

Differences in soluble COD and ammonium when applying ultrasound to primary, secondary and mixed sludge

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ABSTRACT

Ultrasound treatment is often applied to enhance the anaerobic digestion of sludge. Optimal conditions for organic matter solubilisation of primary, secondary and mixed sludge were assessed by implementing ultrasound disruption at different specific energies (from 3,500 to 21,000 kJ/kgTS). The variation in soluble chemical oxygen demand (sCOD) and ammonium nitrogen (NH_4^+ -N) was monitored following the treatment and after a subsequent fermentation (24 hours, 37°C). The effect of the treatment was clearly more pronounced in secondary sludge than in the other types of sludge. Relatively minimum values in solubility were found when applying ultrasound at different energies depending on the sludge (3,500-7,000 kJ/kgTS in primary sludge and 10,500-14,000 kJ/kgTS in secondary sludge). This minimum was not so noticeable in mixed sludge. The addition of inoculum was not required after ultrasound disruption to perform the subsequent fermentation. After this final stage, no general pattern in terms of sCOD was observed. Increases and decreases were conditioned by the coverage of the ultrasound irradiation. NH_4^+ -N values increased notably during the fermentation.

KEYWORDS

Ammonium, COD, mixed sludge, primary sludge, secondary sludge, ultrasound

INTRODUCTION

At a conventional wastewater treatment plant (WWTP), the settleable suspended matter is removed resulting in “primary” sludge, with a concentration of total solids (TS) in the 1-3% range. The water line flows into the bioreactor, where microbial respiration produces “secondary” sludge (0.5-1.5% TS) rich in microorganisms and their extracellular polymeric substances (EPS). After thickening, both types of sludge are frequently mixed, constituting what is known as “mixed” sludge (3-8% TS). The proportion of primary and secondary sludge is expected to determine the properties of mixed sludge. However, the former undergo thickening processes for a certain period of time and hence not all the characteristics of mixed sludge are a clear combination of those of primary and secondary sludge. The characterisation of a type of sludge helps wastewater engineers to choose an affordable and suitable form of handling. Sludge management is considered a critical step because of the high-budget demand (Kavitha *et al.* 2014). Technical progress has assumed this precept, launching novel strategies in composting, aerobic digestion and anaerobic digestion (Tchobanoglous *et al.* 2014). The lower costs of the microbiological alternatives compared to other technologies often make them interesting to industry (Hasegawa *et al.* 2000). As regards biological implementation, the bioavailability of nutrients is an essential factor (Appels *et al.* 2008). Sewage sludge has been successfully used as a source of carbon for microorganisms that perform denitrification (Ucisik & Henze 2008). However, the “occlusion” of nutrients in cell structures requires their release by means of an effective treatment applied to the microbial networks. Likewise, accelerating the hydrolysis step (which constitutes the rate-limiting step in anaerobic digestion of sewage sludge) by applying a treatment aids the anaerobic sequence (Appels *et al.* 2008).

The treatments employed to release the microbiological material in sludge may be physical, chemical, biological or combinations thereof (Carrère *et al.* 2010; Uma Rani *et al.* 2012a). The application of a physical treatment like ultrasound to sewage sludge transforms electrical power into mechanical energy. This mechanical energy supplied by a sonotrode results in cavitation due to bubble collapse which affects the medium (Erden & Filibeli 2010). As extreme local conditions are caused, radicals and oxidising species appear (e.g. $\text{OH}\cdot$, $\text{HO}_2\cdot$, $\text{H}\cdot$ and H_2O_2). An increase in temperature is therefore inherent to ultrasound disruption due to these species, shear forces and friction generated in the medium. One advantage of ultrasound treatment is the feasibility of its implementation by means of an ultrasonicator, the Specific Energy (E_s) to apply being a key factor. Ultrasound irradiation may be technically expressed in different ways, although E_s is the term generally employed (Pilli *et al.* 2011). The optimum E_s should be related to the purpose of the treatment, whether this be increased solubilisation of organic matter (Zhang *et al.* 2007), enhanced methane production (Luste & Loustarinen 2011), or further post-processing of the substrate, e.g. dewatering (Erden & Filibeli 2010; Devlin *et al.* 2011). There are several reasons explaining the advantages of ultrasonication for the promotion of methane production by microorganisms: 1) Bacterial walls become flawed and permeability to monomers is increased. Even microbiological metabolism is promoted at low-intensity ultrasound implementation

