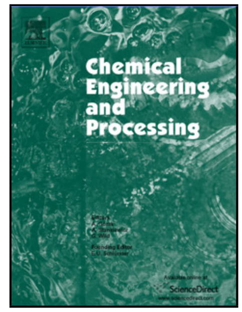

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SHORT TERM EVOLUTION OF SOLUBLE COD AND AMMONIUM IN PRE-TREATED SEWAGE SLUDGE BY ULTRASOUND AND INVERTED PHASE FERMENTATION

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Highlights

NH4-N and sCOD were indicator parameters of hydrolysis (short time evolution)

Ultrasound produced a greater increase in sCOD than IPF for sewage sludge

The optimum ultrasound energy input was around 7000 kJ/kgTS for sewage sludge

Combined pre-treatments did not suppose an advantage

Next research would cover more substrates, BMP and the suitability of these tests

Abstract

Ultrasonication, enzymic hydrolysis and combinations of both pre-treatments were applied to sewage sludge with the aim of enhancing biogas production in the anaerobic treatment. Short-term monitoring of soluble COD (sCOD) and NH4-N (by keeping the pre-treated substrate under anaerobic conditions) was used to compare the pre-treatments. Five ultrasound energy inputs were applied: 3500, 7000, 10500, 14000 and 21000 kJ/kgTS. Enzymic hydrolysis was achieved by promoting endogenous enzyme actions through the incubation of sludge (at 42ºC over 48 hours), resulting in a solid phase (top) and a liquid phase (bottom), in a process known as Inverted Phase Fermentation (IPF). Ultrasonication produced a greater increase in sCOD (up to 532% for 7000 kJ/kgTS) than IPF (up to 324%). When applying both pre-treatments, if ultrasonication was applied first, sludge settlement occurred instead of the usual phase inversion that occurred when IPF was applied alone. When IPF was applied first, ultrasonication was only applied to the solid phase, as it was not necessary to apply it to the liquid phase on account of its high soluble organic matter. However, ultrasonication was not effective when applied to the solid

phase, due to its high solid content. NH_4-N increase was notable in all instances of pre-treatments.

Keywords: Sewage sludge, ultrasound, inverted phase fermentation, COD, ammonium, anaerobic digestion

ABBREVIATIONS

IPF = inverted phase fermentation

sCOD = soluble chemical oxygen demand

tCOD = total Chemical oxygen demand

TS = total solids

VS = volatile solids

1. INTRODUCTION

Substrate biodegradability tests assess biogas production but are very time-consuming. Keeping records of the volume and methane composition of the biogas during the test, analysis of the digestate enables the quantification of biodegradation with respect to the initial parameters in the raw substrate. The efficiency of the biodegradation process is usually measured by the methane yield and organic matter degradation. The theoretical methane yield reaches its maximum at 0.35 litres (273ºK, 101 kPa) per gram of degraded COD [1]. A variety of strategies may thus be developed to improve the biodegradability of some substrates and thus achieve this target value.

Biogas production from a substrate, such as sewage sludge, consists of several stages in which specific micro-organisms are involved in every step of the process. The first and rate-limiting stage is hydrolysis [2, 3, 4]. During this initial stage, the macromolecules, cellular structures and organelles are broken up and transformed into monomers and low molecular weight chemical species, ready to be taken from the environment by bacteria. After hydrolysis, and once the nutrients are bioavailable, biochemical reactions continue among bacterial communities following the well-known sequence: acidogenesis, acetogenesis and methanogenesis [3, 5]. As hydrolysis slows down the entire process, the characteristics of the substrate are crucial when designing a strategy for accelerating biogas production in an anaerobic reactor. Apart from co-digestion, 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 to introducing it into the reactor [6]. Among the most widely employed pre-treatments for upgrading hydrolysis, mechanical (e.g. ultrasonication), thermal, chemical (e.g. acids and bases) and biochemical (e.g. enzymes) are used prior to introducing the substrate into an anaerobic digestor [7, 8]. These pre-treatments will be capable of destroying the floccules in the sludge and hence the cell walls so that the cytoplasm is released into the environment. In summary, all pre-treatments destroy structures until achieving a marked upgrade in the nutrients feeding the acidogenic micro-organisms. This evolution may be observed chemically through an increase in indicative parameters such as soluble chemical oxygen demand (sCOD), as an indicator of the hydrolysis of organic insoluble matter, and ammonium nitrogen (NH4-N), as a specific indicator of protein degradation [9, 10]. The sCOD reports on organic matter which can be easily withdrawn from the environment by bacterial blankets. However, many raw substrates present much higher total COD (tCOD) values than sCOD values. This situation would seem to indicate that these substrates have a practical limitation. This particular case refers to sewage sludge.

Although pre-treatments of sewage sludge are sometimes used before anaerobic digestion, economic issues frequently limit the implementation of a pre-treatment. Therefore, research into topics such as the enhanced efficiency of pre-treatments, suitability in terms of substrate type, and all innovation aimed at the final goal of maximum methane production at the lowest cost and in the shortest time is still a subject of major interest.

Inverted Phase Fermentation is a novel pre-treatment that enhances the endogenous enzymes in sewage sludge by keeping it under anaerobic conditions at 42ºC for 48 hours [11]. This pre-treatment produces an enzymic hydrolysis and a solid liquid separation is observed with a top layer enriched in solids, known as the “Solid Phase (SP)” and a clarified bottom layer, known as the “Liquid Phase (LP)”. This separation occurs due to the flotation of solids caused by the nascent bubbles in the heated sludge.

Short-term monitoring of sCOD and NH4-N (by keeping the pre-treated substrate under anaerobic conditions) may be used to compare the efficiency of the pre-treatments in terms of methane production potential. Sludge is a biological waste mainly made up of bacteria whose cell walls avoid the release of nutrients to the environment and, consequently, the feed cannot be removed 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 a potential reactor. These tests are not the traditional biodegradability tests that monitor biogas production; however, parameters related to biogas production are controlled, while achieving savings in both time and costs. In this paper, the effect on sCOD and NH4-N was compared just after the use of ultrasonication, after the IPF technique, or after a combination of both treatments. Furthermore, both parameters were also measured after 24 hours of further fermentation at 37ºC in order to monitor the short-term evolution of the sludge. In the experiments including IPF, the solid content was measured in the two separate phases obtained.

