound or any other disintegration treatment, can actually trigger some form of enhanced metabolism either. According to Liu and Tay (2001), the hypothesis proposed by Abbassi et al. (2000) requires further investigation because conflicting opinions exist in the literature regarding the effects of oxygen concentrations on sludge production: the range of reductions attributed to increased oxygen concentrations varied from 0 to 66%.

In this study, the ability of ultrasound to reduce floc size was firstly evaluated during the ultrasound potential trials. Results from these trials indicated that ultrasound treatment could reduce floc size by 88% with an energy density of 42 kJ L\(^{-1}\). Secondly, the pilot-scale plant trial at low energy input (trial L) was completed to test the viability of this mechanism based on the actual impact of continuous in-line ultrasound treatment on sludge production during the dynamic studies. The trial L was completed on purpose after the trial M/L (F/M) and trial M/L to ensure that all the biomass in the test lane was reduced in size. As a result, during trial L, the floc size in the control lane was 323.4±43.5 μm, while in the test lane was down to 65.1±6.4 μm. A low energy input (1008 kJ d\(^{-1}\)) and a low energy density (42 kJ L\(^{-1}\)) was then used during trial L to prevent the re-aggregation of the biomass while minimising the release of lysate due to sludge solubilisation and the impact on the biological activity. The rationale was to trigger only the mechanism based on the enhanced metabolism by floc breakage and limit the implementation of the other two. As a result, during trial L, it was possible to create appropriate conditions to investigate the actual impact on sludge production due to floc breakage:
The average floc size in the test lane was 65.1±6.4 µm, 5 times smaller than the average floc size in the control lane.

The daily average lysate release was as low as 8.5 g lysate COD d⁻¹, on average 5.3 and 12.9 times lower than during the trials M and trial H, respectively. Therefore the effects of lysis-cryptic growth on sludge production during the trial L were minimal in comparison to the other trials.

Finally, according to the results from the respirometric trials, the use of an energy density of 42 kJ L⁻¹ was not able to trigger any change in the biological activity. Therefore, no effects on sludge production due to increased maintenance metabolism were meant to occur during the trial L.

During trial L, instead of a decrease in the sludge production, a 9.4 % increase was observed (Figure 5-25 in the Dynamic studies chapter). Even removing from that figure the 5.2 % offset found during the baseline, the net increase would be still around 4 %. The 9.4 % increase in sludge production found in this study is in agreement with the results found by Strünkmann et al. (2006). At low energy inputs, they also found an increase in sludge production in the test lane between 8 and 28 %. Strünkmann et al. (2006) proposed the following two hypothesis to justify the increase in sludge production observed during their study at low energy inputs:

- An enhancement effect on microbial growth due to the increased oxygen, substrate and nutrients supply to the microorganisms within the floc triggered by the extension of the available surface caused by floc breakage.
- An enhancement effect on microbial growth due to the availability in the test reactor of cell lysate.

However, Strünkmann et al. (2006) did not undertake any specific investigations to support or negate the validity of those hypotheses. Furthermore, in relation to the first hypothesis, the increased oxygen diffusion within the floc was proposed by Abbassi et al. (2000) to justify the opposite effect, i.e. the 22% decrease in sludge production observed in their study by increasing the dissolved oxygen concentration in the mixed liquor.
If both Strünkmann et al. (2006) and Abbassi et al. (2000) are right, it seems that the effects on the biomass metabolism are completely different depending on the conditions used to increase the diffusion of oxygen within the floc. Further research, which goes beyond the scope of this study, is therefore needed to understand how the biomass metabolism is affected by changes in the floc size and/or increases in the dissolved oxygen concentration during the activated sludge process.

Nevertheless, the experimental evidence found in the study undertaken by Strünkmann et al. (2006) and in this one, indicated that a reduction in the floc size due to the use of ultrasound at low energy inputs did not lead to sludge reduction. Therefore, the enhanced metabolism by floc breakage is either not a viable sludge reduction mechanism or it cannot be implemented by ultrasound treatment.

### 8.3 Sludge reduction due to increased maintenance metabolism by induced stress

Enhanced maintenance metabolism is a well known mechanism capable of reducing sludge production (Low and Chase, 1999b; Liu and Tay, 2001; Wei et al., 2003). This mechanism is generally implemented by increasing the biomass concentration while keeping a constant supply of substrate, i.e. by decreasing the food to microorganism (F/M) ratio. These conditions enhance maintenance phenomena involving bacterial reactions like death, lysis and endogenous metabolism (Canales et al., 1994; Henze et al., 2002) and can lead to a situation in which the amount of energy provided by the supply of substrate equals the maintenance demand. The low sludge production occurring in membrane bioreactors (MBR) operated at high SRTs and mixed liquor concentrations is based on this principle.

Nevertheless, Rai et al. (2004) suggested that any form of stress induced on microorganisms should be able to increase the portion of substrate used for fuelling maintenance metabolism. Based on this hypothesis, ultrasound treatment could force microorganisms to divert some of the energy from biosynthesis to the maintenance processes required to repair the damages caused by the hydromechanical shear stress and forces triggered by acoustic cavitation. Rai et al. (2004) tested the hypothesis that the stress induced by ultrasound treatment could trigger this mechanism using respirometry and confirmed the presence of enhanced metabolic needs in terms of
increased oxygen consumption in the treated sludge. Rai et al. (2004) did not complete any dynamic trial to verify the actual effects of enhanced maintenance metabolism on sludge production. They estimated the potential degree of sludge reduction on a theoretical basis, taking into account the amount of oxygen consumed and the COD removed during respirometric trials (equation (2.17) in the Literature review) on RAS samples treated with ultrasound. According to their theoretical approach, they estimated a 14 % degree of sludge reduction at around 32 kJ L⁻¹, this reduction went up to ~29% at 256 kJ L⁻¹. Liu et al. (2005) also investigated the effects of stress on sludge production. In their study, they found that shear stress caused by increasing agitation rates from 50 to 800 rpm within a stirred batch reactor led to 30 % sludge reduction. 