(Xie *et al.* 2009). 2) Ultrasound leads to the breakdown of EPS, converting them into an available substrate for bacteria (Nabarlatz *et al.* 2010). 3) The levels of E_s make ultrasound disruption selective for bacterial cultures (More & Ghangreakar 2010). Whereas a low E_s promotes methane production, a high E_s worsens the yield. A threshold seems to appear, above which, methane production decreases. If the purpose of the treatment is to promote hydrolysis, this is achieved at low E_s . Kim & Lee (2012) observed that the excessive hydrolysis of waste activated sludge (i.e. 50%) caused by ultrasound irradiation led to poorer anaerobic performance. This response was probably due to partial transformation of volatile solids into inhibitory and/or inert compounds.

The success of the treatments may be measured through the solubilisation of organic matter, with soluble chemical oxygen demand (sCOD) and ammonium nitrogen ($\text{NH}_4^+\text{-N}$) being considered suitable indicators. The ratio of organic matter filtered (the soluble fraction) to total organic matter (the total Chemical Oxygen Demand, tCOD) was used to estimate the ease with which bacteria can overcome the steric inaccessibility of particulate organic matter (Eskicioglu *et al.* 2006). $\text{NH}_4^+\text{-N}$, on the other hand, is the product of degradation of nitrogen present in macromolecules such as proteins (Broderick 1987). Thus, evaluation of ammonia can be used to monitor sludge solubilisation. This also supposes an example of inorganic species solubilisation by ultrasound treatment (Tyagi *et al.* 2014). Both parameters (the sCOD/tCOD ratio and $\text{NH}_4^+\text{-N}$) have accordingly been used to assess this type of hydrolysis (Negral *et al.* 2013). Although the literature contains many studies on the hydrolysis of secondary sludge, mainly on biomethanisation (Appels *et al.* 2008), papers discussing the effect of applying treatments to primary and mixed sludge are scarce. This is relevant in terms of refining the selection criteria for the fractions that should be treated and to what degree. King & Forster (1990) studied EPS extraction by ultrasound disruption of flocs; however, they described a subsequent re-flocculation phenomenon. Mao *et al.* (2004) observed that primary sludge was more easily solubilised, while Tyagi *et al.* (2014) reported that ultrasound application produced more disintegration in secondary sludge than in primary and mixed sludge. The latter finding could possibly be explained by the barrier of deflocculation in secondary sludge that must be overcome before cellular lysis (Gayathri *et al.* 2015). Moreover, the need to interrupt the hydrolysis step may arise if the hydrolysate is intended to be used as a carbon source in other processes (Salsabil *et al.* 2009). This explains the interest in presenting the results of an optimisation of hydrolysis for each type of sludge. In this context, the aims of this paper are to: a) promote hydrolysis in the three aforementioned types of sludge by applying ultrasound disruption; b) determine and compare the optimal conditions for solubilisation of the different types of sludge produced at a WWTP; and c) assess the evolution of short-term hydrolysis after applying fermentation subsequent to ultrasound disruption.

MATERIALS AND METHODS

Materials

Experiments were carried out with fresh sludge from a WWTP with an average flow rate of $21,600 \text{ m}^3/\text{day}$ (85,000 population equivalent), removal efficiencies of >85%

suspended solids (SS) and >90% 5-day biochemical oxygen demand (BOD₅). The plant operates in a conventional manner with production of mixed sludge as a mixture of primary and secondary sludge. It is located in Asturias, a coastal region in the north of Spain. Primary sludge is thickened by gravity and secondary sludge, by flotation. All sludge samples were taken prior to dewatering and were grouped by type of sludge (Table 1).

Equipment employed

The ultrasound equipment was a Hielscher UP400S. This system operates at 24 kHz, with a maximum power of 400 W, pulse adjustable to 0-100%, and is equipped with an H22 titanium sonotrode with a 22 mm tip diameter. The system treated up to 2 litres in each run.