2. METHODS

2.1 Materials

Experiments were carried out with fresh sludge from the sewage treatment plant of an industrialized town, with an average flow rate of 3210 m^3/h. The wastewater undergoes a pre-treatment followed by a high rate activated sludge process (solid retention time of around one day). As there is no primary treatment, only one type of sludge is produced. Ferric chloride (100-300 mg/L of sludge) was sometimes added to improve settling in the secondary tank. All tests with the sludge were performed prior to the addition of a flocculant for dewatering.

2.2 Equipment employed and analytical methods

The ultrasonication apparatus 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, tip diameter 22 mm. The system is capable of treating up to 2 litres in each run.

Soluble COD was determined following Method 5220 (closed reflux colorimetric method) of the Standard Methods for the Examination of Water and Wastewater [9] on a Perkin Elmer Lambda 35 Visible-UV system. Samples were centrifuged (3500 rpm, 15 minutes) and filtered through 1.2 µm pore filter paper [11]. Total COD was determined without centrifuging or filtrating. NH4-N was determined using an Orion 95-12 selective electrode for ammonium. Total solids (TS) and volatile solids (VS) were determined following Method 2540 of the Standard Methods for the Examination of Water and Wastewater [9]. pH was determined with a Crison pH 25 pH-meter. All analytical determinations were performed in triplicate.

2.3 Methods

All sludge samples were characterized on reception at the laboratory. Samples were kept in a refrigerator at 4°C for a maximum of 1 day before applying the pre-treatment, whilst the sludge was being characterized.

Ultrasonication was applied to three samples of the sludge. Five energy inputs were monitored per sludge sample: 3500 (<1-3 minutes), 7000 (2-7 min), 10500 (3-14 min), 14000 (5-20 min) and 21000 kJ/kgTS (8-37 min). Minor deviations from these values were observed due to different TS in the initial sludge samples and equipment sensitivity. The specific energy (Es) applied to the sludge was calculated as:

$Es\ [kJ/kgTS] = P\ t / V\ TS_0$

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. sCOD and NH4-N were determined just after ultrasonication. 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. sCOD and NH4-N were analysed again after fermentation.

IPF was performed with three samples of the sludge, promoting endogenous enzymes at 42°C for 48 hours under anaerobic conditions [11]. Around 1 litre of fresh sludge was introduced into plastic bottles filled to the cap. An outlet hose was connected from the bottle to a beaker containing water to achieve anaerobic conditions. Samples of both separated phases (bottom liquid phase and upper solid phase) were taken after 48 hours to determine sCOD, NH4-N, TS and VS. After the subsequent fermentation of the two phases, sCOD, NH4-N, TS and VS were analysed once again.

The combined pre-treatment was tested in two ways: applying ultrasonication and then IPF (U+IPF), and applying IPF and then ultrasonication (IPF+U) to the solid phase. The liquid phase did not undergo ultrasonication after IPF because almost all the COD was present as sCOD. Both combined pre-treatments were tested in one sample of sludge. The energy input applied in the combined pre-treatments was around 7000 kJ/kgTS. This value was chosen in keeping with the results obtained in previous ultrasonication tests. In all the combined pre-treatments, aliquots were taken after each stage to determine sCOD, NH4-N, TS and VS.

3. RESULTS AND DISCUSSION

The composition of the sludge samples employed in the experiments is shown in Table 1. Great variability can be observed: the total solid content varies between 68.6 and 22.2 g/L, although volatile solids represent around 80% of the total solids (the values ranged between 75-84%). In accordance with the variability in solid content, the COD was also found to be highly variable for the different samples, presenting a maximum value of 91 g/L and a minimum of 18 g/L. As is usual in sewage sludge, the sCOD/tCOD ratio was very low (between 0.04-0.10). With the exception of sample 08/08, which is the sample with very low solid and COD content, the values of the tCOD/VS ratio ranged between 1.6 and 2.2.

3.1 Ultrasound pre-treatment

As already stated in Section 2.3, five different energy inputs were applied to the sludge (ranging between 3500 and 21000 kJ/kgTS). Table 2 presents the increase in sCOD and NH4-N with respect to the initial values in the fresh sludge samples and the evolution of the sCOD/tCOD ratio. The results are also represented in graphic form for the energy input of 7000 kJ/kgTS (Figures 1 and 2).

Water evaporation during ultrasonication (due to the temperature effect) may distort the effects of ultrasound pre-treatment [12]. Ultrasonication pre-treatment produces an increase in temperature: the longer the sonication time, the higher the temperature reached (up to 98°C). The longest ultrasonication times were, in fact, for the sludge samples with more TS in the fresh sludge. As temperature increased during ultrasonication, the sCOD/tCOD ratio may be a more accurate indication of the effect of the pre-treatment rather than the absolute values of sCOD. The reason is that a similar effect can be expected due to water evaporation in sCOD and tCOD. In short, the undesirable variation in tCOD caused by the side effect of the temperature would be assumed using this ratio. This procedure of using ratios has been already used elsewhere to overcome similar problems [12, 13, 14].

In general, the higher the energy input, the higher the increase in sCOD up to a specific energy applied of 14000 kJ/kgTS. However, the opposite effect was observed for the highest energy input (21000 kJ/kgTS). In the case of sample 15/08, this decrease began from 10500 kJ/kgTS on. Luste and Luostarinen [12], when applying ultrasound to dairy cattle slurry, observed that there was a threshold in Es (9000 kJ/kg TS) that, when surpassed, resulted in a decrease in the sCOD/VS ratio. A notable gap was observed in the sludge when applying ≈ 7000 kJ/kgTS with respect to the lowest energy input (≈ 3500 kJ/kgTS). For energy inputs higher than 7000 kJ/kgTS, increases in sCOD were not generally as pronounced as in the rest of the energy inputs. The highest upgrades in sCOD were achieved in sample 08/08, the most diluted sludge (2.2% TS), though the one with the highest VS/TS ratio.

