ENHANCING BIOGAS PRODUCTION WITH COMBINED ULTRASOUND AND ENZYMATIC HYDROLYSIS TREATMENT

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Abstract

One of the aims of the "END-O-SLUDG" EU FP7 project focuses on the pre-treatment of sludge to enhance biogas production in anaerobic digestion. Different pre-treatments were studied within this context. This paper presents the results of applying ultrasound cell disruption (24 kHz, 3500 – 20000 kJ/kg TS) and Enzymatic Hydrolysis (42°C, 48 hours) as single treatments and in combination. Tests were performed on primary, secondary and combined sludge from two wastewater treatment plants. Each pre-treatment was followed by 24 hours of fermentation at 37°C to monitor the short-term improvement in performance. The results are discussed in terms of the variations in soluble chemical oxygen demand (sCOD) and ammonia. An increase in sCOD is interpreted as enhanced conversion of the organic load and hence greater energy recovery since the sCOD provides the carbon source for the microorganisms that produce biogas. As this parameter increases the level of recalcitrant compounds is also reduced accordingly as a consequence of the pre-treatments prior to sludge digestion. Ammonia is another parameter considered an indicator of biomass degradation, particularly of proteins.

Keywords

Sewage sludge, ultrasound, enzymatic hydrolysis, COD, ammonium, anaerobic digestion

Introduction

Methane production occurs in nature as a result of the degradation of organic matter by anaerobic microorganisms. This process may be intensified and biogas collected when it takes place in chemical reactors (Duda and Oliveira 2009; Sperling and Oliveira 2009; Castrillón *et al.* 2011). Depending on the operational conditions of the reactor, there is a list of parameters such as temperature, acidity, alkalinity or the substrate type that need to be controlled to favour the bacterial metabolism enhancing biogas production (Barbosa Correa *et al.* 2003). The theoretical methane yield reaches its maximum with 0.35 l per gram of degraded COD (Wu *et al.* 1993). A variety of strategies may thus be developed to improve the biodegradability of a substrate and achieve this target value. An upgrade in the methane yield may be achieved either by selecting a culture of microorganisms (e.g. operating under thermophilic rather than mesophilic conditions) or by pre-treating the substrate prior introducing it into the reactor. Chemical, biochemical (e.g. enzymatic hydrolysis intensification) and mechanical (e.g. ultrasound disruption) techniques are used to condition the feed (Bougrier *et al.* 2006; Bruni et al. 2010).

An idea of the rapid availability of a substrate to microorganisms is given by the sCOD. The sCOD reports about organic matter which can be easily withdrawn from the environment by bacterial blankets. Nevertheless, many raw substrates present total COD values much higher than sCOD. This situation would seem to indicate that these substrates have a practical limitation. This particular case refers to the sludge from wastewater treatment plants (WTP). Sludge is a biological waste mostly made up of bacteria whose cell walls avoid the release of nutrients to the environment and, consequently, the feed cannot be withdrawn by biogas producers in reactors. This approach is managed by quick tests that check the increase in sCOD after applying a pre-treatment to the feed. The sCOD is measured just after the pre-treatment and one day later so as to monitor the short-term evolution under anaerobic conditions and the same temperature as in the potential reactor. Although the principal concern of the pre-treatments applied to substrates rich in bacterial walls is to break up these structures, ammonium nitrogen (NH4-N) gives an idea of the degree of protein degradation (Broderick 1987; APHA 1998). These

tests are not the traditional biodegradability tests monitoring biogas production; however, parameters related to biogas production are controlled, while saving both time and costs. This concept has been employed here and the results of pre-treated sludge from two WTP are reported. The tested pre-treatments comprise ultrasound disruption, enzymatic hydrolysis (EH) through the novel technique of Inverted Phase Fermentation® (IPF) and combinations of these techniques.

Experimental procedure

Materials

Experiments were carried out with fresh sludge (which was always pre-treated in under 24 hours after collection) from two WTP in Spain:

WTP A is characterized by an average flowrate of 3210 m^3/h, treating wastewater from 450,000 population equivalents, the majority of which is of industrial origin. It only produces one type of sludge, which comes from biological treatment without previous primary settlement, the SRT is very short (around one day), and the removal efficiency is >75% SS and >65% BOD_5. Ferric chloride (100-300 mg/L of sludge) is sometimes added to improve settling. All tests with the sludge from this WTP were performed with this sludge type prior to conditioning in order to be dewatered in a centrifuge.

