



# Controlled Sewage Sludge Alkaline Fermentation to Produce Volatile Fatty Acids to be Used for Biological Nutrients Removal in WWTPs

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## ABSTRACT

In municipal wastewater treatment plants (WWTPs), the disposal of sewage sludge accounts for 20–50% of capital and operating expenses. Therefore, technologies that can simultaneously reduce sludge and generate carbon source for improving biological nutrients removal are in demand. The application of sewage sludge alkaline fermentation can provide the external carbon source that is required to remove nutrients via nitrite for the separate treatment of the supernatant produced from the anaerobic digestion of sewage sludge. Existing studies on sewage sludge fermentation use caustic soda or other chemicals to achieve the desirable pH for alkaline fermentation; this practice increases the cost. In this work, the anaerobic supernatant produced from sewage sludge was used to adjust the pH in the fermentation reactor, while the impact of temperature (30 °C and 55 °C), pH (8–11), retention time (1–8 days) and initial total solids (TS = 4.5 and 6.5%) concentration of sludge was examined. The highest volatile fatty acids (VFAs) concentration was achieved at a retention time of 6 days, sludge TS = 6.5%, pH = 10–11 and temperature of 55 °C. The addition of pre-aerated anaerobic supernatant achieved similar VFAs as the one of similar pH in which caustic soda adjustment took place.

*Keywords:* Alkaline fermentation; sewage sludge; short-chain fatty acids; nutrients removal

## 1. INTRODUCTION

### 1.1 Increasing sewage sludge valorisation

The extensive application of anaerobic digestion for sewage sludge treatment results in the production of high quantities of anaerobic effluents that must be properly managed within the wastewater treatment plant (WWTP). Such effluents are characterized by high nutrients content, since organic nitrogen and organic phosphorus forms are hydrolyzed during the anaerobic digestion process. The usual practice

implemented in WWTPs is to return the anaerobic supernatant to the inlet of the sewage treatment line. Although this liquid stream is a small proportion of influent flow, it increases significantly the nitrogen and phosphorus load (by 15–30%). The separate post-treatment of the anaerobic supernatant can relieve the sewage treatment line from such loads, avoiding N and P overloads. Thus, the application of innovative bioprocesses that will increase the on-site biological valorisation of wastewater and sewage sludge is a major challenge.

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## **1.2 Biological nutrients removal via the nitrite pathway**

Nitritation/denitritation (or partial nitrification/denitrification) is accomplished through the oxidation of ammonium to nitrite and its subsequent reduction to gaseous nitrogen. This process has the advantages of lower oxygen and organic carbon requirements, less sludge production and less CO<sub>2</sub> emissions compared to the conventional nitrification/denitrification process (Gustavsson, 2010). The denitrifying via nitrite biological phosphorus removal (DNBPR) offers the possibility to integrate phosphorus and nitrogen removal in a robust process in which the nitrite denitrification and phosphate uptake simultaneously occur under anoxic conditions. The low carbon to nitrogen (C/N) ratio of the supernatant requires the addition of external carbon source to achieve effective heterotrophic denitrification (Andreottola et al., 2012) and does not favour the conventional complete nitrification/denitrification path.

## **1.3 Need for external carbon source**

Anaerobic effluents originating from the digestion of sewage sludge have very low content of biodegradable organic matter. Furthermore, their highly nitrogenous character means that an external carbon source must be added to accomplish the processes of denitrification and enhanced biological phosphorus removal (EBPR). Thus, nitritation/denitritation can be coupled to certain short-chain fatty acids (SCFAs) to enhance the DNBPR. Bio-processes via nitrite allow greater residual polyhydroxyalkanoates (PHA) in activated sludge. The excess sludge may be rich in P (up to 10%) and in PHA (up to 15%), an optimal condition for its ex situ valorisation as a fertilizer or in the field of bioplastics. The SCFAs composition has also been reported to affect the performance of EBPR (Feng et al., 2009). Recent results have shown that carbon sources

that contain a mixture of SCFAs can improve DNBPR (Ji and Chen, 2010). A key factor in determining the cost-effectiveness of biological phosphorus removal is the relative amount of organic material (i.e. carbon source) that can be used by the polyphosphate accumulating organisms (PAOs). The process requires significant concentration of volatile fatty acids (VFAs) and readily biodegradable COD (rbCOD). The VFA/PO<sub>4</sub>-P ratio should be at least 4 (Neethling et al., 2005) and the rbCOD/PO<sub>4</sub>-P ratio around 15; otherwise the performance of the EBPR decreases (Barnard et al., 2005; EPA, 2008). The addition of chemically synthesized SCFAs (i.e. acetic and/or propionic acid) to improve biological nutrients removal increases the operating cost of the process, as the price of the commonly used synthetic carbon sources has increased significantly over the last decade. Significant cost savings and carbon footprint reduction can arise when liquid effluent derived from organic waste is supplied as carbon source.