Even at the lowest energy applied, ultrasound pre-treatment led to a higher increase than thermal treatment alone at 37ºC for 24 hours under anaerobic conditions (fermentation). Fermentation after ultrasonication increased the sCOD in the sample with the lowest COD and the lowest tCOD/VS ratio (i.e. 08/08) for all Es. In the sample with the highest tCOD/VS and sCOD/tCOD ratios (15/08), the sCOD decreased for all Es. For those experiments in which fermentation decreased the sCOD, a removal of sCOD linked to CO2 production took place; whereas if the sCOD increased with fermentation, the hydrolysis of organic matter would continue prior to the intensification of biogas production.

It should be borne in mind that a higher sCOD does not necessarily result in higher biogas production. For instance, in their work with grease trap sludge, Luste et al. [13] pointed to the presence of an excessive amount of long-chain fatty acids as the cause for the non-reflection of higher biogas production with high sCOD. Ge et al. [15] found that part of the sCOD could not be attributed to organic acids. Moreover, sCOD increased, thereby confirming hydrolysis. However, this did not lead to an increased conversion to organic acids. The reasons put forward by these authors included higher bacterial concentrations and enzyme activities, which could explain higher hydrolysis and no upgrade in sludge degradability.

As regards the evolution of the sCOD/tCOD ratio, a positive effect on biomass solubilisation can be observed in all the ultrasonicated samples. The ratio underwent a 2- to 5-fold increase after ultrasonication. The subsequent fermentation increased the ratios with the exception of some cases; for instance, when applying 7000 kJ/TS to sample 15/08, the ratio 0.26 (the maximum of all ultrasonication experiments) decreased to 0.22 after the fermentation step. It is worth noting that the maximum ratios both after ultrasonication and fermentation seemed to be around 7000 kJ/kgTS. This observation explained the reason of taking this Es as the most efficient to be employed in the subsequent combined pre-treatments.

Ultrasonication achieved an increase in NH4-N, although no common pattern could be extracted for all samples. The sample with the highest tCOD but the lowest initial sCOD/tCOD ratio experienced much higher increments in NH4-N than the other two samples. Note that NH4-N also underwent a significant increase after fermentation in most of the samples and energies applied.

3.2 Inverted phase fermentation pre-treatment

Figure 3 shows the variations obtained in sCOD, NH4-N, TS and VS when applying IPF to different samples of sludge in terms of the percentage increase with respect to the initial values in the fresh samples. Figure 4 shows the evolution of the VS/TS ratio. As expected [11], a separation of phases occurred, leading to a top solid phase with higher concentrations of COD, NH4-N, TS and VS.

IPF always increased sCOD and NH4-N in both the solid and liquid phase. The sample with the highest increases in sCOD and NH4-N was the one with the lowest initial sCOD and tCOD (17/05). TS and VS increased in the solid phase, but decreased in the liquid phase with respect to the initial values of the sludge samples. Once again, the greatest variations occurred in sample 17/05.

As to the VS/TS ratio (Figure 4), it can be seen that ratios in the two phases of all the samples decreased when compared to the initial values. This behaviour matches the biogas production linked to VS degradation during the IPF. This is related to the process itself, as IPF uses the nascent gas to accomplish solid-liquid separation. Sample 17/05 showed the highest differences with respect to the initial value of the sludge.

sCOD and NH4-N usually decreased in the solid phase and increased in the liquid phase after fermentation. These effects could probably be related to a slight dilution and collapse of the solid phase into the liquid phase. This phenomenon would be in line with the depressurization caused by the sampling of the phases after IPF and the removal of biogas from the solid phase, resulting in a loss in buoyancy and hence partial sinking of some of the solid phase into the liquid phase. This explanation may be reasonable with regards to the expectable rapid biodegradability of the liquid phase, as this phase had an extremely low solids concentration. As will be seen in following paragraphs and in line with the literature [11], most of the tCOD was present as sCOD in the liquid phase, opposite of what was observed in the solid phase. In summary, a substrate that can be so easily biodegraded (the liquid phase) should only increase its organic load as the result of an external contribution (the solid phase).

In terms of the behaviour of the VS/TS ratio after fermentation, a reduction in the solid and liquid phases with respect to the values just after IPF is to be expected. Anaerobic fermentation would explain the degradation of part of the VS. What actually occurred in the two phases of the studied samples was a decrease or no change in the ratio, with the exception of a slight increase in the liquid phase of sample 17/05 (Figure 4).

3.3 Combined pre-treatments

Figure 5 presents the variations in sCOD, NH4-N, TS and VS (expressed as the percentage increase with respect to the initial values), while Figure 6 shows the sCOD/tCOD and VS/TS ratios of the combined pre-treatments applied to one sample of sludge. The energy input for these assays was always around 7000 kJ/kgTS, a value chosen after assessing previous results from ultrasonication pre-treatments alone.

3.3.1 Ultrasound + Inverted phase fermentation

When applying ultrasound pre-treatment followed by IPF, the liquid solid separation resulted in a top layer of “liquid phase” and a bottom layer of “solid phase”, the opposite behaviour to what is usual. Moreover, the obtained “solid phase” was less concentrated than when applying IPF as the sole treatment.

The sCOD of the liquid phase in this combined treatment increased notably with respect to the value in the liquid phase when applying IPF alone. However, the sCOD of the solid phase remained at almost the same level as in IPF alone.

The sCOD slightly increased in the liquid phase after fermentation in the U+IPF pre-treatment, but decreased in the solid phase. Apart from biogas production, a dilution of part of the solid phase into the liquid phase might be the reason for this result.

As can be seen in Figure 6, as a result of the high increase in sCOD, the sCOD/tCOD ratio increased from 0.07 in the untreated sludge to 0.23 after applying ultrasound and to 0.95 in the liquid phase obtained after applying IPF.

NH4-N increased notably in the combined pre-treatment. This behaviour was observed at the end of the pre-treatment and after the subsequent fermentation; hence a thermal influence was always observed. This fact would point to the variable influence of ultrasonication on protein degradation, as this depends on the form in which the proteins are present in the ultrasonicated fluid [16].