WTP B is characterized by an average flowrate of 900 m^3/h, treating wastewater from 85,000 population equivalents. Three types of sludge are produced here: primary (thickened by gravity), secondary (thickened by flotation) and combined sludge (a mixture of primary and secondary sludge), and the removal efficiency is >87% SS and >97% BOD. The tests were carried out with primary, secondary and combined sludge from this WTP, prior to the addition of coagulants in order to be dehydrated in a filter press.

Equipment and analytical methods

The ultrasound disruption apparatus was a Hielscher UP400Se. This system operates at 24 kHz, with a maximum power of 400 W, pulse adjustable to 0-100%, an H22 sonotrode made of titanium, tip diameter 22 mm, and is capable of treating up to 2 litres in each run.

EH is achieved by the novel technique IPF, which requires the incubation of the sludge samples at 42°C for 48 hours.

The sCOD was determined following Method 5222 (closed reflux colorimetric method) of the Standard Methods for the Examination of Water and Wastewater (APHA 1998) on a Perkin Elmer Lambda 35 Visible-UV system. Each sample was centrifuged (3500 rpm, 15 minutes) and filtered with 1.2 µm pore filter paper (Le *et al.* 2008).

NH4-N was determined using an Orion 95-12 selective electrode for ammonium. TS were determined following Method 2540B of the Standard Methods for the Examination of Water and Wastewater (APHA 1998). All analytical determinations were performed in triplicate.

Methods

All sludge samples were characterized on reception at the laboratory: soluble COD, NH4-N and Total Solids being determined. Samples were kept in a fridge at 4°C until application of the pre-treatment.

Ultrasound disruption was applied to: 3 samples of the sludge from WTP A; and to 4 samples of primary sludge (with marked differences in the solid content, as a result of the different thickening yields), 3 samples of secondary sludge and 3 samples of combined sludge from the WTP B. Five energy inputs per sludge were monitored: 3500 (<1-3 minutes), 7000 (1-7 min), 10500 (2-14 min), 14000 (3-21 min) and 21000 kJ/kgTS (5-37 min). Minor deviations from these values were observed due to different TS in the initial sludge samples and equipment sensitivity. A 200ml sludge aliquot was employed in every assay. A 200ml aliquot without sonication was assumed as blank for these tests. sCOD and NH4-N were determined just after sonication. The recipients containing the samples were then sealed and flushed with N_2 to remove the air present in the container, thus achieving anaerobic conditions over the subsequent 24 hours of fermentation.

EH was performed with: 3 samples of the sludge from WTP A; and 4 samples of primary sludge (with marked differences in the solid content), 3 samples of secondary sludge and 3 samples of combined sludge from the WTP B. EH was achieved by promoting endogenous enzymes in the sludge. This process is based on the Inverted Phase Fermentation (IPF) technique, which results in the separation of two phases from the raw sludge: the top layer known as the “solid phase” and the bottom layer known as the “liquid phase”. IPF requires a temperature of 42°C for 48 hours and anaerobic conditions (Le *et al.* 2008). Around 1 litre of fresh sludge was introduced in plastic bottles filled to the cap. An outlet hose was connected from the bottle to a beaker containing water to achieve anaerobic conditions and to collect part of the solid phase pushed by the incipient pressure into the bottle. Samples of each phase were taken after 48 hours to determine sCOD and NH4-N.

The combined pre-treatment was tested in two ways: first, applying ultrasound disruption and then EH (U+EH); and second, applying EH and then ultrasound disruption (EH+U) to the solid phase and also to a mixture of solid and liquid phases, maintaining the initial proportion of phases obtained after EH. The liquid phase did not undergo ultrasound disruption after EH because almost all the total COD is present as soluble COD. Ultrasound disruption followed by EH was applied to one sludge sample from the WTP A and one primary, secondary and combined sludge sample from WTP B. The EH+U pre-treatment was tested in one sludge sample from WTP A and one primary sludge sample from the WTP B. The energy input in all the combined pre-treatments was always around 7000 kJ/kgTS. This value was chosen in keeping with the results obtained in previous sonication tests. In all the combined pre-treatments, aliquots were taken after each stage of the combined pre-treatment and from each produced phase or mixture of phases. These aliquots were then analyzed to determine sCOD and NH4-N.

