

# Differences in soluble COD and ammonium when applying inverted phase fermentation to primary, secondary and mixed sludge

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## ABSTRACT

Primary, secondary and mixed sludge were treated by inverted phase fermentation. This treatment results in solid–liquid separation of sludge after endogenous enzymatic hydrolysis (anaerobic conditions: 42°C, 48 hours). The soluble chemical oxygen demand (sCOD) was increased in the solid phase up to 1,800%, 21,300% and 260% in primary, secondary and mixed sludge, respectively. The corresponding increase in sCOD in the liquid phase accordingly reached values of up to 440%, 5,100% and 140%. Phase separation led to an enrichment of volatile solids in the solid phase (89–358% primary sludge, 80–102% secondary sludge and 29–133% mixed sludge).  $\text{NH}_4^+$ -N values increased notably after the endogenous enzymatic hydrolysis itself. To investigate the short-term evolution following the treatment, the variation in sCOD,  $\text{NH}_4^+$ -N and solids was also monitored after keeping the hydrolysate at 37°C under anaerobic conditions for 24 hours. This stage showed no generalized pattern in terms of sCOD.

**Key words** | ammonium, COD, inverted phase fermentation, mixed sludge, primary sludge, secondary sludge

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## LIST OF ACRONYMS

F	fermentation
IPF	inverted phase fermentation
LP	liquid phase
M	mixed sludge
$\text{NH}_4^+$ -N	nitrogen concentration measured as the $\text{NH}_4^+$ form
P	primary sludge
sCOD	soluble chemical oxygen demand
sCOD/tCOD	ratio between soluble chemical oxygen demand and total chemical oxygen demand
S	secondary sludge
SP	solid phase
tCOD	total chemical oxygen demand
tCOD/Vs	ratio between total chemical oxygen demand and volatile solids
TSs	total solids
VSs	volatile solids
VS/TS	ratio between volatile solids and total solids

## INTRODUCTION

The production of biosolids in wastewater treatment in Europe during the second half of the past decade exceeded 10 Mt dry solids/year (European Commission 2010). It is no wonder that such quantities may constitute a financial burden for wastewater management entities (Murray *et al.* 2008). A positive consequence, however, has been the development of extensive research in this field. Water treatment manuals include several processes for managing biosolids or sewage sludge (Tchobanoglous *et al.* 2014). A variety of strategies are implemented depending on both the nature of the wastewater and the process itself.

One of the most frequent treatments applied to sewage sludge is anaerobic digestion. The notable reduction in volume produced by the mineralization of organic matter supports this choice. Of the three types of sludge produced in a conventional wastewater treatment plant (WWTP), mixed sludge (i.e., a combination of primary and secondary sludge) is usually digested. However, the biodegradability of secondary sludge is limited because of the abundance of complex microbial material (Appels *et al.* 2008). This

limitation is related to kinetics. Sludge hydrolysis constitutes the rate-limiting step, slowing down the biodegradation rate (Skiadas *et al.* 2005). By overcoming this first barrier to biodegradation, the entire process is thus speeded up. Several treatments conceived as pre-treatments to digestion are then implemented to resolve this issue (Carrère *et al.* 2010). A hydrolysed substrate favours any biological treatment applied to sludge, not just anaerobic digestion. For example, Ucisik & Henze (2008) proved the use of sludge as a carbon source in biological nitrogen and phosphorus removal.