Table 1. Characterisation of initial sludge samples (mean±standard deviation)

Sample	sCOD (mg/L)	tCOD (mg/L)	sCOD/tCOD	NH ₄ ⁺ -N (mg/L)	TS (g/L)	VS (g/L)	VS/TS	tCOD/VS (g/L)/(g/L)	pH
P1*	791±158	12,283±503	0.06	29±0	17.00±0.17	10.01±0.18	0.59	1.23	6.8
P2	6,597±174	50,368±911	0.13	136±2	37.78±0.74	26.72±0.40	0.67	1.89	n.a.
P3	940±2	48,501±323	0.02	17±0	59.03±0.21	36.02±0.16	0.61	1.35	5.7
P4	1,013±13	60,884±1,414	0.02	38±3	78.40±0.31	43.00±0.10	0.55	1.42	5.5
S1	56±5	24,933±815	<0.01	6±0	31.94±1.65	21.92±1.17	0.69	1.14	6.7
S2	185±55	42,104±4,138	<0.01	5±0	45.27±0.29	31.06±0.31	0.69	1.36	6.5
S3	51±13	39,339±2,712	<0.01	10±1	47.55±0.07	32.21±0.13	0.68	1.22	6.7
M1	5,236±50	47,903±1,494	0.11	369±3	39.76±0.10	27.43±0.08	0.69	1.75	6.0
M2	4,270±199	40,732±1,629	0.10	330±9	35.29±0.15	23.19±0.09	0.66	1.76	6.2
M3	3,776±73	45,333±1,346	0.08	263±3	47.05±0.11	30.17±0.08	0.64	1.50	6.0

P: primary sludge; S: secondary sludge; M: mixed sludge; n.a.: not available

*: This sample was collected prior to thickening

Experimental procedure

Hydrolysis was monitored by measuring the sCOD and NH₄⁺-N after each ultrasound irradiation and after 24 hours of fermentation at 37°C. No addition of inoculum was required for the fermentation stage to occur. Values for “NH₄⁺-N” in this manuscript represent the nitrogen concentration measured as the NH₄⁺ form. Total COD was determined as complementary information.

Total COD, soluble COD, NH₄⁺-N, total solids (TS), volatile solids (VS) and pH were determined following the Standard Methods for the Examination of Water and Wastewater (APHA 1998), as in a previous paper (Marañón *et al.* 2013). All analytical determinations were performed in triplicate.

All sludge samples were characterised on reception at the laboratory. In order to work with sludge as fresh as possible, each trial was completed before beginning a new test.

Thus each sample was taken on a different date from the others. Samples were kept in a refrigerator at 4°C for a maximum of 1 day before applying the treatment.

Ultrasound disruption was applied to three samples of secondary and mixed sludge and to four samples of primary sludge due to the differences found in its solid content. In line with previous research (Negral *et al.* 2013), five energy inputs were assayed per sludge sample: 3,500 (<1-4 minutes), 7,000 (2-7 min), 10,500 (3-13 min), 14,000 (4-20 min) and 21,000 kJ/kgTS (6-34 min). The specific energy (E_s) applied to the sludge was calculated as:

$$E_s \text{ [kJ/kgTS]} = P t / V TS_0$$

where P = ultrasound power, t = time of exposure of the sample to ultrasound, V = volume of the sample treated, and TS_0 = initial total solids

The temperature of the sludge sample was measured after ultrasonication. A 200 ml sludge aliquot was employed in every assay, always performing a blank test for the sake of comparison.

RESULTS AND DISCUSSION

The results in Table 1 show that the values of the typical physicochemical parameters fall within the usual range found for secondary and mixed sludge (Tchobanoglous *et al.* 2014). The greatest variability was found for primary sludge, even beyond the expected differences between the thickened samples and the unthickened sample (i.e. P1). This finding may be explained by two facts: the WWTP receives influent sewage from a combined sewer, and samples were taken in late spring/early summer, when rainfall is highly variable in the Atlantic climate region where the plant is located.

Tables 2-4 present the variations in tCOD and the sCOD/tCOD ratio with respect to their initial values after ultrasound disruption and after 24 hours of further fermentation at 37°C. As the interest in any treatment lies in the disintegration/solubilisation of the raw material (Barjenbruch & Kopplow 2003), Figure 1 shows the evolution in the concentrations of sCOD versus the applied E_s just after ultrasonication and subsequent fermentation.