When comparing TS and VS in the liquid and solid phases in the combined U+IPF pre-treatment and in IPF alone, the liquid-solid separation was more efficient in the sole pre-treatment. This result counterweighed the achievements of the higher sCOD/tCOD ratios obtained. A dilution of part of the solid phase into the liquid phase might accordingly be responsible for these findings. The sCOD/tCOD ratio showed the effect of biogas production, as it decreased after the fermentation step.

The value of the VS/TS ratio when applying the combined pre-treatment was similar to or below the initial value in the untreated sludge and also when compared to the IPF alone, due to the degradation linked to the production of biogas. The VS/TS ratio reached 0.68 in the liquid phase after U+IPF, whereas this ratio only reached 0.59 in the liquid phase after IPF alone. This situation might point to either a differential distribution of VS and fixed solids in the U+IPF pre-treatment (which was unlikely bearing in mind the similar ratios of the solid phases in U+IPF and IPF alone), or to the fact that the organic matter removal and biogas production in the liquid phase were higher when applying IPF alone. TS and VS behaved in a similar way after fermentation, both increasing slightly in the liquid phase and decreasing in the solid phase.

3.3.2 Inverted phase fermentation + ultrasound

A limited effect was observed when applying the combined IPF+U pre-treatment. As previously mentioned, ultrasonication was applied after IPF to the solid phase, as most of the organic matter in the liquid phase is soluble and hence there is no need for hydrolysis.

When applying this combined pre-treatment, the sCOD of the solid phase increased around 100 % more than when applying IPF alone. Although this combined pre-treatment resulted in an important increase in sCOD, ultrasonication of the solid phase, containing 126 gTS/L, supposes a problem, making ultrasonication of such a concentrated fluid unfeasible. This is in agreement with the diminished efficiency of ultrasonication with the increase in solids. Ultrasonication of the solid phase was accordingly 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 ultrasonication decrease with increasing TS [16, 17]. Due to the high solid content, the effect of ultrasonication was restricted to the sludge portion touching the tip of the sonicator. Moreover, the time needed for any given energy input was prolonged, thus increasing the temperature. To reduce the duration of this assay, only 28 grams of solid phase were ultrasonicated to achieve an energy input of 7000 kJ/kgTS with a final temperature of 64ºC at the end of the 8 minutes that ultrasonication of that amount of solid phase lasted.

When applying this combined pre-treatment, solids increased after fermentation in the solid phase while the VS/TS ratio remained steady, apparently due to a higher effect of water evaporation than either that of biogas production or removal of solids, whereas the sCOD decreased. This anomalous behaviour would provide proof of the inefficiency of the process: the effect of ultrasonication of a sample with a high solid content would be limited to the nearest sludge sample in direct contact with the sonicator tip. The reduction in sCOD after fermentation would indicate biogas production. A deterioration of the sCOD/tCOD ratio for this pre-treatment was observed with respect to ultrasonication of the sludge alone.

As regards to NH4-N, it increased notably after this combined pre-treatment and after the subsequent fermentation, as was also observed in the other combined pre-treatment.

4. CONCLUSIONS

1. The immediate measurement of COD, solids and NH4-N just after ultrasonication and IPF and 24 hours of further fermentation of sewage sludge samples indicated differential responses to the pre-treatments applied. These measurements might thus be suitable for assessing the rapid responses of these pre-treated substrates.

2. The ultrasonication pre-treatment produced a greater increase in sCOD than IPF in the studied sludge (from a wastewater treatment plant which treats domestic wastewater, but which also has a high load of industrial wastewater).

3. Decreases in sCOD and NH4-N were sometimes observed after subsequent fermentation of the pre-treated samples. This might be explained by removal of organic compounds and ammonia. When no decrease was observed, this might mean that the pre-treatment did not produce sufficient hydrolysis and so hydrolysis continued during fermentation. In the case of IPF, a solubilisation of phases might also be involved in this phenomenon.

4. From the results obtained, the combined pre-treatments did not confer an advantage with respect to pre-treatments alone. They required a longer time and a higher energy input and, consequently, higher economic costs to maintain or even degrade the goals achieved by pre-treatments alone.

5. Future research would cover other types of substrates so that both the suitability of these tests and the relation between solubilisation and biodegradability may be more broadly verified.

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References

[1] W.M. Wu, L. Bhatnagar, J.G. Zeikus, Performance of anaerobic granules for degradation of pentachlorophenol, Appl. Environ. Microbiol. 59 (1993) 389-397.

[2] P. Zhang, G. Zhang, W. Wang, Ultrasonic treatment of biological sludge: floc disintegration, cell lysis and inactivation, Bioresour. Technol. 98 (2007) 207-210.

[3] L. Appels, J. Baeyens, J. Degrève, R. Dewil, Principles and potential of the anaerobic digestion of waste-activated sludge, Prog. Energy Combust. Sci. 34 (2008) 755-781.

[4] Y.H. Lee, Y.C. Chung, J.Y. Jung, Effects of chemical and enzymic treatments on the hydrolysis of swine wastewater, Water Sci. Technol. 58 (2008) 1529-1534.

[5] L.W. Hulshoff Pol, S.I. de Castro Lopes, G. Lettinga, P.N.L. Lens, Anaerobic sludge granulation, Water Res. 38 (2008) 1376-1389.

[6] L. Castrillón, Y. Fernández-Nava, P. Ormaechea, E. Marañón, E., Optimization of biogas production from cattle manure by pre-treatment with ultrasound and co-digestion with crude glycerin, Bioresour. Technol. 102 (2011) 7845-7849.

[7] C. Bougrier, C. Albasi, J.P. Delgenès, H. Carrère, Effect of ultrasonic, thermal and ozone pre-treatments on waste activated sludge solubilisation and anaerobic biodegradability, Chem. Eng. Process. 45 (2008) 711-718.

[8] E. Bruni, A.P. Jensen, I. Angelidaki, Comparative study of mechanical, hydrothermal, chemical and enzymic treatments of digested biofibers to improve biogas production, Bioresour. Technol. 101 (2010) 8713-8717.

[9] APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, D.C. 1998.

[10] G.A. Broderick, Determination of protein degradation rates using a rumen in vitro system containing inhibitors of microbial nitrogen metabolism, Br. J. Nutr. 58 (1987) 463-475.