Enzymatic treatment uses enzymes to catalyse the hydrolysis of different substrates. Hydrolases are substrate-specific, so lipases, proteases and glucosidases (i.e., enzymes that catalyse the hydrolysis of lipids, proteins and carbohydrates, respectively) can be listed among these enzymes (Burgess & Pletschke 2008). Enzyme implementation may be achieved by direct addition to sludge (Davidsson *et al.* 2007) or by endogenous promotion of these enzymes naturally present in sludge (Le *et al.* 2008). This promoting of hydrolases in turn requires the enhancement of their microbial producers. In a review of the state of the art of mechanical, thermal, chemical and enzymatic (or 'biological') treatments carried out in 2001, Müller (2001) distinguished enzymatic treatments as the best. The scoring criterion this author used was based on low energy consumption, resistance to wear, reliability of operation, extent of research experiences and stage of development for WWTP application. Enzymatic treatment scored the same or higher in all fields compared to other treatments, except in terms of the extent of research experiences, where it obtained an average score. Mayhew *et al.* (2002) described the 'enzymic hydrolyser' as a technology pending the granting of a patent which was of major innovative interest. Disappointingly, after experimenting with carbohydrase in sludge, Barjenbruch & Kopplow (2003) found that the formation of foam during digestion was not prevented by the enzyme and could only be avoided by means of thermal treatment. Dursun *et al.* (2006) published the results of the application of commercial enzyme mixtures at a laboratory scale and at a WWTP. These authors found that the enzymes destroyed the gel structure of sludge, intrinsically formed by extracellular polymeric substances (EPSs). In effect, Davidsson *et al.* (2007) reported that the enzymatic activity of sludge was associated with its 'solid phase' (i.e., particles or flocs). Burgess & Pletschke (2008) published a review of the literature on enzymatic treatment, detailing the mechanisms of action of various types of enzymes in terms not only of the substrate which they catalyse, but also of the location

in the sludge where they are found. Seven years after Müller's assessment, Le *et al.* (2008) presented the already approved 'inverted phase fermentation' (IPF) patent. This technology consists of increasing the endogenous enzymatic activity of the sludge by heating to 42 °C for 48 hours. As it is a thermal process, it circumvents the problems reported by Barjenbruch & Kopplow (2003). This process takes advantage of the generation of nascent bubbles that appear in the sludge to thicken it, at the same time as performing hydrolysis. Gas bubbles were one of the causes of foaming reported by Barjenbruch & Kopplow (2003). The uniqueness of the treatment developed by Le *et al.* (2008) is that, at the end of the process, a 'solid phase, SP' floats above a clarified 'liquid phase, LP'. The SP reaches TS concentration factors of up to 2.8 (defined as TS in the SP versus TS in the initial sludge). Owing to the enzymatic hydrolysis, the SP and the LP present a higher content in soluble organic matter than the sludge. The LP, rich in soluble species such as volatile fatty acids, is characterized by a notable hydrolysis assessed in terms of soluble chemical oxygen demand (sCOD) (Negral *et al.* 2013). Obtaining these distinct phases has several advantages: different treatment for the phases, thickening of sludge in the SP without the use of additives, enhancement of enzymatic hydrolysis to increase sCOD and 99.9% destruction of *Escherichia coli*.

The scientific literature has reported the advantages of treating secondary sludge before digestion (Appels *et al.* 2008; Tyagi *et al.* 2014). However, treatment of primary sludge has also produced promising results (Ferreiro & Soto 2003; Ucisik & Henze 2008). The research presented in this paper seeks to: (a) study the implementation of endogenous enzymatic hydrolysis with phase separation; (b) monitor the solubilization (sCOD and  $\text{NH}_4^+\text{-N}$ ) of primary, secondary and mixed sludge following the treatment; and (c) assess the short-term response of these three types of hydrolysed sludge at 37 °C anaerobically.

## MATERIALS AND METHODS

### Materials

Experiments were carried out with fresh sludge from a municipal WWTP with an average flow rate of 21,600 m<sup>3</sup>/day (85,000 population equivalent), removal efficiencies of >85% SS and >90% BOD<sub>5</sub>. The plant operates in a conventional manner with production of mixed sludge as a mixture of primary and secondary sludge from a suspended activated sludge process. Primary sludge is thickened by gravity and

secondary sludge, by flotation. Although  $\text{FeCl}_3$  and lime are added to the sludge before being dewatered in a filter press, all the samples of thickened mixed sludge were taken prior to dewatering.