One aspect to be taken into consideration in this treatment is the solids content of the sludge. Although the use of the E_s applied to the substrate enables normalisation of the sludge in terms of its solids content, this content is described as a key factor for optimising the treatment (Carrère *et al.* 2010; Pilli *et al.* 2011). The yield of ultrasound disruption is limited by the physical properties of the fluid to be treated. For instance, sludge containing high levels of solids supposes a problem, making ultrasonication of this concentrated fluid unfeasible. Ultrasonication of sludge with a high solids content is unsatisfactory because of the hindering effects of cavitation (Carrère *et al.* 2010). Moreover, the optimum solids content reported by Show *et al.* (2007) does not match

the optimum for hog manure found by Elbeshbishy *et al.* (2011). The latter authors reported that increasing TS up to 9% in hog manure was more effective than in primary and waste activated sludge. As the best E_s remains within a broad range strongly dependent on the substrate and its characteristics, the optimum solids content thus seems to be substrate specific (Show *et al.* 2007; Negral *et al.* 2013).

Primary sludge

sCOD can be seen to increase with increasing E_s (Figure 1). Three out of the four samples are seen to behave in a unique manner, presenting a more or less pronounced inflexion point in solubility after 3,500-7,000 kJ/kgTS. The decrease in solubility with higher energies may be explained by the combination and re-flocculation of the many substances that had been disintegrated (Bougrier *et al.* 2005; Naddeo *et al.* 2009). A further increase in the applied energy may cause these aggregates to re-solubilise, thus obtaining an asymptotic value. The asymptote appeared without the characteristic inflexion point in the unthickened sample (P1), which had a lower particulate matter content to solubilise (< 2% TS). Different authors have observed this asymptotic value with other treatments (Naddeo *et al.* 2009; Uma Rani *et al.* 2012b).

Table 2. Increases in tCOD with respect to initial values and evolution of the sCOD/tCOD ratio after ultrasound treatment and subsequent fermentation of primary sludge

Sample	Treatment	Percentage increase with respect to initial tCOD						
		kJ/kgTS	Untreated	3,500	7,000	10,500	14,000	21,000
P1	Ultrasonication		0	33	37	45	45	45
	+Fermentation		25	48	33	43	40	41
P2	Ultrasonication		0	15	19	29	25	30
	+Fermentation		-4	15	16	14	18	22
P3	Ultrasonication		0	50	61	47	34	61
	+Fermentation		32	43	57	56	54	58
P4	Ultrasonication		0	-3	15	77	13	49
	+Fermentation		-31	18	6	48	-2	43

Sample	Treatment	Evolution of sCOD/tCOD						
		kJ/kgTS	Untreated	3,500	7,000	10,500	14,000	21,000
P1	Ultrasonication		0.06	0.14	0.19	0.21	0.23	0.24
	+Fermentation		0.10	0.11	0.18	0.15	0.15	0.03
P2	Ultrasonication		0.13	0.17	0.21	0.17	0.17	0.18
	+Fermentation		0.18	0.18	0.20	0.19	0.18	0.18
P3	Ultrasonication		0.02	0.06	0.06	0.07	0.07	0.06
	+Fermentation		0.09	0.12	0.11	0.12	0.13	0.12
P4	Ultrasonication		0.02	0.12	0.08	0.09	0.14	0.11
	+Fermentation		0.13	0.11	0.13	0.09	0.13	0.10

tCOD values increased when applying ultrasound (Table 2). This may be the result of water evaporation related to the thermal effect of ultrasound (Luste & Luostarinen 2011), caused by the production of radicals, shear forces and friction generated by the dissipation of ultrasound waves. Therefore, the more pronounced the ultrasound irradiation (i.e. the greater the energy applied), the higher the temperature reached by the treated sludge. The rise in temperature also reinforces the disintegration caused by the treatment (Uma Rani *et al.* 2014), but it also has another effect on COD: the more volatile organic matter may be removed, as already reported by different researchers (Zhang *et al.* 2007; Naddeo *et al.* 2009; Erden & Filibeli 2010). This was a reasonable explanation, as an intense odour was perceived from the highly irradiated samples. The problem caused by water evaporation was solved using the sCOD/tCOD ratio, which counterbalances the loss in solvent in both the soluble and insoluble COD in a similar way (Luste & Luostarinen 2011). However, the effect of the selective removal of volatile components by ultrasonication leads to a decrease in this ratio.