[11] M.S. Le, R. Hu, S. Tyrrel, E. Cartmell, Inverted Phase Fermentation for digestion intensification, Aqua Enviro 13th European Biosolids and Organic Resources Conference, Manchester, UK. 2008.

[12] S. Luste, S. Luostarinen, Enhanced methane production from ultrasound pre-treated and hygienized dairy cattle slurry, Waste Manage. 31 (2011) 2174-2179.

[13] S. Luste, S. Luostarinen, M. Sillanpää, Effect of pre-treatments on hydrolysis and methane production potentials of by-products from meat-processing industry, J. Hazard. Mater. 164 (2009) 247-255.

[14] S. Pérez-Elvira, M. Fdz-Polanco, F.I. Plaza, G. Garralón, F. Fdz-Polanco, Ultrasound pre-treatment for anaerobic digestion improvement, Water Sci. Technol. 60 (2009) 1525-1532.
[15] H. Ge, P.D. Jensen, D.J. Batstone, Pre-treatment mechanisms during thermophilic-mesophilic temperature phased anaerobic digestion of primary sludge, Water Res. 44 (2010) 123-130.
[16] E. Elbeshbishy, S. Aldin, H. Hafez, G. Nakhla, M. Ray, Impact of ultrasonication of hog manure on anaerobic digestability, Ultrason. Sonochem. 18 (2011) 164-171.
[17] S. Pilli, P. Bhunia, S. Yan, R.J. LeBlanc, R.D. Tyagi, R.Y. Surampalli, Ultrasonic pretreatment of sludge: A review, Ultrason. Sonochem. 18 (2011) 1-18.

CAPTIONS FOR TABLES

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

U: ultrasound; IPF: inverted phase fermentation; U+IPF: ultrasound followed by inverted phase fermentation; IPF+U: inverted phase fermentation followed by ultrasound

Table 2. Increases in sCOD and NH4-N with respect to initial values and evolution of the sCOD/tCOD ratio after ultrasound pre-treatment and subsequent fermentation of sludge

CAPTIONS FOR FIGURES

Figure 1. Increases in sCOD and NH4-N with respect to initial values after ultrasound (U) pre-treatment (7000 kJ/kgTS) and subsequent fermentation of sludge (F).

Figure 2. Evolution of the sCOD/tCOD ratio after ultrasound (U) pre-treatment (7000 kJ/kgTS) and subsequent fermentation of sludge (F).

Figure 3. Behaviour of sludge samples after IPF and subsequent fermentation (F). Variation with respect to initial sCOD, NH4-N, TS and VS. LP= Liquid phase. SP= Solid phase.

Figure 4. Evolution of the VS/TS ratio after IPF and subsequent fermentation (F). LP= Liquid phase. SP= Solid phase.

Figure 5. Percentage variation when applying combined pre-treatments to the sludge: ultrasound plus inverted phase fermentation (U+IPF) or inverted phase fermentation plus ultrasound (IPF+U), followed by subsequent fermentation (F).

Figure 6. Evolution of the sCOD/tCOD and VS/TS ratios when applying combined pre-treatments to the sludge: ultrasound plus inverted phase fermentation (U+IPF) or inverted phase fermentation plus ultrasound (IPF+U), followed by subsequent fermentation (F).

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

Sample	sCOD (mg/L)	tCOD (mg/L)	sCOD/tCOD	NH4-N (mg/L)	TS (g/L)	VS (g/L)	VS/TS	tCOD/VS (g/L)/(g/L)	pH	Pre-treatments
31/07	4002	91165	0.04	89	61.72	50.25	0.81	1.81	5.3	U
08/08	905	18189	0.05	86	22.20	18.56	0.84	0.98	6.0	U
15/08	6164	60207	0.10	119	33.44	27.00	0.81	2.23	5.3	U
28/03	7847	82039	0.10	368	68.63	51.28	0.75	1.60		IPF
09/05	6159	72738	0.09	424	54.67	43.77	0.80	1.66	6.3	IPF
17/05	5313	62987	0.08	280	47.49	37.08	0.78	1.70	5.6	IPF
01/10	3969	54972	0.07	147	40.35	30.62	0.76	1.80	5.7	U+IPF; IPF+U

U: ultrasound; IPF: inverted phase fermentation; U+IPF: ultrasound followed by inverted phase fermentation; IPF+U: inverted phase fermentation followed by ultrasound

Table 2. Increases in sCOD and NH4-N with respect to initial values and evolution of the sCOD/tCOD ratio after ultrasound pre-treatment and subsequent fermentation of sludge

Sample	Treatment	Percentage increase with respect to initial sCOD					
	kJ/kgTS	Untreated	3500	7000	10500	14000	21000
31/07	Ultrasonication	0	242	261	253	289	245
	+ Fermentation	183	94	347	325	254	255
08/08	Ultrasonication	0	464	532	554	590	519
	+ Fermentation	409	700	822	880	933	927
15/08	Ultrasonication	0	81	119	123	104	99
	+ Fermentation	33	76	84	101	70	66

Sample	Treatment	Percentage increase with respect to initial NH4-N					
	kJ/kgTS	Untreated	3500	7000	10500	14000	21000
31/07	Ultrasonication	0	496	180	426	386	276
	+ Fermentation	486	526	636	576	548	479
08/08	Ultrasonication	0	55	51	48	29	24
	+ Fermentation	86	327	310	243	404	340
15/08	Ultrasonication	0	13	45	45	32	35
	+ Fermentation	76	241	92	79	24	31

Sample	Treatment	Evolution of the sCOD/tCOD ratio					
	kJ/kgTS	Untreated	3500	7000	10500	14000	21000
31/07	Ultrasonication	0.04	0.23	0.22	0.21	0.21	0.18
	+ Fermentation	0.21	0.14	0.33	0.28	0.25	0.19
08/08	Ultrasonication	0.05	0.15	0.17	0.16	0.17	0.16
	+ Fermentation	0.16	0.25	0.28	0.29	0.30	0.31
15/08	Ultrasonication	0.10	0.22	0.26	0.23	0.22	0.20
	+ Fermentation	0.17	0.22	0.22	0.24	0.21	0.20