### Experimental procedure and analytical methods

The hydrolysis was monitored by measuring the sCOD,  $\text{NH}_4^+\text{-N}$  and solids after IPF and after a final stage (24 hours) at 37 °C under anaerobic conditions. Total COD (tCOD), soluble COD,  $\text{NH}_4^+\text{-N}$ , total solids (TSs), volatile solids (VSs) 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 characterized on reception at the laboratory. Samples were kept in a refrigerator at 4 °C for a maximum of 1 day before applying the treatment, while the sludge was being characterized.

IPF was performed with three samples of secondary and mixed sludge, but four samples of primary sludge were considered due to dissimilar thickening. IPF was achieved by promoting endogenous enzymes at 42 °C for 48 hours under anaerobic conditions (Le *et al.* 2008). Approximately 1 litre of fresh sludge was introduced into plastic bottles filled to the cap. An outlet hose connected the bottle to a beaker containing water to achieve anaerobic conditions. Samples of both separated phases (bottom LP and upper SP) were taken after 48 hours to determine sCOD,  $\text{NH}_4^+\text{-N}$ , TS and VS. These parameters were determined once again after the final stage at 37 °C. A 400-litre volume

J.P. Selecta Dry-Big oven was used to maintain the chosen temperature throughout the experiments. Incubation was performed under quiescent conditions. No pH adjustment was required, as microbial inhibition did not occur during the experiments.

### RESULTS AND DISCUSSION

As reported by Le *et al.* (2008), IPF is suitable for use with sludge streams with initial solids concentrations in the 2–5% TS range. Although most of the primary sludge samples had higher solids concentrations than these values (see Table 1), application of the treatment was possible throughout all the assays.

Figures 1–3 present the percentage variations in sCOD,  $\text{NH}_4^+\text{-N}$  and VS with respect to their initial values after IPF and after the final stage at 37 °C. The VS/TS ratio is also provided in each stage of every experiment. The reason for providing the solids content in the experiments is that, besides solubilizing solids, the IPF alters the distribution of particulate matter between the two phases thus generated. After removing aliquots to characterize the two phases, these are kept in the same container during the final stage at 37 °C for 24 hours to minimize disturbance.