The monitoring of hydrolysis of all the different types of pre-treatments was prolonged 24 hours under fermentation at 37°C.

Results and discussion

Table 1 presents the sCOD, NH4-N and TS of the fresh sludge samples and the pre-treatments that were carried out. Great variability can be observed in the composition of sludge samples from WTP A. This variability was more limited in the different types of sludge samples from WTP B; an exception arose with the primary sludge samples, mainly due to the different level of thickening. As expected, the lowest sCOD and NH4-N were found for secondary sludge samples, which were mostly biological waste from the bacterial growth in the biological treatment of the WTP.

Table 1: Characterization of initial sludge samples and pre-treatments applied

Sample	CODs (mg/L)	NH4-N (mg/L)	TS (g/L)	Pre-treatments
A 31/07	4002	89	61.72	Ultrasound (U)
A 08/08	905	86	22.20	U
A 15/08	6164	119	33.44	U
A 28/03	7847	368	68.63	Enzymatic Hydrolysis (EH)
A 09/05	6159	424	54.67	EH
A 17/05	5313	280	47.49	EH
A 01/10	3969	147	40.35	U+EH; EH+U (Solid Phase & Mixture of phases)
PB 13/06	791	29	17.00	U
PB 12/09	6597	136	39.78	U
PB 11/07	940	17	59.03	U&EH
PB 18/07	1013	38	78.40	U&EH
PB 30/05	1482	51	83.08	EH
PB 06/06	542	36	26.27	EH
PB 05/09	4045	99	72.55	U+EH; EH+U (Solid Phase & Mixture of phases)
SB 11/07	56	6	31.94	U&EH
SB 25/07	185	5	45.27	U&EH
SB 08/08	51	10	47.55	U
SB 27/06	375	6	40.74	EH
SB 19/09	561	45	48.71	U+EH
CB 30/05	5236	369	39.76	U
CB 06/06	4270	330	35.29	U&EH
CB 13/06	3776	263	47.05	U
CB 16/05	5019	452	40.45	EH
CB 23/05	5050	450	36.43	EH
CB 26/09	7437	435	40.79	U+EH

PB, SB and CB= primary, secondary and combined sludge from WTP B, respectively

Ultrasound pre-treatment

The ultrasound disruption pre-treatment was carried out with the sludge samples shown in Tables 2a and 2b. Five different energy inputs were applied (3,500 – 21,000 kJ/kgTS). These tables present the percentage increase in sCOD and NH4-N with respect to the initial values in the fresh sludge samples.

In general, the higher the energy input, the higher the increase in sCOD. However, there was a notable gap when applying ≈ 7000 kJ/kgTS with respect to the lowest energy input (≈ 3500 kJ/kgTS). For energy inputs higher than 7000 kJ/kgTS, minor increases in sCOD were observed. Without a doubt, the best improvements in sCOD are for the secondary sludge in comparison to the others. This behaviour had already been reported elsewhere (Mao *et al.* 2004) and was expected bearing in mind the low sCOD and the characteristics of this type of sludge. No common pattern was observed in sCOD after subsequent fermentation of the sonicated samples.

On the basis of the results shown in Table 2b, a flawed test with the PB 18/07 sample can be suggested in the NH4-N determination. NH4-N decreased in this sample just after sonication, which is an unusual event with respect to the rest of the results. Nevertheless, this parameter increased again after further fermentation; this is somewhat in line with the increase in NH4-N after fermentation in the other types of sludge. It is worth noting that the NH4-N underwent a greater increase due to fermentation than when applying ultrasound disruption, except for some samples of sludge from WTP A and primary sludge from the WTP B.