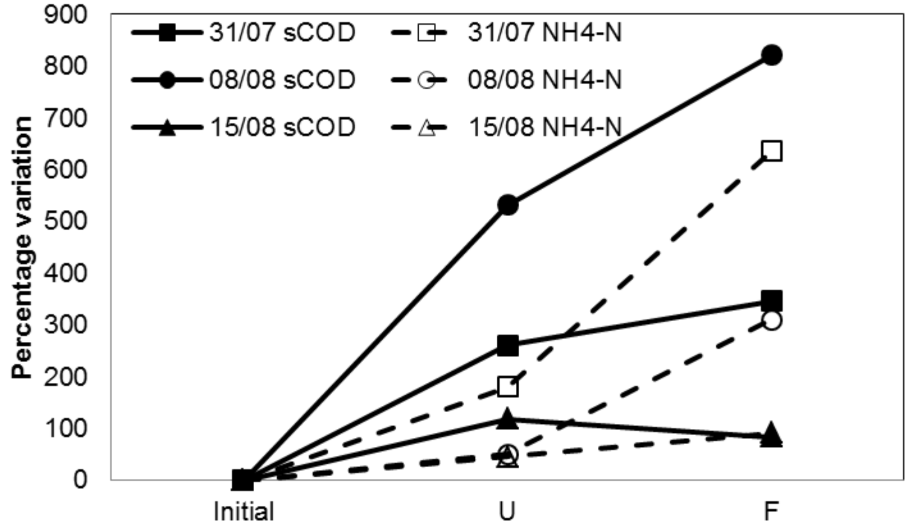

Figure 1. Increases in sCOD and NH4-N with respect to initial values after ultrasound (U) pre-treatment (7000 kJ/kgTS) and subsequent fermentation of sludge (F).

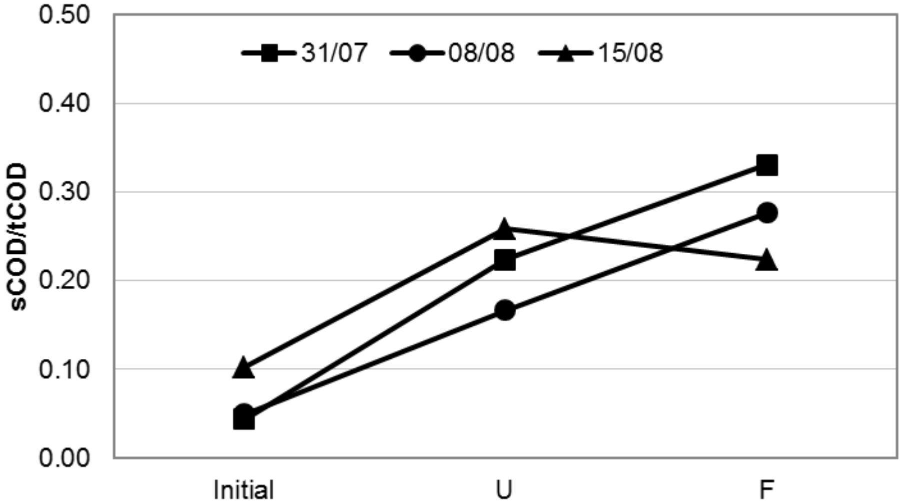

Figure 2. Evolution of the sCOD/tCOD ratio after ultrasound (U) pre-treatment (7000 kJ/kgTS) and subsequent fermentation of sludge (F).