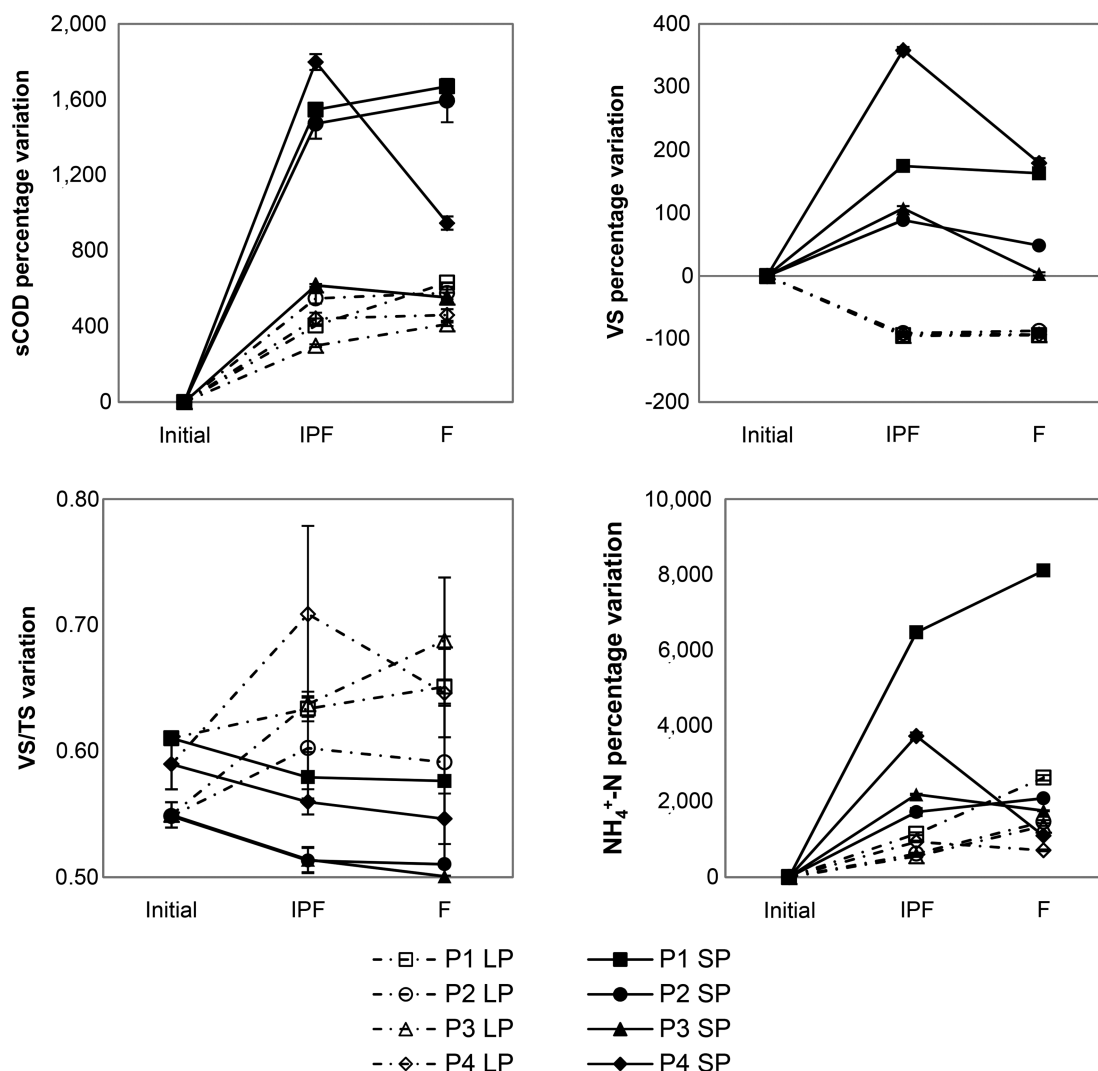
#### Primary sludge

The greatest increases in sCOD during IPF (600–1,800%) were observed for the SP (Figure 1). These results were to be expected, seeing as the SP contains most of the particulate matter and therefore drags most of the bacteria with

**Table 1** | Characterization of initial sludge samples (mean  $\pm$  standard deviation of triplicates)

Sample	sCOD (mg/L)	tCOD (mg/L)	sCOD/tCOD	$\text{NH}_4^+\text{-N}$ (mg/L)	TS (g/L)	VS (g/L)	VS/TS	tCOD/VS	pH
P1	940 $\pm$ 2	48,501 $\pm$ 323	0.02	17 $\pm$ 0	59.03 $\pm$ 0.21	36.02 $\pm$ 0.16	0.61	1.35	5.7
P2	1,013 $\pm$ 13	60,884 $\pm$ 1,414	0.02	38 $\pm$ 3	78.40 $\pm$ 0.31	43.00 $\pm$ 0.10	0.55	1.42	5.5
P3	1,482 $\pm$ 105	62,872 $\pm$ 1,027	0.02	51 $\pm$ 1	83.08 $\pm$ 1.26	45.65 $\pm$ 0.94	0.55	1.38	6.1
P4	542 $\pm$ 169	16,836 $\pm$ 344	0.03	36 $\pm$ 1	26.27 $\pm$ 0.78	15.49 $\pm$ 0.38	0.59	1.09	6.6
S1	56 $\pm$ 5	24,933 $\pm$ 815	<0.01	6 $\pm$ 0	31.94 $\pm$ 1.65	21.92 $\pm$ 1.17	0.69	1.14	6.7
S2	185 $\pm$ 55	42,104 $\pm$ 4,138	<0.01	5 $\pm$ 0	45.27 $\pm$ 0.29	31.06 $\pm$ 0.31	0.69	1.36	6.5
S3	375 $\pm$ 15	34,615 $\pm$ 1,173	0.01	6 $\pm$ 0	40.74 $\pm$ 2.41	28.93 $\pm$ 1.83	0.71	1.20	6.6
M1	4,270 $\pm$ 199	40,732 $\pm$ 1,629	0.10	330 $\pm$ 9	35.29 $\pm$ 0.15	23.19 $\pm$ 0.09	0.66	1.76	6.2
M2	5,019 $\pm$ 192	44,650 $\pm$ 1,045	0.11	452 $\pm$ 14	40.45 $\pm$ 0.11	27.31 $\pm$ 0.11	0.68	1.63	6.1
M3	5,050 $\pm$ 217	36,848 $\pm$ 241	0.14	450 $\pm$ 9	36.43 $\pm$ 0.03	24.84 $\pm$ 0.16	0.68	1.48	5.9