In disagreement with the previous studies, Camacho et al. (2002b), using a high pressure homogenizer, did not detect the presence of increased maintenance metabolism, even when working at high pressure (700 bars). The biological inactivation detected at high energy densities (> 80% at around 162 kJ L⁻¹) was permanent and was attributed to cell lysis. In their study, they found a 24 % reduction using the high pressure homogeniser. Camacho et al. (2002b) attributed the 24 % reduction in sludge production to the lysis-cryptic growth mechanism based on sludge solubilisation.

In this study, the presence of enhanced maintenance metabolism was investigated by evaluating and comparing changes in the biological activity of RAS samples exposed to ultrasound treatment during the ultrasound potential trials and the pilot-scale plant trials. The biological activity was measured in terms of their specific oxygen uptake rates (SOUR) and specific oxygen uptake (SOU) using respirometry.

The effects of enhanced maintenance metabolism on sludge production were then evaluated by comparing changes in the sludge production during three pilot-scale plant trials. The three pilot-scale plant trials were completed using ultrasound operational conditions designed to trigger changes in the biological activity of the treated RAS, based on the results of the respirometric trials.
8.3.1 Investigating the presence of enhanced maintenance metabolism using respirometry

In the literature, it was not possible to find a common, well-defined procedure to evaluate the presence of enhanced maintenance metabolism. According to Camacho et al. (2002b) and Rai et al. (2004), temporary biological inactivation followed by recovery of the initial activity and/or an increase in the oxygen consumption in the treated sludge samples could indicate the presence of increased maintenance metabolism that might eventually lead to some form of growth reduction.

In this study, using respirometry, the occurrence of temporary biological inactivation was evaluated by the profile analysis of the SOUR curves. The increase in oxygen consumption was then evaluated by the quantitative analysis of the increase in the SOU in the RAS samples exposed to ultrasound treatment in comparison to the untreated RAS samples.

The ultrasound treatment was applied using the Ultrawaves probe at 42, 84 and 168 kJ L\(^{-1}\). To evaluate the short-term and long-term effects of ultrasound treatment, three different types of exposure were considered on the RAS samples used during the respirometric trials:

- RAS samples collected from the control lane and treated for the first time just before the respirometric trial
- RAS samples previously exposed to ultrasound treatment and treated again before the respirometric trial. These RAS samples were collected from the test lane during the pilot-scale plant trials and the previous exposure occurred 8.5 days before collection
- RAS samples previously exposed to ultrasound treatment but not treated again before the respirometric trial. These RAS samples were collected from the test lane during the pilot-scale plant trials and the previous exposure occurred from 8.5 to 50.8 days before collection.

No increased maintenance metabolism was found in the RAS samples treated at 42 and 84 kJ L\(^{-1}\) and in the RAS samples treated at 168 kJ L\(^{-1}\) that were previously exposed to ultrasound treatment.
Only the RAS samples collected from the control lane and treated at 168 kJ L\(^{-1}\) for the first time had a SOUR profile indicating the occurrence of temporary biological inactivation coupled with a significant 8.5 % increase in the SOU in comparison to the untreated RAS samples. These results suggested that increased maintenance metabolism occurred only at high energy densities and that RAS samples already exposed to ultrasound treatment were less affected by it.

### 8.3.2 Evaluating the effects of enhanced maintenance metabolism during the pilot-scale plant trials

To detect the presence of increased maintenance metabolism in the RAS samples treated at 168 kJ L\(^{-1}\) during the respirometric trials does not mean that its presence would eventually lead to a reduction in the sludge production during the activated sludge process. The actual impact of increased maintenance metabolism on sludge production could only be investigated during the pilot-scale plant trials.

However, the problem is that floc breakage, sludge solubilisation and enhanced maintenance metabolism are all triggered by sludge disintegration at the same time and, theoretically, each of them can affect sludge production. Therefore, the goal was to find a way to differentiate the effects of increased maintenance metabolism on sludge production from the other two.

To achieve this goal, three pilot-scale plant disintegration trials were completed using the same medium energy input (trials M at 6048 kJ d\(^{-1}\)) but applying different energy densities: 42 kJ L\(^{-1}\) (trial M/L), 84 kJ L\(^{-1}\) (trial M/M) and 168 kJ L\(^{-1}\) (trial M/H). The use of the same energy input at different energy densities implied the treatment of proportionally lower amounts of RAS: 10 % at 42 kJ L\(^{-1}\), 5 % at 84 kJ L\(^{-1}\) and 2.5 % at 168 kJ L\(^{-1}\).

These operational conditions were used during the three pilot-scale plant trials to highlight the effects of increased maintenance metabolism on sludge production by exploiting the different relationships between floc breakage, sludge solubilisation, maintenance metabolism versus energy density observed during the ultrasound potential trials.

More specifically, the selection of the operational conditions to highlight the effects of increased maintenance metabolism was based on the three following assumptions.
The first assumption was related to the effects of floc breakage. The ultrasound potential trials indicated that 88% of the floc reduction occurred already at 42 kJ L\(^{-1}\). Therefore, the effects of floc breakage on sludge production during the three pilot-scale plant trials at 42, 84 and 168 kJ L\(^{-1}\) were supposed to be similar because the use of higher energy densities was not supposed to vary the floc size in the test lane significantly.

The second assumption was related to the effects of sludge solubilisation. The ultrasound potential trials indicated that the lysate release increased for increasing energy densities with an almost linear relationship. Therefore, the effects of sludge solubilisation on sludge production were supposed to be similar during the three pilot-scale plant trials at 42, 84 and 168 kJ L\(^{-1}\) because the higher amounts of lysate release at higher energy densities were compensated by the proportional lower amounts of RAS treated.