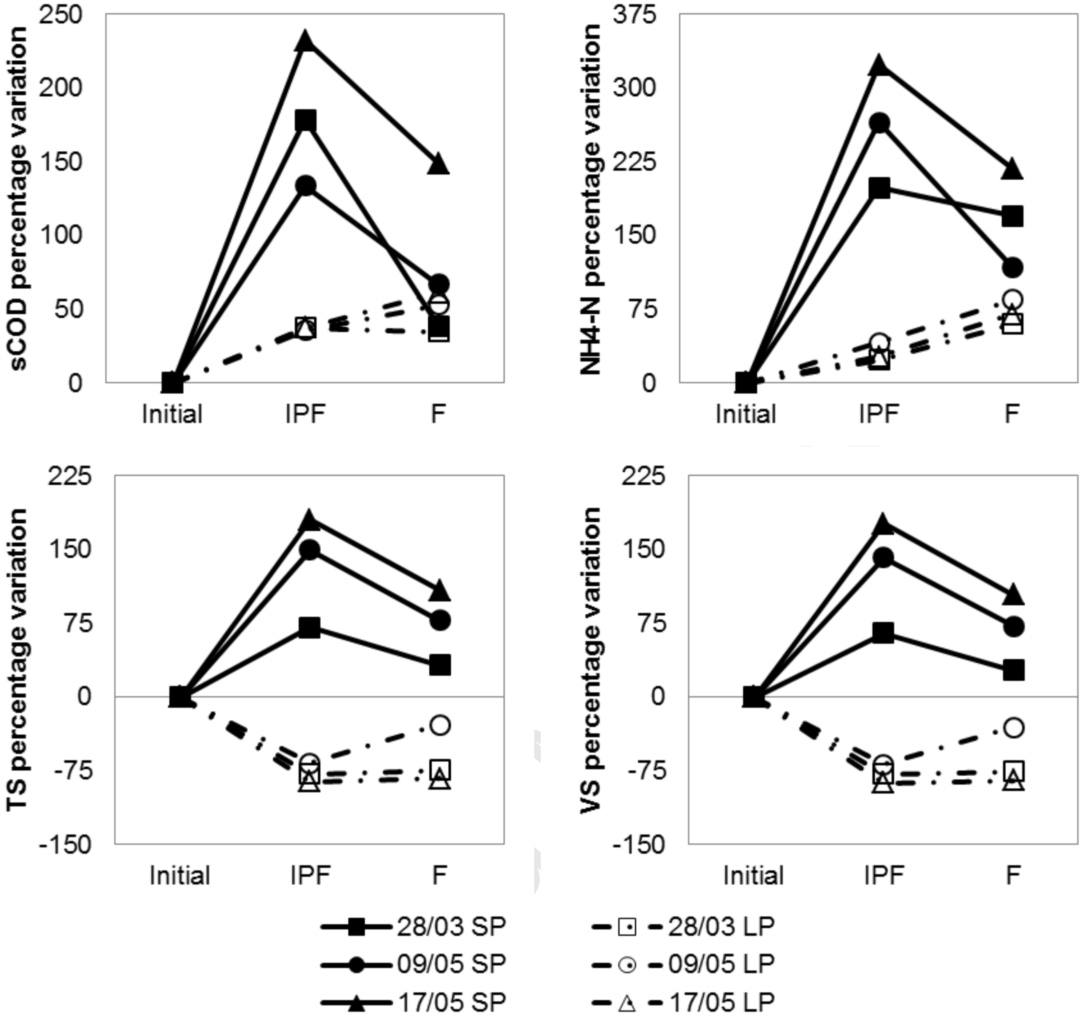

Figure 3. Behaviour of sludge samples after inverted phase fermentation (IPF) and subsequent fermentation (F). Variation with respect to initial sCOD, NH4-N, TS and VS. LP= Liquid phase. SP= Solid phase.

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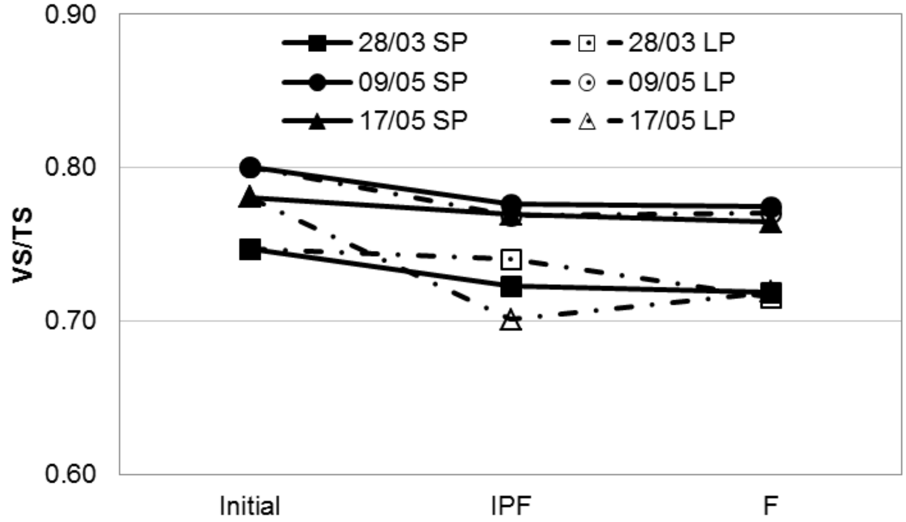
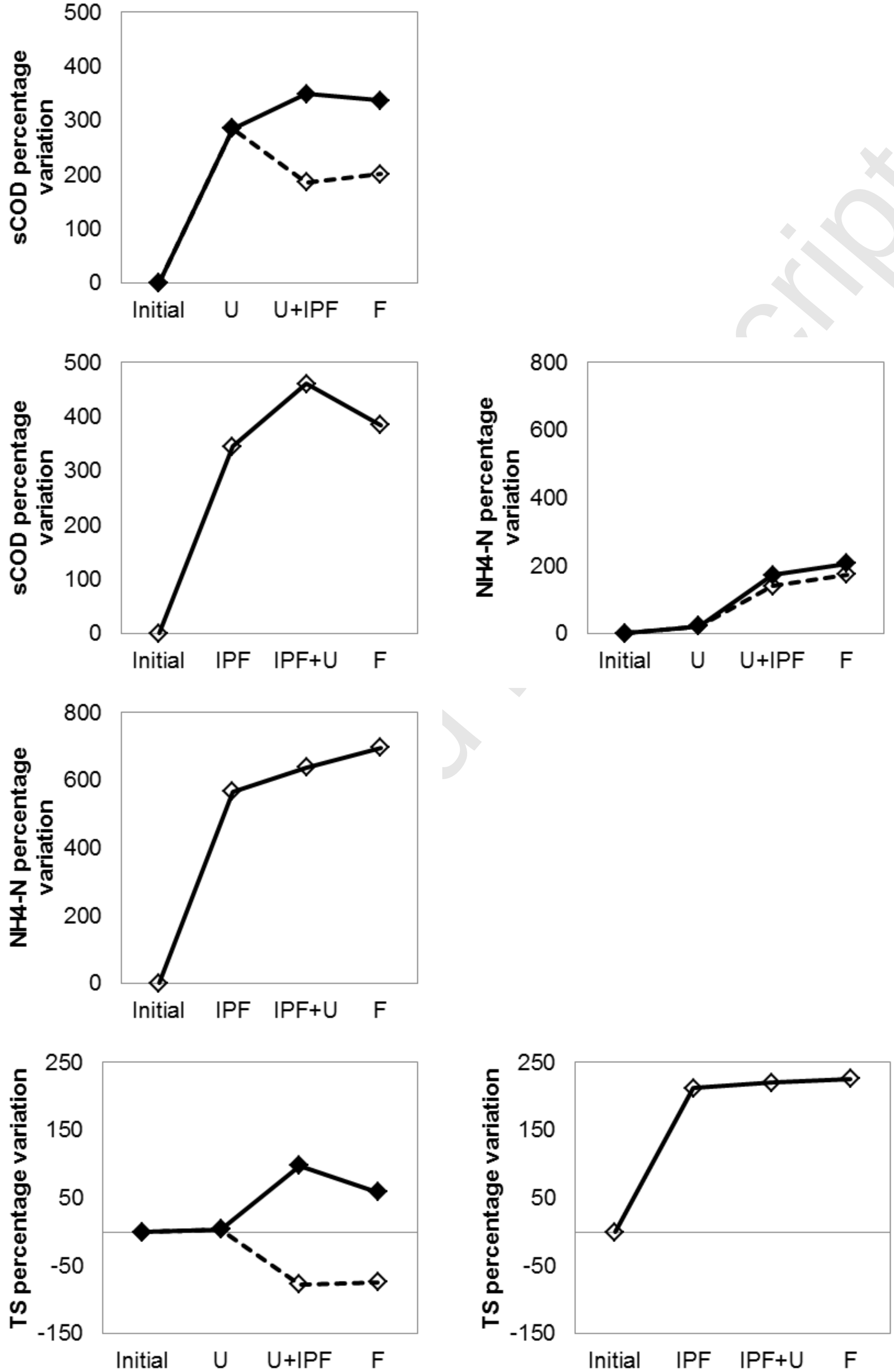