P: primary sludge; S: secondary sludge; M: mixed sludge.



**Figure 1** | Behaviour of primary sludge samples after IPF and the final stage at 37 °C (F). Evolution of sCOD,  $\text{NH}_4^+\text{-N}$ , VS and the VS/TS ratio. LP: liquid phase, SP: solid phase.

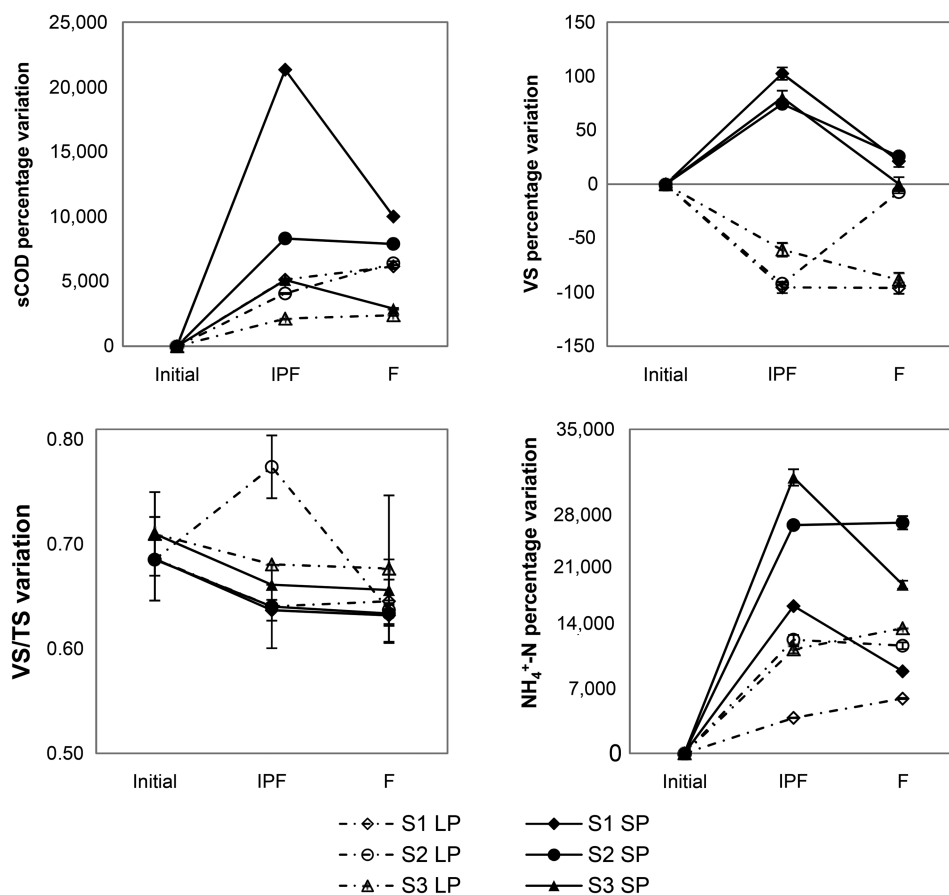
hydrolytic enzymes (Le et al. 2008). The maximum increase was observed for the sludge sample with the lowest initial sCOD (i.e., P4). There was also notable solubilization in the LP (300–550%), corroborating the occurrence of hydrolysis in both streams (Negral et al. 2013).