The third assumption was related to the effects of maintenance metabolism. The ultrasound potential trials indicated the presence of increased maintenance metabolism only in the RAS samples treated at 168 kJ L\(^{-1}\). Therefore, the effects of increased maintenance metabolism were supposed to affect sludge production only during the pilot-scale plant trial at 168 kJ L\(^{-1}\) (trial M/H).

According to these assumptions, if the sludge reduction mechanism based on increased maintenance metabolism by induced stress was viable, the degree of sludge reduction was supposed to be higher in the trial 168 kJ L\(^{-1}\) than in the trials at 42 and 84 kJ L\(^{-1}\).

The results from the pilot-scale plant trials indicated that the assumptions made on the similar floc size reductions and lysate releases during the three disintegration trials were reasonably accurate. During the three disintegration trials at 42, 84 and 168 kJ L\(^{-1}\), the floc size in the test lane varied between 62.2 and 93.0 µm, with a ratio between the standard deviation and the average of 21.1%. The daily average release of lysate varied between 36 and 46.7 g lysate COD d\(^{-1}\), with a ratio between the standard deviation and the average of 13.6%.

However, the third assumption related to an increase in the degree of sludge reduction during the trial at 168 kJ L\(^{-1}\) due to the presence of increased maintenance metabolism was wrong. The degrees of sludge reduction (RSP) achieved during these trials were
also similar: 13.4, 17.2 and 14.5 % for the trials at 42, 84 and 168 kJ L\(^{-1}\), respectively, with the gradient of their linear fit close to zero. Furthermore, the constancy during the trials at 42, 84 and 168 kJ L\(^{-1}\) of the ratio RSP/ΔS\(_{\text{Lys}}\) suggested a strong relationship between the degree of sludge reduction and the release of cell lysate regardless of the energy density used.

Considering that floc breakage did not lead to sludge reduction, the similar degrees of sludge reduction achieved during the trials at 42, 84 and 168 kJ L\(^{-1}\) and the lack of an increase in sludge reduction at 168 kJ L\(^{-1}\), could be explained with the following three scenarios:

- The increased maintenance metabolism cannot be triggered by cavitation-based technology, as Camacho et al. (2002b) suggested in their study
- The increased maintenance metabolism can be triggered at 168 kJ L\(^{-1}\) but only in RAS treated with ultrasound for the first time. Therefore, its effects on sludge production are lost in RAS continuously treated with ultrasound, as it happens during the pilot-scale plant trials and, eventually, at full-scale
- The increased maintenance metabolism was triggered during the trial M/L at 168 kJ L\(^{-1}\). However, the contribution to sludge reduction due to the increased maintenance metabolism was not as important as the one due to the lysis-cryptic growth mechanism based on sludge solubilisation. Therefore, the increased maintenance metabolism does not play a significant role in sludge reduction at the energy densities investigated.

Even if the increased maintenance metabolism might occur at higher energy densities, i.e. higher than 168 kJ L\(^{-1}\), its contribution could not be exploited at full-scale because so high energy densities are never used. For example, recommended energy densities for the use of the Ultrawaves equipment at full-scale are around 15 kJ L\(^{-1}\).

### 8.4 Sludge reduction due to lysis-cryptic growth by sludge solubilisation

Both the enhanced metabolism and the increase in maintenance metabolism were not found to be viable sludge reduction mechanisms in this study. The mechanism of enhanced metabolism was excluded due to the increase in sludge production during the trial L. Either the mechanism of the increased maintenance metabolism could be
excluded or its role was not significant, due to the similar degrees of sludge reduction observed during the disintegration trials at medium energy input. Therefore, the remaining viable sludge reduction mechanism was the lysis-cryptic growth by sludge solubilisation.

Lysis-cryptic growth was explicitly considered at least as one of the main mechanism of sludge reduction in most of the studies found in the literature (Camacho et al., 2002b; Cao et al., 2006; Strünkmann et al., 2006). To investigate the potential of the lysis-cryptic growth, it was crucial to evaluate the amount of cell lysate released by the ultrasound treatment and its biodegradability. During this study, the increase of cell lysate released by ultrasound treatment was almost linear between 0 and 168 kJ L$^{-1}$ (Figure 5-16). The biodegradability of the cell lysate released by sludge treated at 42 kJ L$^{-1}$ was 78% (Figure 5-17). These results were in agreement with those from Andreottola et al. (2007) and Rocher et al. (1999).

Andreottola et al. (2007) found an almost linear increase in the release of cell lysate between 0 and 300 kJ L$^{-1}$ and a biodegradability of 82%. They also found that the cell lysate biodegradability increased exponentially after 130 kJ L$^{-1}$: in their study, the higher was the release of lysate COD, the higher was its biodegradability. Rocher et al. (1999) found that up to 75% of the lysate was biodegradable.

The well-documented release of cell lysate during sludge disintegration and its biodegradability support the viability of the lysis-cryptic growth as a sludge reduction mechanism.

However, in theory, the higher the amount of cell lysate released, the higher should be the degree of reduction achievable. On the contrary, in this study, lysis-cryptic growth could justify the degrees of sludge reduction observed during the disintegration trials at medium energy inputs, i.e. trials M, but it could not explain the limited degree of sludge reduction achieved during the trial at high energy input, the trial H.