Two relevant aspects regarding sonication pre-treatment should be considered. First, water evaporation during sonication (due to the temperature effect) may distort the effects of ultrasound pre-treatment. The sonication pre-treatment was coupled to an increase in temperature: the longer the sonication time, the higher the temperature reached (up to 98°C). The longest sonication times were in fact for the sludge samples with more TS in the fresh sludge. Second, another important issue to consider regarding these tests is that the sonicated samples were not inoculated after the pre-treatment, which could possibly be affecting the bacterial producers of biogas.

Figure 1 shows the variation observed in sCOD and NH4-N with ultrasound disruption pre-treatments. A common pattern was observed: the increase in sCOD after sonication was generally followed by a reduction after fermentation, while the increase in NH4-N after sonication continued after fermentation.

Table 2a: Increase with respect to initial sCOD (%) after ultrasound pre-treatment and subsequent fermentation

Sample	Increase with respect to initial CODs (%)					
kJ/kgTS	Untreat.	3500	7000	10500	14000	21000
A 31/07		242	261	253	289	245
+ferment.	183	94	347	325	254	255
A 08/08		464	532	554	590	519
+ferment.	409	700	822	880	933	927
A 15/08		81	119	123	104	99
+ferment.	33	76	84	101	70	66
PB 13/06		181	307	377	428	449
+ferment.	87	154	264	231	229	-33
PB 12/09		54	89	69	64	75
+ferment.	35	58	77	63	63	68
PB 11/07		402	408	402	402	404
+ferment.	514	821	822	871	908	909
PB 18/07		608	471	834	866	904
+ferment.	445	686	739	738	661	754
SB 11/07		7734	11545	12868	16871	16943
+ferment.	3876	7356	7653	8631	8036	10324
SB 25/07		4494	6916	7097	7896	9587
+ferment.	1646	2900	3180	3470	3988	3725
SB 08/08		22768	34184	35171	32729	39032
+ferment.	11634	21426	24794	28601	29693	28483
CB 30/05		125	136	139	133	134
+ferment.	62	113	136	135	136	136
CB 06/06		136	232	270	256	251
+ferment.	99	141	168	244	186	273
CB 13/06		240	368	334	327	340
+ferment.	168	277	284	286	300	360

Untreat.= untreated sludge, without ultrasound disruption

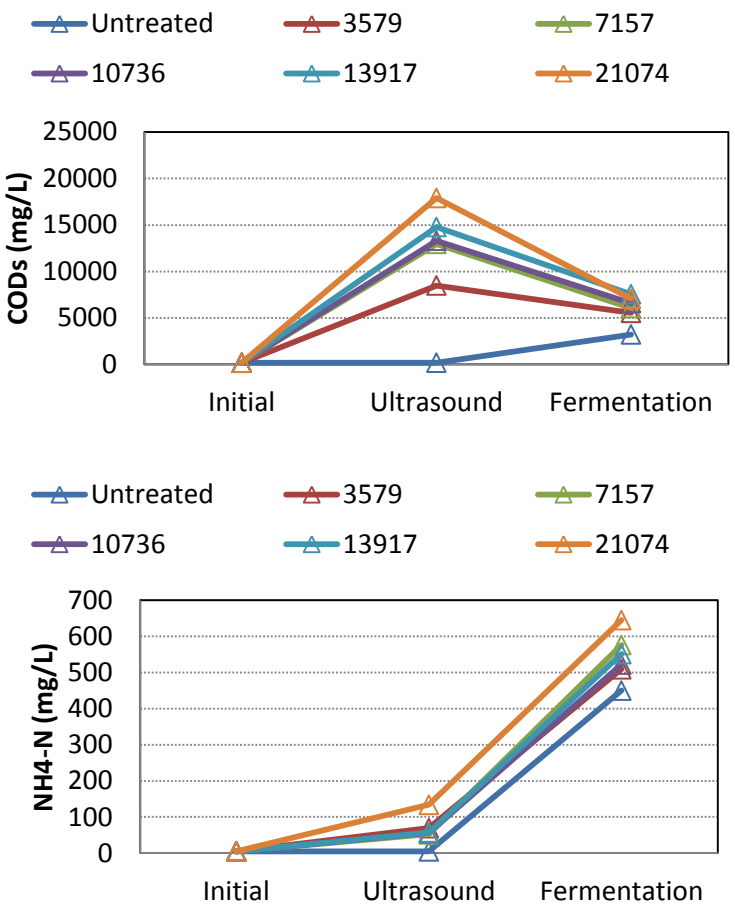

Figure 1: Evolution of CODs and NH4-N by ultrasound pre-treatment applied to the secondary sludge sample SB 25/07 from the WTP B (the numbers in legends indicate the energy input applied in kJ/kgTS)