The distribution of VS during IPF once again reached its maximum value for the SP of sample P4, being very similar for all samples in the LP, showing decreases of between 91 and 95%. The concurrence of the best performance for P4 should not be taken as coincidental. In fact, the VS/TS ratio was the highest in the LP (0.71). Thus, although it was not the lowest in the SP (0.56), it represented the largest difference between the phases of the same sample. P4 is the only primary sludge sample in which TS are in the 2–5% range set by Le et al. (2008) for successful IPF.

The study of the VS/TS ratio shows that IPF also shifted the distribution of solids. The increase in the VS/TS ratio in the LP with respect to its value in the SP is explained by the dragging to the SP of all kinds of matter, especially non-volatile particulate substances. In contrast, already dissolved substances or those that could be more easily dissolved remain in the LP.

IPF led to changes in  $\text{NH}_4^+\text{-N}$  concentration.  $\text{NH}_4^+\text{-N}$  is the product of degradation of macromolecules such as proteins (Broderick 1987). This was the reason for the high increases in  $\text{NH}_4^+\text{-N}$ , both in the SP (1,700–6,400%) and in the LP (550–1,150%).

The subsequent stage at 37 °C showed no common pattern in the four samples. P1 and P2 did not seem to have completed their hydrolysis during IPF, which continued



**Figure 2** | Behaviour of the secondary sludge samples after IPF and final stage at 37 °C (F). Evolution of sCOD, NH<sub>4</sub><sup>+</sup>-N, VS and the VS/TS ratio. LP: liquid phase, SP: solid phase.