During the trial H, the average daily amount of lysate released was ~2.4 higher than during the trials M but the degree of sludge reduction was similar: 16.2% for the trial H, 15.4%, on average, for the trials M. A possible explanation for the lower degree of sludge reduction observed during the trial H could be related to the characteristics of the cell lysate. Cell lysate for its nature is a complex mix of extracellular and intracellular compounds. Some compounds might be more or less biodegradable than
others. When the activated sludge microbial community is exposed to high amounts of lysate caused by high energy inputs, it might tend to consume and metabolise the highly biodegradable compounds first. Higher biodegradable compounds are also generally characterised by higher yields (Bitton, 2005). Therefore, in the presence of high amounts of lysate, the overall re-growth of bacteria on their autochthonous substrate might be higher than expected. This would lead to lower levels of biomass mineralisation and, hence, to lower degrees of sludge reduction.

The presence of this reduction-limiting mechanism at high energy inputs could justify:

- The similar degrees of sludge reduction detected during trials M and trial H., i.e. higher release of cell lysate does not automatically lead to higher degrees of sludge reduction
- The relatively low values of sludge reduction found in this study, 22 % (including the 5 % offset observed during the baseline), and in the studies undertaken by Strünkmann et al. (2006) and Camacho et al. (2002b), 24 %

On the other hand, this limiting mechanism fails to explain the agreement on the much higher levels of sludge reduction reported by the authors investigating the use of ozone (Table 8-1).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sludge reduction [％]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yasui and Shibata (1994)</td>
<td>100</td>
</tr>
<tr>
<td>Sakai et al (1997)</td>
<td>66</td>
</tr>
<tr>
<td>Kamiya and Hirotsuji (1998)</td>
<td>70</td>
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<tr>
<td>Délérès et al. (2002)</td>
<td>70</td>
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<tr>
<td>Boehler and Siegrist (2004)</td>
<td>46</td>
</tr>
<tr>
<td>Salhi et al. (2003)</td>
<td>100</td>
</tr>
<tr>
<td>Saktaywin et al. (2006)</td>
<td>60</td>
</tr>
<tr>
<td>Huysmans et al. (2001)</td>
<td>50</td>
</tr>
</tbody>
</table>

A possible explanation of the difference between the average degrees of sludge reduction achieved in this and the other studies using ultrasound systems or high pressure homogenisers and those achieved in studies using ozone might be found in the work of Paul et al. (2006), Camacho et al. (2005) and Yoon and Lee (2005). Paul
et al. (2006) discussing the sludge reduction mechanisms of ozone, highlighted the fact that ozone could implement lysis-cryptic growth in two ways by:

- Solubilising sludge biomass, with subsequent mineralisation of part of it, as ultrasound does
- Increasing the biodegradability of the inert organic solid fraction, to enhance even further the overall mineralisation process. Ultrasound might be limited in achieving this increase.

According to their study, depending on the sludge retention time and the influent inert COD particulate fraction, up to 60% of the sludge VSS could be composed of inert organic materials. In a situation like this, without increasing the biodegradability of the inert organic material, it would be impossible to achieve high level of sludge reduction. Ozone is supposed to be able to increase biodegradability by causing the incorporation of oxygen in molecules of inert material (Salhi et al., 2003). Studies using lab-scale plant fed with synthetic sewage might observe higher degree of reduction because synthetic sewage generally contains little inorganic material (Zhang et al., 2007a). A decrease in the ratio VSS/TSS ratio was seen as the sign of increased levels of mineralization of the sludge in the biological reactor and was reported by various authors in Table 8-1. On the contrary, an increase in that ratio was observed in this study (Figure 5-14 in the Dynamic studies chapter).

Camacho et al. (2005) also tried to understand sludge reduction mechanisms implementing a model, developed from ASM1, targeting the main phenomena impacting sludge production during their investigation of the potential of thermal treatment for sludge reduction. They found that using high stress factors, i.e. increasing the contact time between sludge and the thermal disintegration unit, the sludge reduction achieved was lower than expected. Using the model to back up their theory, they reached the conclusion that the reason for the lower degree of reduction detected was due to an increase production/accumulation of inert particulate compounds when thermal treatment was more frequently applied. Thermal treatment failure to affect the chemical molecular structure, whose modification could increase the biodegradability of the particulate organic matrix, led to the increased production/accumulation of inert particulate compounds, which decreased its potential
for sludge reduction. Nevertheless, using the thermal treatment, sludge reductions up to 60% were achieved by Camacho et al. (2005), around 55% by Paul et al. (2006). These degrees of sludge reduction are higher than those achieved with ultrasound treatment.

It is well known and accepted that sludge disintegration by ultrasound is mainly due to the mechanical effects of cavitation and not to its radical effects (Neis et al., 2001; Tiehm et al., 2001). On the contrary, according to Paul et al. (2006), ozone success to reach high degree of reduction is based on its oxidising effects, capable of enhancing the biodegradability of inert compounds. If thermal treatment can only partially achieve this enhancement of biodegradability (Camacho et al., 2005), it is unlikely that ultrasound disintegration, which is based on its mechanical action, can be more efficient in this conversion than the thermal one.

Yoon and Lee (2005) implemented a model to prove what Salhi et al. (2003) and Paul et al. (2006) suggested on the base of their experimental work. According to their model, a sludge disintegration unit can be characterised by its ability to solubilise biomass and by its efficiency to convert non-biodegradable particulates to biodegradable particulates. The result was that sludge reduction was much more influenced by the conversion efficiency than the solubisation ratio.