Table 3 shows the sCOD and NH4-N results of the IPF and subsequent fermentation in the sludge samples in terms of the percentage increase with respect to the initial values in the fresh sludge samples. EH always increased the sCOD and NH4-N in both the solid and liquid phase.

For sludge from the WTP A, sCOD and NH4-N always decreased in the solid phase after fermentation, but no common pattern was found in the liquid phase. For primary sludge from the WTP B, fermentation led to different results (an increase or decrease in sCOD and NH4-N). This fact might be related to the diversity of the primary sludge samples. For secondary sludge from WTP B, sCOD always decreased in the solid phase and increased in the liquid phase after fermentation. For combined sludge from the WTP B, both sCOD and NH4-H generally decreased in both phases after fermentation.

As expected (Le *et al.* 2008), sCOD and NH4-H were more concentrated in the solid phase than in the liquid phase. This occurred just after the EH pre-treatment and after the subsequent fermentation.

Table 2b: Increase with respect to initial NH4-N (%) after ultrasound pre-treatment and subsequent fermentation

Sample	Increase with respect to initial NH4-N (%)					
kJ/kgTS	Untreat.	3500	7000	10500	14000	21000
A 31/07		496	180	426	386	276
+ferment.	486	526	636	576	548	479
A 08/08		55	51	48	29	24
+ferment.	86	327	310	243	404	340
A 15/08		13	45	45	32	35
+ferment.	76	241	92	79	24	31
PB 13/06		56	79	82	80	80
+ferment.	421	615	688	713	709	674
PB 12/09		51	46	45	49	60
+ferment.	117	199	71	46	49	52
PB 11/07		176	150	70	64	57
+ferment.	1133	1859	1859	1935	1755	2133
PB 18/07		-45	-80	-3	-17	-6
+ferment.	732	928	445	231	133	187
SB 11/07		589	877	657	476	441
+ferment.	5467	7160	6986	6671	6287	5694
SB 25/07		1222	897	962	994	2449
+ferment.	8461	9583	10858	9830	10382	12170
SB 08/08		1029	989	839	773	759
+ferment.	3996	5724	5674	5724	6094	6004
CB 30/05		91	88	109	119	277
+ferment.	263	266	187	190	164	156
CB 06/06		24	35	40	41	43
+ferment.	282	352	318	288	336	300
CB 13/06		50	47	53	63	52
+ferment.	238	266	190	197	203	207

Untreat.= untreated sludge, without ultrasound disruption

Enzymatic hydrolysis pre-treatment (Inverted Phase Fermentation, IPF)

Table 3: Increase with respect to initial CODs and NH4-N (%) after enzymatic hydrolysis pre-treatment and subsequent fermentation

Sample	Increase with respect to initial CODs (%)		Increase with respect to initial NH4-N (%)	
Phase	Liquid	Solid	Liquid	Solid
A 28/03	37	178	23	199
+ferment.	35	39	60	170
A 09/05	36	134	41	265
+ferment.	53	67	85	118
A 17/05	38	232	27	324
+ferment.	61	149	70	218
PB 11/07	405	1147	1148	6474
+ferment.	631	1670	2631	8111
PB 18/07	548	1473	622	1723
+ferment.	577	1595	1465	2087
PB 30/05	298	617	552	2188
+ferment.	411	553	1358	1758
PB 06/06	442	1798	934	3731
+ferment.	460	947	711	1095
SB 11/07	5140	21337	3838	15914
+ferment.	6164	10016	5927	8875
SB 25/07	4073	8315	12278	24656
+ferment.	6401	7891	11631	24914
SB 27/06	2134	5109	11234	29806
+ferment.	2396	2908	13522	18220
CB 06/06	137	136	305	446
+ferment.	133	140	229	248
CB 16/05	130	255	181	301
+ferment.	108	135	128	155
CB 23/05	109	261	133	312
+ferment.	91	111	114	162

Figure 2 shows the evolution of sCOD in sludge samples from the WTP A which underwent EH pre-treatment by applying IPF. For these sludge samples, the behaviour of NH4-N ran parallel to that of the sCOD.