## **1.4 Generation of SCFAs through sludge fermentation**

Fermenting sludge to generate SCFAs seems to be a sustainable process to produce an external carbon source with favourable characteristics that can be used in the subsequent biological short-cut nutrients removal process, exhibiting the additional advantage of decreasing the amount of sludge to be disposed of. Research is focusing on the investigation of sludge alkaline fermentation for enhancing the composition of the external carbon source (Zheng et al., 2010). In fermentation, the pH plays an important role in the hydrolysis of sludge and the production of SCFAs from excess sludge. Under alkaline conditions, the yield of SCFAs can be significantly enhanced (Yuan and Weng, 2006). Recent studies have demonstrated enhanced SCFAs production and inhibition of methanogenic activity (resulting in less SCFAs

consumption) under alkaline conditions (Wu et al., 2010). NaOH and Ca(OH)<sub>2</sub> are widely used for alkaline sludge treatment, which can affect waste activated sludge (WAS) hydrolysis, acidification and dewatering ability (Kim et al., 2003). Therefore, the reagent used for the pH adjustment in alkaline fermentation influences the effectiveness of the process. The optimum pH range 9–11 was reached using NaOH and Ca(OH)<sub>2</sub> (Su et al., 2013). This technique is not economically and environmentally sustainable and enhances the salinity of the carbon source, thus decreasing the rates of nitrification/nitrification. Furthermore, the sludge dewatering characteristics and the separation of the produced fermentation liquid from sludge can be adversely affected by the use of NaOH (Su et al., 2013). Recent studies have shown that the use of WAS fermented liquid as carbon source results in the reduction of nitrous oxide (N<sub>2</sub>O) and nitric oxide (NO) production during the via nitrite processes (Zhu and Chen, 2011).

Feng et al. (2009) examined the influence of solids retention time (SRT) and temperature on sludge hydrolysis and continuous production of SCFAs during WAS fermentation at alkaline pH. The increase of SRT and temperature increased the hydrolysis of WAS and benefited the production of SCFAs, influencing the proportions of acetic acid and propionic acid in the fermented liquid. SRT affects not only the concentration but also the composition of VFAs. At pH = 10 the change in SRT was found to be more influential for acetic acid and propionic acid, and less influential for the slightly long-chain fatty acids (i.e. n-butyric, iso-butyric, n-valeric, iso-valeric acids). Bouzas et al. (2002) demonstrated that SRT above 6 d did not significantly improve the VFA yields; however, an important decrease of VFA was observed with a SRT of 4 d.

## 1.5 PHAs synthesis by fermentation liquid and waste carbon

The SCFAs produced during fermentation can be utilized and polymerized into PHA by bacteria when there is an essential growth-limiting component, such as N, P or oxygen limitation with excess carbon source (Keshavarz and Roy, 2010). Since PHAs are truly biodegradable (Yu and Si, 2001), they are regarded as potential substitutes for traditional petrochemically produced plastics. However, PHA products are ten times more expensive to produce than polyethylene products (Lee, 1996). The polyethylene product largely depends on the cost of raw materials, such as pure cultures and substrate media (Kasemsap and Wantawin, 2007). The increase in the price of crude oil is a notable driving force for extended research on biopolymers production from alternative sources. The versatility of PHAs has made them 'good candidates' for the study of their potential in a variety of areas from biomedical/medical fields to food, packaging, textile and household material (Keshavarz and Roy, 2010). To conclude, fermenting excess sludge to generate carbon source for PHA production and P removal by activated sludge can be a sustainable process (Tong and Chen, 2007).

## 1.6 Objective of current work

In this work, anaerobic supernatant of sewage sludge was used to adjust the initial pH in the fermentation process of sewage sludge. The impact of several parameters on the fermentation process were examined including the temperature, the pH, the retention time, the initial total solids (TS) concentration of the sewage sludge, the use of pre-aerated and non-aerated anaerobic supernatant to control the pH.