The degrees of sludge reduction observed in this study were around 20% and are in agreement with those provided by Strünkmann et al. (2006) and Camacho et al. (2002b). The reliability of the results achieved in this study is based on the use of arguably the best experimental set-up and the longest experimental phase among the studies investigating the potential of cavitation-based technologies for sludge reduction. Based on these results, the main contribution of this study was to provide reliable evidence of the limitation in the degrees of sludge reduction achievable with ultrasound, especially in comparison to ozone, and indicate that this limitation might be due to two main reasons:

- Decreased ability to mineralise cell lysate at increasing energy inputs due to the increased consumption of the most biodegradable part of it. This would lead to an overall re-growth of bacteria on their autochthonous substrate higher than expected.
• Limited efficiency in the conversion of non-biodegradable particulates to biodegradable particulates. Solubilisation can be high but there is little change in the sludge molecular chemistry to make inert compounds more biodegradable. This leads to the production/accumulation of inert particulate compounds, which decreases and limits ultrasound potential for sludge reduction in comparison to technologies like ozone, characterised by higher conversion efficiency.
Chapter 9: Conclusions, implications for water utilities and future work

9.1 Conclusions

This project has extended the understanding of the potential of ultrasound for sludge reduction by investigating the effects of ultrasound treatment on sludge biomass, sludge production and the performance of the activated sludge process using a combination of batch and dynamic trials. The main conclusions arising from this study are the following:

- Within the range of energy densities investigated (0-184 kJ L\(^{-1}\)), ultrasound equipment operating at high intensity (~50 W cm\(^{-2}\)) was able to achieve a degree of SCOD release 3.8 higher than ultrasound equipment operating at low intensity (7 W cm\(^{-2}\)).
- At 42 kJ L\(^{-1}\), the relationship between the SCOD release and the solids content of sludge treated with high intensity ultrasound equipment was almost linear (R-square value: 0.99), at least up to solids concentration of 5 % DS.
- At 184 kJ L\(^{-1}\), the average floc reduction achieved treating RAS samples from three different WWTPs (2 CAS and 1 BNR) using high intensity ultrasound equipment varied from 28 to 84 %. On average, 69 % of the total floc reduction was achieved at 46 kJ L\(^{-1}\).
- At 184 kJ L\(^{-1}\), the degree of SCOD release achieved treating RAS and ML samples from five different WWTPs (3 CAS, 1 OD and 1 BNR) using high intensity ultrasound equipment varied from 22.7 to 36.8% and was on average 28.2±6.5 %.
- Using high intensity ultrasound equipment, no significant differences were found in the SCOD release from RAS samples collected from CAS, OD and BNR WWTPs, at least within the range of 10 WWTPs (3 CAS, 6 OD, 1 BNR) investigated in this study.
- Overall, the performance of the activated sludge process was preserved during the dynamic disintegration trials. The TCOD and NH\(_4\) removals in the test lane were significantly decreased only at high energy inputs, using 84 kJ L\(^{-1}\) and treating
12.5% of the RAS. Total phosphorus and total suspended solids removals were not significantly affected. Effluent turbidity was only slightly affected. Settleability was not compromised even if there was a 1.8-fold increase in the sludge volume index of the RAS in the test lane. However, on average, there was a 5.4-fold and 4.2-fold increase in the RAS capillary suction times and specific resistances to filtration (SRF), respectively, which indicated an overall decrease in the dewaterability.

- The batch disintegration trials completed on RAS samples collected from the pilot-scale plant indicated that ultrasound treatment, by causing floc breakage, sludge solubilisation and changes in the biological activity, was theoretically able to implement the following three sludge reduction mechanisms: enhanced metabolism by floc breakage, lysis-cryptic growth by biomass solubilisation and increased maintenance metabolism by induced stress. Floc size was reduced by 88% at 42 kJ L\(^{-1}\), the degree of SCOD release increased almost linearly from 11 to 36% between 42 to 168 kJ L\(^{-1}\), changes in the biological activity that might trigger a stress response were observed only at 168 kJ L\(^{-1}\).

- The degrees of sludge reduction varied from -9.4 to 17.2% during the dynamic disintegration trials. Results from the dynamic studies suggested that lysis-cryptic growth was the main mechanism behind the observed sludge reduction. Based on modelling, lysis-cryptic growth could justify 98% of the sludge reduction observed. The relatively low degrees of sludge reduction achieved by ultrasound, in comparison to those reported in the literature for ozone (>50%), suggested that there might be a limit to the degree of reduction achievable with ultrasound. This limit might be explained by: (1) a decreased ability of the microbial biomass to mineralise cell lysate at increasing energy inputs due to the increased consumption of the most biodegradable part of it and (2) the limited efficiency of the ultrasound treatment in the conversion of non-biodegradable particulates to biodegradable particulates.

### 9.2 Implications for water utilities

Batch disintegration trials indicated that the highest degrees of sludge solubilisation were achieved using ultrasound equipment operating at high power intensities (~50 W.
cm$^2$) on sludge thickened up to a concentration of 5 % DS. The use of high intensity ultrasound systems and sludge thickening before disintegration are therefore recommended during full-scale operations.

A pilot-scale plant fed with settled sewage and comprised of two 1.2 m$^3$ lanes, operated as a test and control, was used for dynamic trials. A high intensity 1 kW ultrasound system used at full-scale for sludge disintegration was integrated into the test lane of the pilot-scale plant to allow the continuous in-line ultrasound treatment of the RAS. This set-up was used to replicate the configuration adopted at full-scale and allow a more reliable scale-up of the results.

Taking into account the 5.2 % biological variability observed between the control and test lanes during the baseline, the maximum degree of sludge reduction achieved without affecting the overall performance of the activated sludge process was 20 %, using 6048 kJ d$^{-1}$ at 42 kJ L$^{-1}$ with a stress factor of 0.28 d$^{-1}$. At these operational conditions, TCOD and NH$_4$ removals were not affected. However, a 5.5-fold increase in the capillary suction time and a 3.6-fold increase in the specific resistance to filtration in the RAS from the test lane indicated a detrimental impact on the dewaterability.

The use of higher energy inputs is not recommended because it did not lead to a significant decrease in sludge production and negatively affected the TCOD and NH$_4$ removal.