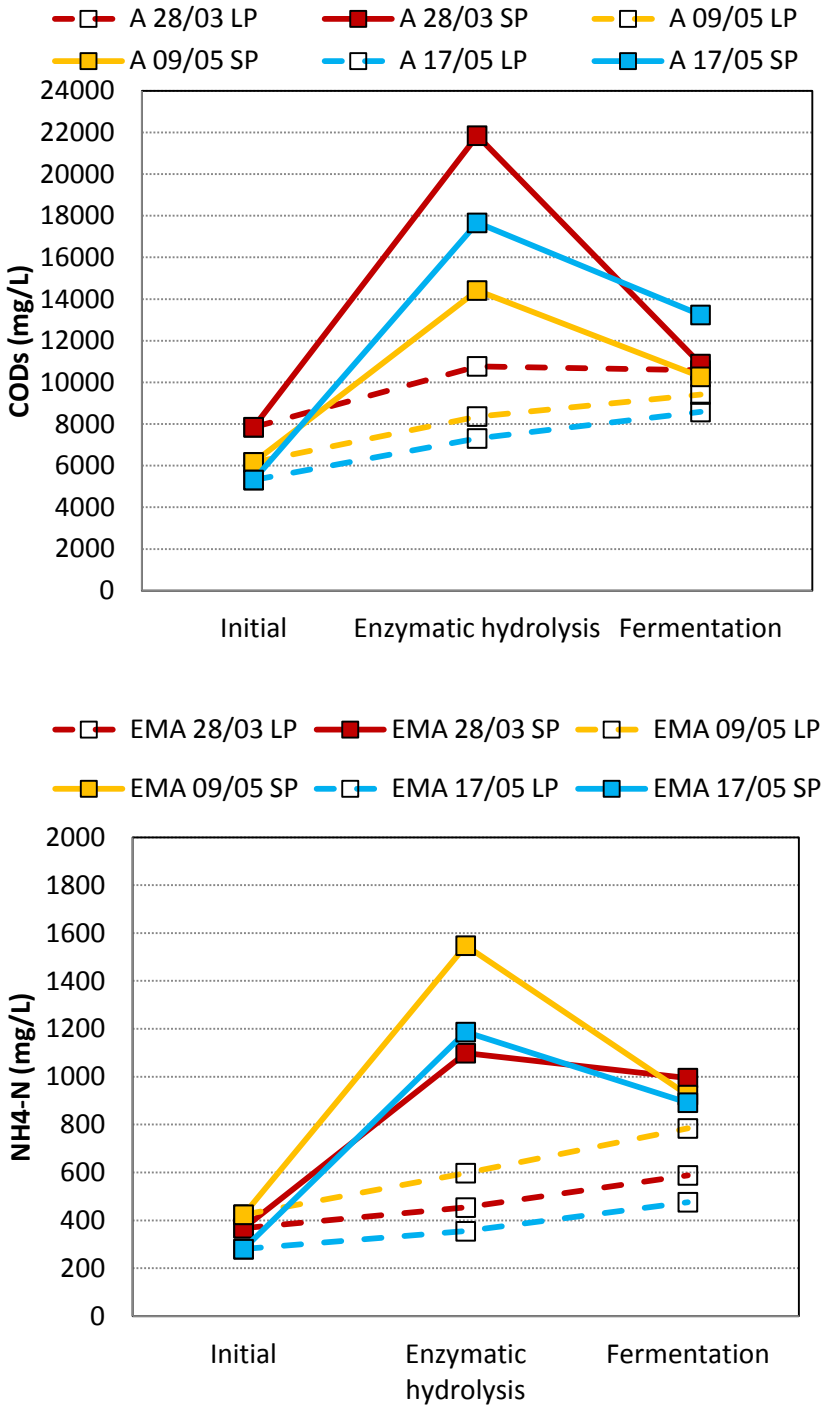

Figure 2: Evolution of CODs and NH4-N by enzyme action pre-treatment applied to the sludge samples from WTP A