477
478 Figure 4. Evolution of the VS/TS ratio after inverted phase fermentation (IPF) and subsequent
479 fermentation (F). LP= Liquid phase. SP= Solid phase.
480



sCOD percentage variation
0
100
200
300
400
500
Initial
U
U+IPF
F
sCOD percentage variation
IPF
IPF+U
NH4-N percentage variation
600
800
TS percentage variation
250
150
50
-50
-150

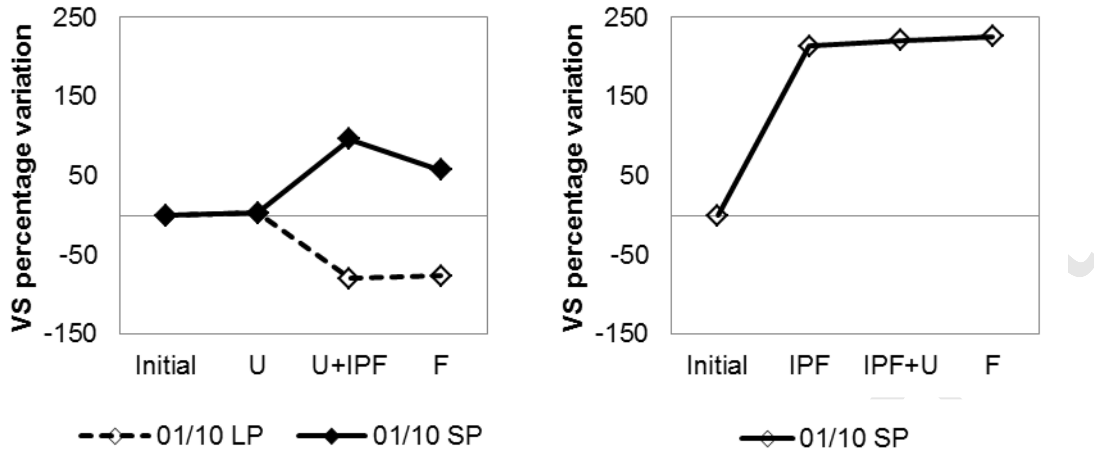


Figure 5. Percentage variation when applying combined pre-treatments to the sludge: ultrasound plus inverted phase fermentation (U+IPF) or inverted phase fermentation plus ultrasound (IPF+U), followed by subsequent fermentation (F).

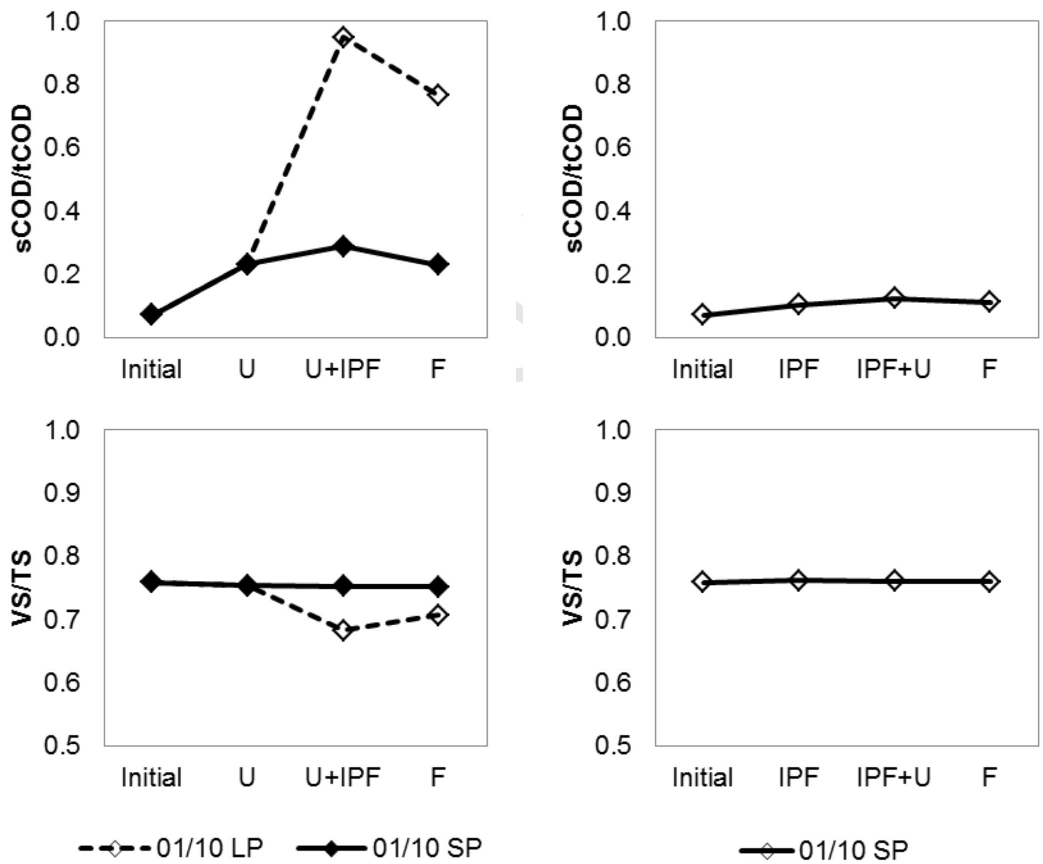


Figure 6. Evolution of the sCOD/tCOD and VS/TS ratios when applying combined pre-treatments to the sludge: ultrasound plus inverted phase fermentation (U+IPF) or inverted phase fermentation plus ultrasound (IPF+U), followed by subsequent fermentation (F).