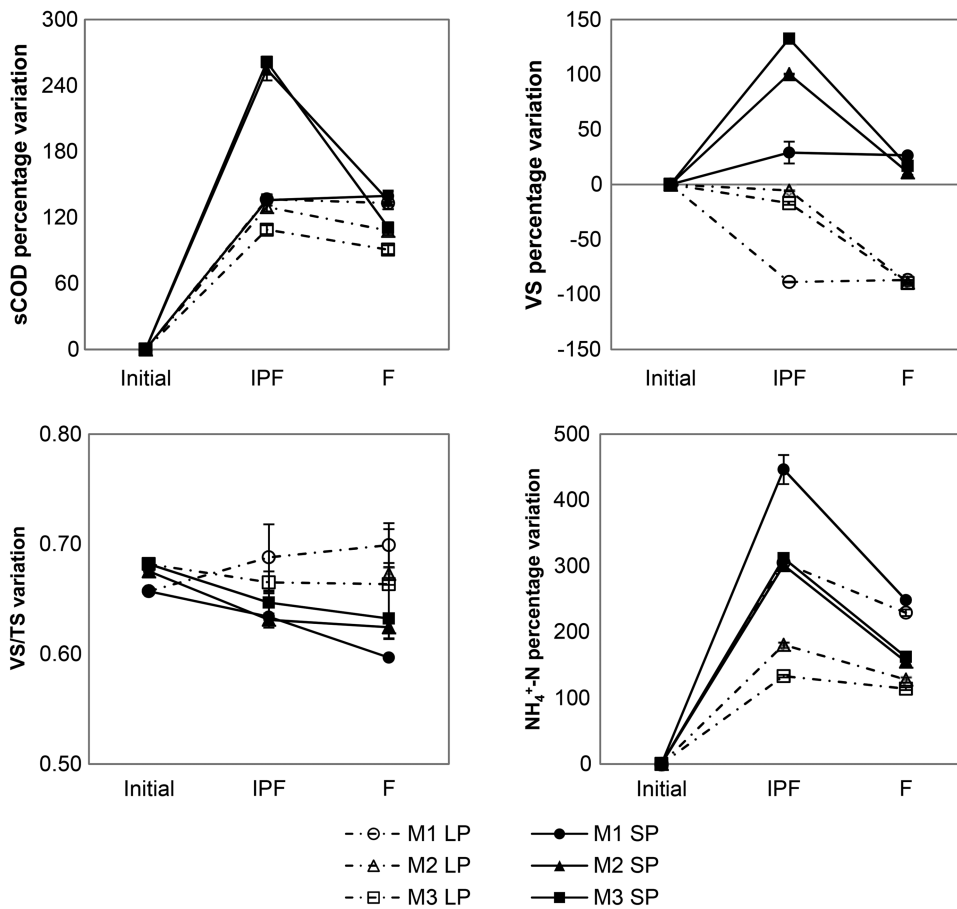
during the next step. The reason for hydrolysis continuing may be the initial acidity of the two samples (i.e., pH 5.7 and 5.5). According to Siegrist *et al.* (2002), inhibition of the hydrolysis of amino acids and sugars occurs at a pH below 5.5. Decreases in sCOD, VS and the VS/TS ratio may be explained by the mineralization of organic matter. The fact that this does not always occur might be motivated by the collapse of part of the SP into the LP. The increase in NH<sub>4</sub><sup>+</sup>-N is explained by the continued hydrolysis of particulate proteins in the SP (e.g., P1 and P2). In fact, Davidsson *et al.* (2007) established that the enzyme activity of the sludge lies in the 'solid phase'. The increase in NH<sub>4</sub><sup>+</sup>-N in the LP would occur, as stated, due to the partial collapse of the SP into the LP.

### Secondary sludge

As in the case of primary sludge, the greatest increases in sCOD in the IPF stage were observed in the SP (5,100–21,300%) (Figure 2). Similarly, the maximum percentage

rise in the SP was observed for the sludge sample with the lowest initial sCOD (i.e., S1). Note that the scale of the sCOD graph for secondary sludge is one order of magnitude greater than that of the other types of sludge. As expected, IPF showed intense hydrolytic activity in the secondary sludge, as had previously occurred with other treatments (Carrère *et al.* 2010; Negral *et al.* in press). However, the variation in VS in the SP did not reach the same percentages as in the primary sludge (358%), remaining at increases of 100% or lower. This is explained by the nature of the secondary sludge and is related to the fact that much of the organic matter is associated with the microorganisms (i.e., cells and EPS), initially non-soluble organic matter (Appels *et al.* 2008). Hence, the percentage increases are quite large for sCOD, though not for VS, as the initial values are very low for sCOD, but not for VS.

The VS/TS ratio decreased in all samples and phases as a result of the mineralization of organic matter, except in LP S2. The mineralization of organic matter (nascent bubbles of CO<sub>2</sub>) is observed where VS mainly disappeared, i.e., in the



**Figure 3** | Behaviour of the mixed sludge samples after IPF and final stage at 37 °C (F). Evolution of sCOD, NH<sub>4</sub><sup>+</sup>-N, VS and the VS/TS ratio. LP: liquid phase, SP: solid phase.

LP. The fact that the VS/TS ratio in the LP of sample S2 increased from 0.69 to 0.78 would seem to indicate a preferential accumulation of VSs compared to the SP, with a ratio of 0.64. Moreover, the mineralization of organic matter was not as noticeable in the IPF as it was following the final stage at 37 °C, when the VS/TS ratios of the two phases decreased below 0.64.