Combined pre-treatments: U+EH and EH+U

Tables 4a and 4b present the sCOD and NH4-N results (expressed as the percentage increase with respect to the initial values) of the combined pre-treatments. The energy input for these assays was always around 7000 kJ/kgTS, a value chosen after assessing the previous results from the sonication pre-treatments. Although higher energy inputs produced higher sCOD, the energy cost was balanced so as to make the final decision.

Only one sample per type of sludge was tested due to the restricted effect observed with the combined pre-treatments. The sonication of the solid phase was unsatisfactory as a result of the high values of TS that hindered the effects of cavitation. According to the literature, this was to be expected as the effects of sonication decrease with the increase in TS (Elbeshbishy *et al.* 2011; Pilli *et al.* 2011). Due to the high solid content, the effect of sonication was restricted to the sludge portion touching the tip of the sonicator. Moreover, the time needed for a given energy input was prolonged, thus increasing the temperature.

In order to solve the drawback of limited cavitation in the solid phase, a mixture of solid and liquid phases was sonicated. The mixture maintained the proportion of phases produced after EH. Nevertheless, the sonication of a mixture of phases was the opposite of that achieved by the IPF.

Furthermore, in the case of ultrasound treatment followed by EH, the two phases separation resulted in a top layer of “liquid phase” and a bottom layer of “solid phase”, the opposite behaviour to the usual. The current “solid phase” was less concentrated than in the solid phase resulting from a simple IPF. This anomalous behaviour of the IPF appeared in all the sludge samples with the exception of the primary sludge from WTP B.

sCOD increased slightly with the combined pre-treatments only in the case of the primary sludge from the WTP B. The sCOD of the other sludge samples decreased.

NH4-N increased notably with the two types of combined pre-treatments. This behaviour was observed at the end of the pre-treatments and after the subsequent fermentation; in other words, a thermal influence was always observed. This fact would be in keeping with the premise idea of the variable influence of ultrasound disruption on protein degradation, as this depends on the form that the proteins are present in the sonicated fluid (Elbeshbishy *et al.* 2011).

Table 4a: Increase with respect to initial CODs (%) after combined pre-treatments and subsequent fermentation (+ferment.): ultrasound pre-treatment followed by enzymatic hydrolysis (U+EH) and enzymatic hydrolysis followed by ultrasound pre-treatment of the solid phase and a mixture of the solid and liquid phases (EH+U)

Sample	Increase with respect to initial CODs (%)							
Pre-treatment	U	U+EH		EH			EH+U	
Phase		LP	SP	LP	SP	Mix.	SP	Mix.
A 01/10	285	186	350	84	346	156	461	346
+ferment.		201	337				385	296
PB 05/09	177	246	272	103	479	228	552	282
+ferment.		323	323				585	294
SB 19/09	3601	2545	2999					
+ferment.		2252	2911					
CB 26/09	136	94	128					
+ferment.		87	127					

Table 4b: Increase with respect to initial NH4-N (%) after combined pre-treatments and subsequent fermentation (+ferment.): ultrasound pre-treatment followed by enzymatic hydrolysis (U+EH) and enzymatic hydrolysis followed by ultrasound pre-treatment of the solid phase and a mixture of the solid and liquid phases (EH+U)

Sample	Increase with respect to initial NH4-N (%)							
Pre-treatment	U	U+EH		EH			EH+U	
Phase		LP	SP	LP	SP	Mix.	SP	Mix.
A 01/10	21	140	173	139	567	241	639	272
+ferment.		175	207				696	377
PB 05/09	170	509	459	282	1440	651	1918	836
+ferment.		519	466				1784	671
SB 19/09	159	1262	2207					
+ferment.		1390	2538					
CB 26/09	2	40	103					
+ferment.		98	154					

Figure 3 shows the sCOD and NH4-N evolution of sludge samples from the WTP A which underwent the combined pre-treatments.