The sCOD/tCOD ratio of the treated samples was higher for all the applied energies. Therefore, the losses in water and volatile substances did not hinder the obtaining of a much more soluble substrate. The variation in NH_4^+ -N concentration when applying ultrasound (Figure 2) followed a similar pattern to the COD, with the exception of sample P4, in which a decrease in NH_4^+ -N was observed regardless of the energy applied. Figure 1 shows that the most pronounced decrease in solubilised matter occurred for this sample, especially for a E_s of 7,000 kJ/kgTS, for which the lowest sCOD/tCOD ratio was obtained (0.08), as well as the maximum decrease in NH_4^+ -N. It is therefore highly likely that ammonium will be affected by re-aggregation of the solubilised matter.

Subsequent fermentation of the primary sludge after ultrasonication showed no clear pattern for sCOD. However, organic matter solubilisation appears to have continued during fermentation when no significant solubilisation was achieved by applying ultrasound. Similarly, if substantial solubilisation was achieved by applying ultrasound, the subsequent fermentation was accompanied by a certain degree of mineralisation of the organic matter.

What is unquestionable is that the effect of fermentation was much more influential in solubilising nitrogen than ultrasound was, regardless of the energy applied, as can be observed by the greater increase in NH_4^+ -N in the sample not treated by ultrasound. Biomolecules containing nitrogen (mainly proteins) were hence far less sensitive to ultrasound than to heating at 37°C for one day.

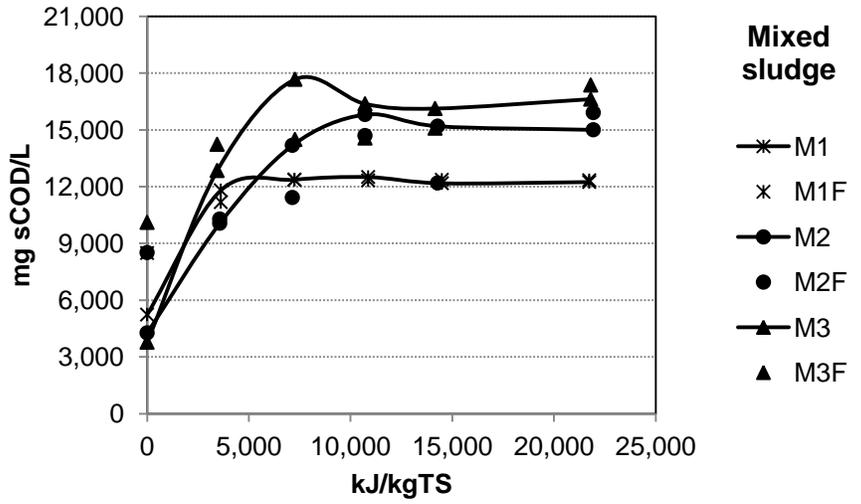
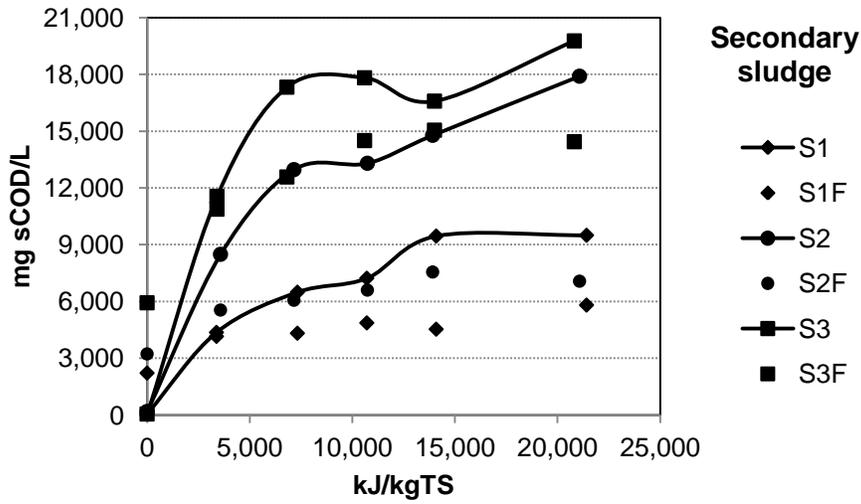
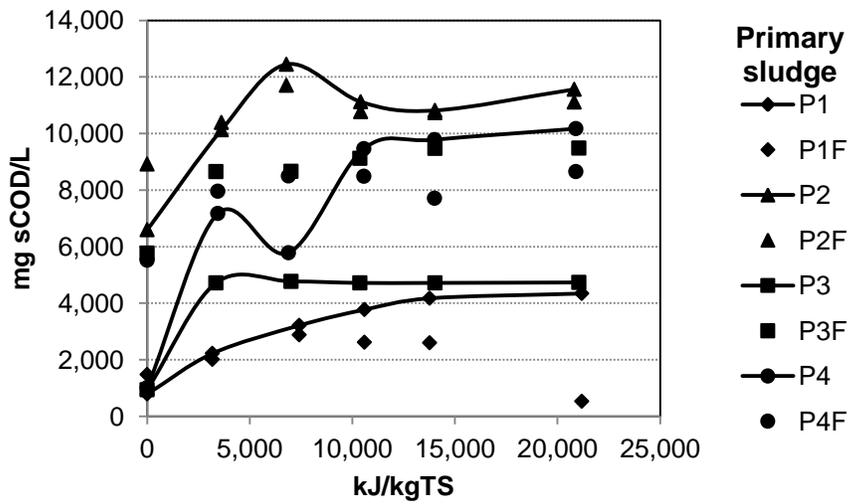