An accurate and well-established cost analysis model is not available in the literature. In this study, the economical analysis was performed scaling-up the results from the dynamic trials to a full-scale scenario. The following main assumptions were considered: (1) the use of the 5-kW full-scale configuration for the ultrasound system, (2) the disintegration of RAS after being thickened to a concentration of 6-7 % DS, (3) a 20 % decrease in sludge production and (4) a cost for sludge treatment and reuse of £ 423 per tonne DS.

In accordance with these assumptions, the cost analysis indicated that sludge reduction by ultrasound disintegration is not economically viable, with a breakeven cost for the sludge treatment and reuse of £ 961 per tonne DS.
As a conclusion, the application of ultrasound treatment for sludge reduction should be carefully evaluated case by case and, in general, might be advisable only where specific conditions might occur, such as:

- High costs for sludge treatment and reuse (> £961 per tonne DS) and the need to control operational problems such as bulking sludge and/or provide readily biodegradable carbon source to enhance denitrification
- Prevention of an enlargement of an activated sludge plant as a result of lower sludge production

### 9.3 Future work

Further research should build on the results of this study and focus on fundamental aspects of sludge reduction to understand their role in achieving it. Possible objectives could be:

- Assess the conditions necessary to trigger some form of enhanced maintenance metabolism. In this study, sign of enhanced metabolism could be found only at very high energy densities, i.e.: 168 kJ L\(^{-1}\). According to Camacho et al. (2002a) maintenance metabolism was found associated with thermal treatments rather than mechanical ones. It would be interesting to evaluate their hypothesis by comparing the oxygen uptake and oxygen uptake rates of sludge samples treated with ultrasound against those treated with thermal processes. This comparison could lead to a better understanding of the increased maintenance metabolism as a viable sludge reduction mechanism
- Assess the occurrence of cell lysis. It is commonly accepted that at low energy inputs only flocs are disrupted while cell lysis starts to occur at higher energy inputs, but it is not clear at all when this happens and which is the minimum energy input required to make it happen. DNA-based analyses could be carried out to assess the presence and percentage of cell lysis. The potential of a technique based on the detection of the activity of an enzyme, the glucose-6-phosphate-dehydrogenase (G6PDH), was investigated during this study. This technique, developed by Frølund et al. (1996) and reported by Biggs and Lant (2000), detects the activity of the enzyme G6PDH in the supernatant of a sludge sample. Enzyme G6PDH is normally only found within the cell, therefore its presence in the sludge
supernatant might suggest the occurrence of cell lysis (Biggs and Lant, 2000). A kit for the detection of enzyme G6PDH was purchased from Trinity Biotech Plc (Bray, Ireland) but, due to time constraints, it was never used. The procedure is quite simple and could be used as a starting point for a more comprehensive investigation on cell lysis

- Develop a mathematical model based on ASM1-3 more sophisticated than the one used in this study. The current literature available on the modelling of the RAS disintegration process for sludge reduction assumes that all bacterial cells are broken up and converted into particulates (Yoon and Lee, 2005). At the energy densities generally used at full-scale (~14 kJ L$^{-1}$), the assumption that all the microorganisms present in the sludge during the disintegration process are lysed is not realistic. It would be interesting to model the disintegration process without that kind of simplification to interpret the experimental results and evaluate relationships among process control parameters and their potential effects on sludge reduction in the most realistic way

- Develop a more comprehensive cost analysis model. Too many studies report different conclusions when evaluating the economics behind the application of disintegration processes for sludge reduction. A standardised cost analysis model would be useful for evaluate the best compromise between capital and operational expenditures necessary to implement sludge reduction technologies and savings associated with reduced costs for sludge treatment and reuse. This is particularly important considering that, in general, disintegration costs are still very high and the application of reduction strategies in real situations must be evaluated case by case. A well established model for cost analysis would be able to evaluate site specific applications characterised by a wide variation in sludge reuse/disposal and retrofit costs

- The use of ultrasound treatment for sludge reduction exploiting the lysis-cryptic growth mechanism might be always limited by the high energy costs associated with its implementation. Predation on bacteria could be a possible alternative mechanism. Floc reduction can be achieved by ultrasound at much lower energy levels, therefore ultrasound treatment could be used only to reduce floc size and
promote disperse growth. This sludge disintegration process at lower energy input should then be followed by a predator enhancement stage.
Chapter 10: References


Grönroos, A., Kyllönen, H., Korpijärvi, K., Pirkonen, P., Paavola, T., Jokela, J. and Rintala, J. (2005) Ultrasound assisted method to increase soluble chemical oxygen demand (scod) of sewage sludge for digestion, Ultrasonics Sonochemistry, 12(1-2) 115-120.


Mason, T. J. (2003) Sonochemistry and sonoprocessing: The link, the trends and (probably) the future, Ultrasonics Sonochemistry, 10 175-179.


Chapter 10: References


Chapter 11: Appendices
Appendix I: Results of the multiple comparison tests

In this section, the results from the multiple comparisons tests are presented. The multiple comparison tests were used to assess if the results from the ultrasound potential trials and the pilot-scale plant trials completed during the dynamic studies in chapter 5 were significantly different or not. For this purpose, multiple comparison tests were used in conjunction with the one-way ANOVA test (p-value < 0.05), applying the Tukey's honestly significant difference criterion. This criterion, using the statistical information from the one-way ANOVA test, provided comparison intervals. Two means were significantly different if their comparison intervals were disjoint, and were not significantly different if their intervals overlapped. The application of multiple comparisons tests based on the one-way ANOVA tests implied the following assumptions (Hogg and Ledolter, 1990):

- All measurement populations are normally distributed
- All measurement populations have equal variance
- All measurements are mutually independent.