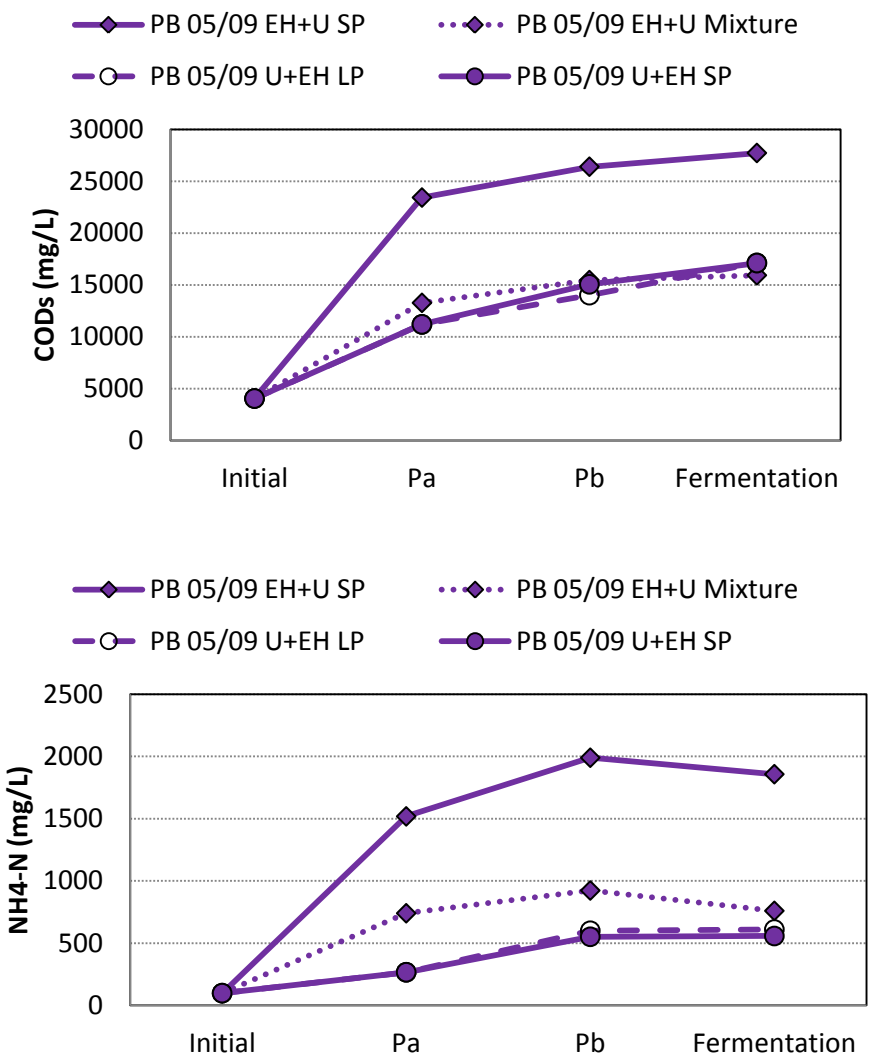

Figure 3: Evolution of sCOD and NH4-N with combinations of pre-treatments applied to the primary sludge sample PB 05/09 from the WTP B (Pa denotes the first pre-treatment and Pb the subsequent pre-treatment).

Conclusions

When ultrasound disruption and EH pre-treatments are compared, it may be concluded that:

1. The ultrasound disruption pre-treatment produced a lesser effect than EH in primary and secondary sludge samples; however, the difference was only slight in the combined sludge samples from WTP B. On the other hand, the ultrasound disruption pre-treatment had a greater influence than EH in the sludge samples from WTP A, which treats a high load of industrial wastewater.
2. In terms of sCOD and NH4-N, the effect of any pre-treatment was clearly more pronounced when applied to the secondary sludge in comparison to the others.

3. Decreases in sCOD and NH4-N were sometimes observed after fermentation. This might be explained by a the removal of organic compounds and ammonia. When this decrease was not noticed, this might mean that the pre-treatment did not produce enough hydrolysis and, consequently, hydrolysis continued during the subsequent fermentation.
4. From the results obtained, the combined treatments did not suppose an advantage with respect to the single treatments.

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