Figure 1. Variation in sCOD with specific energy. "F" denotes the subsequent fermentation (37°C, 24 hours) after ultrasound treatment

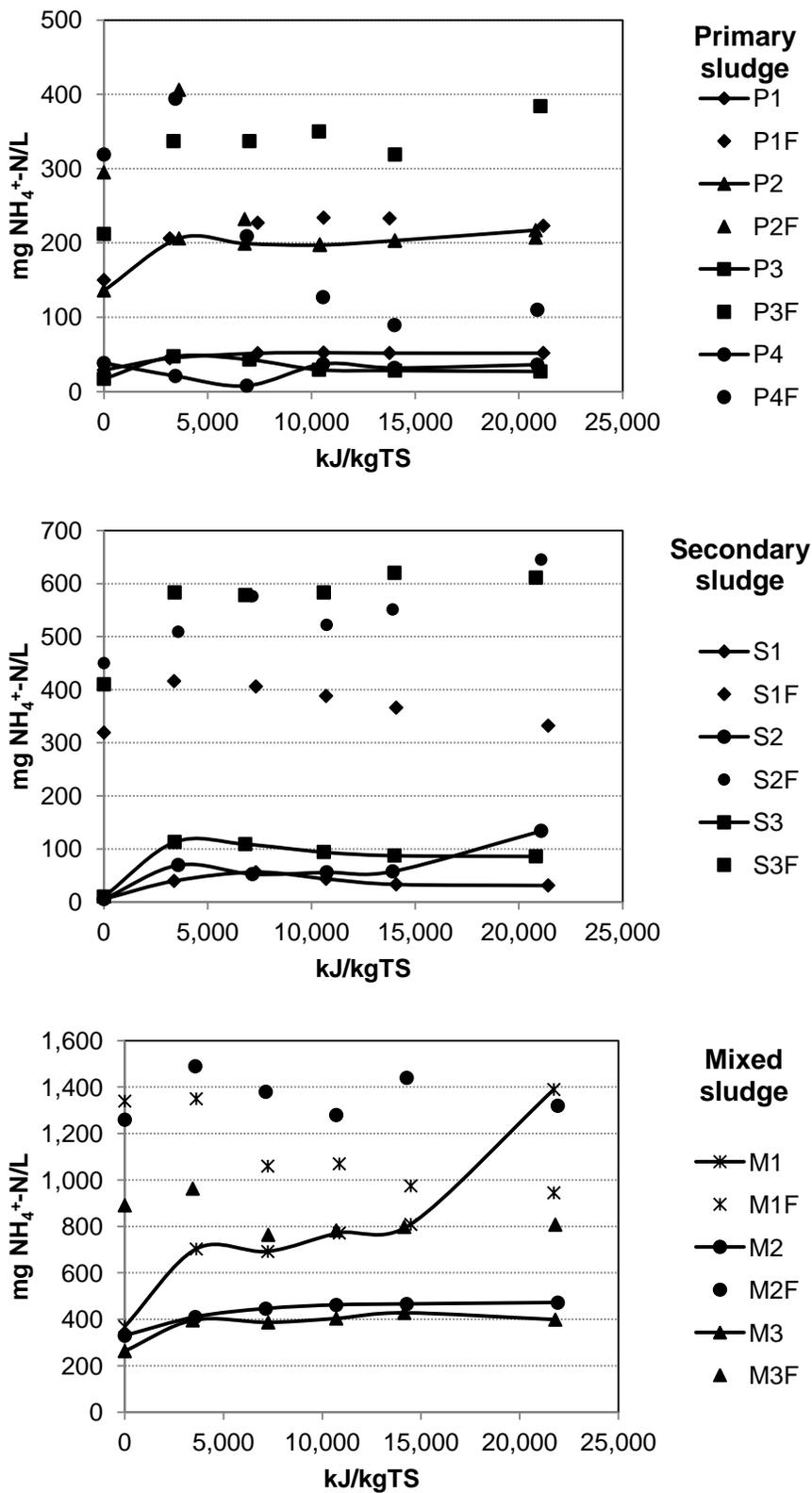


Figure 2. Variation in $\text{NH}_4^+\text{-N}$ with specific energy. "F" denotes the subsequent fermentation (37°C, 24 hours) after ultrasound treatment

Secondary sludge

It is precisely in this sludge where sCOD increased the most (Figure 1), exceeding 19,000% in sample S3 for a $E_s = 21,000$ kJ/kgTS. This is not surprising, as ultrasound disruption has been mainly applied to treat secondary sludge (Carrère *et al.* 2010). The inflexion point in solubilisation that had been observed for 3,500-7,000 kJ/kgTS in the case of primary sludge appeared at 10,500-14,000 kJ/kgTS for secondary sludge.

An increase in tCOD was observed in two samples, S2 and S3, when applying ultrasound due to water evaporation (Table 3). However, tCOD decreased in sample S1 after the treatment. Bearing in mind that the tCOD/VS ratio in this sample was the lowest in all the secondary sludge samples (1.14) and the evolution of its sCOD/tCOD ratio always presented considerably higher values than those observed in the other samples, some volatile compounds may have been removed. This is in line with the slight reduction in the mass of dry solids reported by Uma Rani *et al.* (2014).

Table 3. Increases in tCOD with respect to initial values and evolution of the sCOD/tCOD ratio after ultrasound treatment and subsequent fermentation of secondary sludge

Sample	Treatment kJ/kgTS	Percentage increase with respect to initial tCOD					
		Untreated	3,500	7,000	10,500	14,000	21,000
S1	Ultrasonication	0	-33	-31	-25	-21	-20
	+Fermentation	-35	-35	-36	-36	-36	-37