The ANOVA test is known to be robust to modest violations of the first two assumptions. Tukey's honestly significant difference criterion has been proven conservative for one-way ANOVA tests applied to groups with different numbers of measurements. According to the unproven Tukey-Kramer conjecture, it is also accurate for problems where the quantities being compared are correlated (Hogg and Ledolter, 1990). All tests were implemented using MATLAB®.
Chapter 11: Appendices

A I.1 Effluent soluble COD

The multiple comparison tests were applied to the daily differences in the effluent SCOD concentration between the test and control lanes of the pilot-scale plant. The number of measurements used for each pilot-scale plant trial is given in Table 11-1.

Table 11-1: Number of measurements used for each pilot-scale plant trial

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M/L</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>43</td>
<td>22</td>
<td>28</td>
<td>14</td>
<td>29</td>
<td>23</td>
<td>15</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, only the difference in the trial H was significantly higher than in the baseline (Figure 11-1).

![Multiple comparison test](image)

Figure 11-1: Multiple comparative tests applied to the differences in the soluble COD concentration present in the test and control effluents. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. Only the difference in trial H was significantly higher than in the baseline.

A I.2 Effluent ammonium

The multiple comparison tests were applied to the daily differences in the effluent NH₄ concentration between the test and control lanes of the pilot-scale plant. The
number of measurements used for each pilot-scale plant trial is given in Table 11-2.

Table 11-2: Number of measurements used for each pilot-scale plant trial

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>43</td>
<td>19</td>
<td>19</td>
<td>8</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, only the differences in the trial M/H and trial H were significantly higher than in the baseline (Figure 11-2).

![Multiple comparison test](image)

Figure 11-2: Multiple comparative tests applied to the differences in the NH₄ concentration present in the test and control effluents. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. Only the difference in the trial M/H and trial H were significantly higher than in the baseline.

### A I.3 Effluent soluble total phosphorus

The multiple comparison tests were applied to the daily differences in the effluent soluble total phosphorus concentration between the test and control lanes of the pilot-scale plant. The number of measurements used for each pilot-scale plant trial is given in Table 11-3.
Chapter 11: Appendices

### Table 11-3: Number of measurements used for each pilot-scale plant trial

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>12</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, there were no significant differences among the pilot-scale plant trials (Figure 11-3).

![Multiple comparison test](image)

Figure 11-3: Multiple comparison tests applied to the differences in the total phosphorus concentration present in the test and control effluents. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. No significant differences were found among the pilot-scale plant trials.

### A I.4 Effluent turbidity

The multiple comparison tests were applied to the daily differences in the effluent turbidity between the test and control lanes in the pilot-scale plant. The number of measurements used for each pilot-scale plant trial is given in Table 11-4.
Table 11-4: Number of measurements used for each pilot-scale plant trial

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>43</td>
<td>21</td>
<td>28</td>
<td>14</td>
<td>26</td>
<td>21</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, only the difference in the trial M/L (F/M) was significantly higher than in the baseline (Figure 11-4).

![Multiple comparison test](image)

Figure 11-4: Multiple comparison tests applied to the differences in the effluent turbidity between the test and control lanes. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. Only the difference in the trial M/L (F/M) was significantly higher than in the baseline.

### A 1.5 Effluent total suspended solids

The multiple comparison tests were applied to the daily differences in the effluent total suspended solids concentration between the test and control lanes in the pilot-scale plant. The number of measurements used for each pilot-scale plant trial is given in Table 11-5.
According to the multiple comparison tests, only the difference in the trial M/L (F/M) was significantly higher than in the baseline (Figure 11-5).

### Figure 11-5: Multiple comparison tests applied to the differences in the total suspended solids concentrations present in the test and control effluents. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. Only the difference in the trial M/L (F/M) was significantly higher than in the baseline.

#### A I.6 RAS stirred sludge volume index (SSVI)

The multiple comparison tests were applied to the daily differences in the RAS stirred sludge volume index between the test and control lanes in the pilot-scale plant. The number of measurements used for each pilot-scale plant trial is given in Table 11-6.
Table 11-6: Number of measurements used for each pilot-scale plant trial

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>37</td>
<td>16</td>
<td>17</td>
<td>7</td>
<td>12</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, the differences in all the disintegration trials were significantly higher than in the baseline (Figure 11-6).

![Multiple comparison test](image)

Figure 11-6: Multiple comparison tests applied to the differences in the SSVI values of the RAS samples collected from the test and control lanes. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. In all the disintegration trials, the differences were significantly higher than in the baseline.

### A I.7 RAS capillary suction time (CST)

The multiple comparison tests were applied to the daily differences in the RAS sludge capillary suction time between the test and control lanes in the pilot-scale plant. The number of measurements used for each pilot-scale plant trial is given in Table 11-7.
Table 11-7: Number of measurements used for each pilot-scale plant trial

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M/L</th>
<th>M/L (F/M)</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>42</td>
<td>14</td>
<td>20</td>
<td>8</td>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, the differences in all the disintegration trials were significantly higher than in the baseline (Figure 11-7).

Figure 11-7: Multiple comparison tests applied to the differences in the CST values of the RAS samples collected from the test and control lanes. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. In all the disintegration trials, the differences were significantly higher than in the baseline.

A 1.8 RAS specific resistance to filtration (SRF)

The multiple comparison tests were applied to the daily differences in the RAS specific resistance to filtration between the test and control lanes in the pilot-scale plant. The RAS SRF values for the test and control lanes during the baseline were not available. Therefore, for the multiple comparison tests, the differences between in the RAS SRF values in the control lane during the other pilot-scale trials were used as a reference. The number of measurements used for each pilot-scale plant trial is given...
in Table 11-8.

Table 11-8: Number of measurements used for each pilot-scale plant trial (NA: not available)

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>6</td>
<td>6</td>
<td>NA</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, the differences in all the disintegration trials were significantly higher than in the baseline (Figure 11-8).

![Multiple comparison test](image)

Figure 11-8: Multiple comparison tests applied to the differences in the SRF values of the RAS samples collected from the test and control lanes. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. In all the disintegration trials, the differences were significantly higher than in the baseline.