As regards NH<sub>4</sub><sup>+</sup>-N concentrations, the increases triple the values of those obtained for primary sludge. This fact highlights the extent of the hydrolysis on microbiological structures.

As to the final stage at 37 °C, the sCOD in the SP decreased in all samples, in contrast to what occurred in the LP. VS also decreased in the SP. As regards the LP, VS decreased in two of the samples. However, an increase can be observed in sample S2 due to the collapse of the SP into the LP (Figure 2), although this collapse did not prevent mineralization. This hypothesis is supported by the reduction in the VS/TS ratio in both phases.

### Mixed sludge

As in the other two types of sludge, the greatest increases in sCOD in the IPF stage were observed in the SP (136–261%) (Figure 3). However, sample M1 showed similar upgrades for the two phases (around 135%). The differences found in the mixed sludge with respect to the primary and secondary sludge underscore the idea that the value of the characterization parameters of the mixed sludge cannot be considered a direct combination of the corresponding parameters in the primary and secondary sludge. An initial explanation is that the percentage increases in sCOD are one and two orders of magnitude higher for the primary and secondary sludge, respectively (Figures 1–3). Furthermore, whereas the largest percentage increase in sCOD occurred in the primary and secondary sludge in the sample with the lowest sCOD, the opposite occurred in the mixed sludge (i.e., M3). The mixed sludge presented the highest sCOD values (Table 1). Furthermore, the

mixed sludge always presented an initial sCOD at least double that of any of the values in the primary and secondary sludge in all IPF treatments. Therefore, the mere mixture of primary and secondary sludge seems to cause an 'autolysis' that is further enhanced during IPF.

The percentage change in VS observed in the mixed sludge is lower than the change observed in the primary and secondary sludge. In the SP, the values ranged between 29 and 133% and in the LP, between 5 and 89%. The greatest increases in VS in the SP occurred concurrently with the lowest reductions in VS in the LP. The VS/TS ratio suggests notable mineralization of organic matter, although this is not clear in sample M1.

As regards the variation in  $\text{NH}_4^+\text{-N}$ , the increases observed in all the samples of mixed sludge and in both phases were lower than those observed in the primary and secondary sludge. This is due to the fact that the mixed sludge was more hydrolysed (higher  $\text{NH}_4^+\text{-N}$  concentrations, 330–452 mg/L).

The final stage at 37 °C removed sCOD and VS in the two phases of samples M2 and M3, with a corresponding decrease in the VS/TS ratio. Sample M1 must be considered bearing in mind the evolution of the three parameters in each stage. It appears that the IPF did not reach maximum performance and the dragging of solids continued during the subsequent stage.

There was a general decrease in  $\text{NH}_4^+\text{-N}$  in all samples and phases after the final stage. The hypothesis of assimilatory metabolism may be postulated, considering the mineralization of organic matter, while the occurrence of anammox processes is not very likely (Sun et al. 2011).

## CONCLUSIONS

IPF was able to achieve hydrolysis and solid–liquid separation in primary, secondary and mixed sludge by simply keeping the sludge samples at 42 °C for 48 hours under anaerobic conditions. Although the effect of the treatment was clearly more pronounced when applied to secondary sludge (upgrades of up to 21,000% in sCOD), organic matter solubilization was also notable in primary sludge (up to 1,800% in sCOD) and mixed sludge (up to 260%).

Reductions in sCOD and  $\text{NH}_4^+\text{-N}$  were sometimes observed following a final stage at 37 °C for 24 hours anaerobically. These may be due to metabolic removal of organic compounds and nitrogen ammonium and were more marked in mixed sludge, with the initial highest solubilization. The response to the treatment of mixed sludge,

which was different to that expected from the observed behaviour of primary and secondary sludge, pointed to a previous 'autolysis'. This phenomenon arose when the streams of primary and secondary sludge were mixed at the WWTP.

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