S2	Ultrasonication	0	18	38	28	45	33
	+Fermentation	-40	-39	-39	-38	-38	-51
S3	Ultrasonication	0	61	68	71	34	59
	+Fermentation	-25	18	23	18	4	34

Sample	Treatment kJ/kgTS	Evolution of sCOD/tCOD					
		Untreated	3,500	7,000	10,500	14,000	21,000
S1	Ultrasonication	0.00	0.26	0.38	0.38	0.48	0.47
	+Fermentation	0.14	0.25	0.27	0.30	0.29	0.37
S2	Ultrasonication	0.00	0.17	0.22	0.25	0.24	0.32
	+Fermentation	0.13	0.22	0.24	0.25	0.29	0.34
S3	Ultrasonication	0.00	0.18	0.26	0.26	0.31	0.32
	+Fermentation	0.20	0.24	0.26	0.31	0.37	0.27

Secondary sludge also showed the highest improvements in terms of the hydrolysis of nitrogen compounds. Similar behaviour had already been reported elsewhere (Mao *et al.* 2004). This parameter does not seem to follow any clear pattern when the E_s is increased. The highest concentrations of $\text{NH}_4^+\text{-N}$ were obtained for 3,500, 7,000 and 21,000 kJ/kgTS. This variability could suggest that the re-aggregation of soluble substances also influenced the $\text{NH}_4^+\text{-N}$ concentration for intermediate values of E_s .

The fermentation stage resulted in a reduction in sCOD and tCOD due to mineralisation of the organic matter. This decrease in sCOD was more pronounced when the sCOD following ultrasonication was higher. It may thus be concluded that the higher the sCOD in the ultrasonication stage, the faster the removal of sCOD in the fermentation stage. The upgrade in sCOD achieved in the untreated sample due to fermentation was once again far from the levels obtained in the samples treated by ultrasound disruption. Similar to the other types of sludge, the subsequent fermentation produced a much greater increase in $\text{NH}_4^+\text{-N}$ than ultrasonication.