**A 1.9 RAS volatile fraction**

The multiple comparison tests were applied to the differences in the RAS volatile fraction (i.e. the ratio between the volatile suspended solids and the total suspended solids) between the test and control lanes in the pilot-scale plant. The number of measurements used for each pilot-scale plant trial is given in Table 11-9.
Table 11-9: Number of measurements used for each pilot-scale plant trial

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>40</td>
<td>19</td>
<td>27</td>
<td>13</td>
<td>28</td>
<td>21</td>
<td>15</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, in all the disintegration trials, the differences were significantly higher than in the baseline (Figure 11-9).

Figure 11-9: Multiple comparative tests applied to the differences in the volatile fractions of the RAS samples collected from the test and control lanes in the pilot-scale plant. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. In all the disintegration trials, the differences were significantly higher than in the baseline.

**A I.10 Short-term RAS specific oxygen uptake (SOU)**

The RAS samples used during the short-term respirometric trials were collected from three different locations: (1) the control lane in the pilot-scale plant, (2) the Cotton Valley WWTP and (2) the test lane in the pilot-scale plant. Three distinct sets of multiple comparison tests were completed depending on the collection point of the RAS samples.
The first set of multiple comparison tests were applied to the differences in the specific oxygen uptakes between the untreated and treated RAS samples collected from the control lane of the pilot-scale plant. The RAS samples were treated at 42, 84 and 168 kJ L⁻¹. The number of measurements used for the untreated and treated RAS samples is given in Table 11-10.

**Table 11-10: Number of measurements used for each energy input**

<table>
<thead>
<tr>
<th>Energy density [kJ L⁻¹]</th>
<th>0 (untreated)</th>
<th>42</th>
<th>84</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, only the difference in the RAS samples treated at 168 kJ L⁻¹ was significantly higher than the one for the untreated RAS samples (Figure 11-10).

**Figure 11-10: Multiple comparison tests applied to the differences between the specific oxygen uptakes in the untreated and treated RAS samples.** The RAS samples were collected from the control lane in the pilot-scale plant. Some of the RAS samples were then treated at 42, 84 and 168 kJ L⁻¹ with the Ultrawaves probe. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. Only the difference for the RAS samples treated at 168 kJ L⁻¹ was significantly higher than the one for the untreated RAS samples.
The second set of multiple comparison tests were applied to the differences in the specific oxygen uptakes between the untreated and treated RAS samples collected from the Cotton Valley WWTP. The RAS samples were treated at 42, 84 and 168 kJ L\(^{-1}\). The number of measurements used for the untreated and treated RAS samples is given in Table 11-11.

**Table 11-11: Number of measurements used for each energy input**

<table>
<thead>
<tr>
<th>Energy density [kJ L(^{-1})]</th>
<th>0 (untreated)</th>
<th>42</th>
<th>84</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, none of the differences in the RAS samples treated at 42, 84 and 168 kJ L\(^{-1}\) was significantly higher than the one for the untreated RAS samples (Figure 11-11).

**Figure 11-11: Multiple comparison tests applied to the differences between the specific oxygen uptakes in the untreated and treated RAS samples. The RAS samples were collected from the Cotton Valley WWTP. Some of the RAS samples were then treated at 42, 84 and 168 kJ L\(^{-1}\) with the Ultrawaves probe. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. None of the differences in the treated RAS samples was significantly higher than the one for the untreated RAS samples.**
The third set of multiple comparison tests were applied to the differences in the specific oxygen uptakes between the untreated and treated RAS samples collected from the test lane in the pilot-scale plant. The RAS samples were treated at 42 and 168 kJ L\(^{-1}\). The number of measurements used for the untreated and treated RAS samples is given in Table 11-12.

**Table 11-12: Number of measurements used for each energy input and corresponding p-value**

<table>
<thead>
<tr>
<th>Energy density [kJ L(^{-1})]</th>
<th>0 (untreated)</th>
<th>42</th>
<th>84</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>3</td>
<td>3</td>
<td>NA</td>
<td>3</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, none of the differences in the RAS samples treated at 42 and 168 kJ L\(^{-1}\) was significantly higher than the one for the untreated RAS samples (Figure 11-12).

**Multiple comparison test**

![Chart showing multiple comparison tests](chart.png)

Figure 11-12: Multiple comparison tests applied to the differences between the specific oxygen uptakes in the untreated and treated RAS samples. The RAS samples were collected from the Cotton Valley WWTP. Some of the RAS samples were then treated at 42, 84 and 168 kJ L\(^{-1}\) with the Ultrawaves probe. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. None of the differences in the treated RAS samples was significantly higher than the one for the untreated RAS samples.
A I.11 Long-term RAS specific oxygen uptake (SOU)

The multiple comparison tests were applied to the differences between the specific oxygen uptakes in the RAS samples collected from the test and control lanes during the long-term respirometric trials completed during the pilot-scale plant trials. The number of measurements used for each pilot-scale plant trial is given in Table 11-13.

Table 11-13: Number of measurements used for each pilot-scale plant trial (NA: not available)

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Bas.</th>
<th>L</th>
<th>M/L (F/M)</th>
<th>M/L</th>
<th>M/M</th>
<th>M/H</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

According to the multiple comparison tests, none of the differences investigated was significantly higher than in the baseline. The differences in the disintegration trials at low energy input and low energy density (trial M/L (F/M) and trial M/L) were significantly lower than in the baseline (Figure 11-13).

Figure 11-13: Multiple comparison tests applied to the differences in the RAS specific oxygen uptakes between the test and control lanes. Two means are significantly different if their comparison intervals are disjoint, and are not significantly different if their intervals overlap. The differences in the disintegration trials at medium energy input and low energy density (trial M/L (F/M) and trial M/L) were significantly lower than in the baseline.