Mixed sludge

The application of ultrasound produced an increase in sCOD from 125% to 340% (Figure 1). The lowest increase was observed for the lowest energy input (3,500 kJ/kgTS), whereas no marked differences were obtained for the rest of the E_s in the 7,000-21,000 kJ/kgTS range.

An increase in tCOD was observed (Table 4), being more pronounced for the highest E_s due to the increase in temperature observed in the sample, which led to water evaporation. The rapid effect of sonication on sCOD is revealed in the sCOD/tCOD ratio. The energy inputs led to a two- to three-fold increase in this ratio compared to its initial value.

Table 4. Increases in tCOD and $\text{NH}_4^+\text{-N}$ with respect to initial values and evolution of the sCOD/tCOD ratio after ultrasound treatment and subsequent fermentation of mixed sludge

Sample	Treatment kJ/kgTS	Percentage increase with respect to initial tCOD					
		Untreated	3,500	7,000	10,500	14,000	21,000
M1	Ultrasonication	0	-1	9	8	24	16
	+Fermentation	-12	1	6	10	2	7
M2	Ultrasonication	0	6	11	22	17	21
	+Fermentation	-2	-4	-2	-4	17	-3
M3	Ultrasonication	0	26	27	32	28	40
	+Fermentation	12	14	21	29	25	21

Sample	Treatment kJ/kgTS	Evolution of sCOD/tCOD					
		Untreated	3,500	7,000	10,500	14,000	21,000
M1	Ultrasonication	0.11	0.25	0.24	0.24	0.20	0.22
	+Fermentation	0.20	0.23	0.24	0.23	0.25	0.24
M2	Ultrasonication	0.10	0.23	0.31	0.32	0.32	0.30
	+Fermentation	0.21	0.26	0.29	0.38	0.26	0.40
M3	Ultrasonication	0.08	0.23	0.31	0.27	0.28	0.26
	+Fermentation	0.20	0.28	0.26	0.25	0.27	0.32

The application of ultrasound increased the content in $\text{NH}_4^+\text{-N}$. The energy input did not seem to have a strong effect on this parameter; a higher increase only being observed in sample M1 (up to 277%) when applying the highest E_s . The subsequent fermentation generally produced a reduction in sCOD and tCOD due to losses in CO_2 , and a further increase in $\text{NH}_4^+\text{-N}$.

The sCOD/tCOD ratio experienced slight variations after fermentation depending on the E_s ; however, the variation reached a maximum for the highest E_s . This ratio was always higher than that obtained only by fermentation of the untreated sludge sample. The ratio increased approximately two-fold when fermenting the untreated sample compared to its initial value.

Summarising the results for the different sludge samples, it can be stated that the inflexion point in sCOD was observed in all three types of samples. Only in samples with low solids content and therefore low levels of organic matter to solubilise was this inflexion point barely appreciated. It was observed at lower energies for primary sludge than for secondary sludge, which would explain why the inflexion point was sometimes barely observed in mixed sludge.

CONCLUSIONS

The aim of this study was to obtain the optimum solubilisation of primary, secondary and mixed sludge after ultrasound irradiation (3,500-21,000 kJ/kgTS) and a subsequent fermentation stage (24 hours, 37°C). No addition of inoculum was required for the fermentation stage to occur. Consequently, microbial abatement by the applied energies did not inhibit bacterial activity in primary, secondary or mixed sludge.

An inflexion point in solubility was detected when applying ultrasound at different specific energies depending on the type of sludge (after 3,500-7,000 kJ/kgTS in primary sludge and after 10,500-14,000 kJ/kgTS in secondary sludge). This was not so clearly observed in mixed sludge, the samples of which showed only slight variations in sCOD from 10,500 kJ/kgTS onwards.

Some variations in tCOD during ultrasound treatment were due to the effect of temperature and removal of volatile compounds.

The effect of the treatment (both on sCOD and $\text{NH}_4^+\text{-N}$) was clearly more pronounced when applied to secondary sludge than to the other types of sludge.

Temperature effects influenced the increases in $\text{NH}_4^+\text{-N}$ much more than ultrasound.

Reductions in sCOD and $\text{NH}_4^+\text{-N}$ were sometimes observed after fermentation. These may be due to metabolic removal of organic compounds and nitrogen ammonium and were more pronounced in mixed sludge.

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