## 2. MATERIAL AND METHODS

### 2.1 Source of sewage sludge and anaerobic supernatant

The thickened sewage sludge was obtained from a municipal WWTP located in Treviso province (North Italy), while the anaerobic supernatant was collected from the full-scale anaerobic digester of sewage sludge of the same plant. The thickened sludge consisted of mixed primary and secondary sludge which was thickened at a TS  $\approx$  3.5 – 4.5%.

### 2.2 Sewage sludge fermentation

The impact of temperature, TS concentration, type of solution for pH adjustment on sewage sludge fermentation and SCFAs accumulation were investigated by operating batch reactors, which were made of plexiglas and had a working volume of 1.0 L each. All reactors were stirred at a speed of 100 rpm and the SCFA concentration was measured daily.

**CO<sub>2</sub> stripping:** Anaerobic supernatant samples of 300 mL were introduced into a cylindrical glass tube of 100 mL and were continuously aerated using air diffusers. The pH, alkalinity and ammonium variation with time was recorded, while the temperature was maintained at  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

**Temperature:** Effect of temperature on the fermentation of sewage sludge was examined by operating two batch reactors at  $30 \pm 1^{\circ}\text{C}$ ,  $55 \pm 1^{\circ}\text{C}$ . The pH was always maintained at 8.5 with the addition of pre-aerated anaerobic supernatant by employing an initial sewage sludge/pre-aerated supernatant volume ratio of 0.4. The total duration of the experiments was 15 d. The TS concentration in the thickened mixed sewage sludge was 4%.

**Sludge total solids concentration and use of anaerobic supernatant to control pH:** Influence of the initial TS sludge concentration and of the pH control through the addition of anaerobic supernatant on the alkaline fermentation

process were examined in six batch reactors that were operated in parallel. Two different solids concentrations were tested; 4.5% TS and 6.5% TS. The experiments were performed with and without pH adjustment. The batch tests that were conducted without any control of the pH were the ‘blank’ experiments, while in the other batch reactors the pH was controlled with the addition of pre-aerated and non-aerated anaerobic supernatant in order to maintain the pH at 8.5. In the pre-aerated anaerobic supernatant the CO<sub>2</sub> stripping procedure explained above was followed to increase the pH value. In all the batch fermentation reactors the temperature was kept constant at  $37^{\circ}\text{C}$ . After the addition of the sewage sludge in the reactors, the operation time, the pH and the SCFA concentration were measured every day.

**Effect of pH control using caustic soda:** Four batch fermentation reactors were operated in parallel. In each reactor the pH was adjusted daily at 8, 9, 10 and 11 using NaOH and was monitored for SCFAs production for 8 days at a constant temperature of  $37^{\circ}\text{C}$ .

**Calculation of VFA efficiency:** the VFA efficiency (mg VFA/g VS) was calculated as the VFA concentration (mg/L) minus the initial VFA concentration (mg/L) divided by the initial volatile solids (VS) concentration (g/L) of sewage sludge.

### 2.3 Sampling and analytical methods

Grab samples were taken. Physicochemical characterization of the anaerobic supernatant was conducted. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined using standard methods 2540 D and E respectively, chemical oxygen demand (COD) was determined by the open reflux method (method 5220 B), ammonium nitrogen (NH<sub>4</sub>-N) by distillation and titration (methods 4500 B, C), total Kjeldahl nitrogen (TKN) with the semi-micro-Kjeldahl (method 4500 C), and

alkalinity by the titration standard (method 2320 B). The aforementioned methods are all described in detail in standard methods (APHA, AWWA, WEF, 1998). Phosphates ( $\text{PO}_4\text{-P}$ ) were determined by ion chromatography (Dionex ICS-90 with AG14 and AS14 columns). The sewage sludge was analyzed for TS, total volatile solids (TVS), COD, nitrogen and phosphorus according to standard methods. VFAs in the fermentation liquid (C2–C7) were analyzed by gas chromatography (Column: Nukol 15 m, 0.53 ID; temperature: 85–125 °C, 30 °C/min; carrier:  $\text{N}_2$ , 5 mL/min).

### 3. RESULTS AND DISCUSSION

#### 3.1 Substrate characteristics (anaerobic supernatant and sewage sludge)

The anaerobic supernatant was collected from the full-scale anaerobic digester of sewage sludge in Carbonera (Italy) and physicochemical analysis was conducted (Table 1a). The mixture of primary and activated sludge was collected after the thickener and the main physicochemical characteristics are summarized in Table 1b. The concentration of the total solids in the thickened mixed sludge (i.e. primary and waste activated sludge) was in the range of 3.5–4.5%.

**Table 1a** Characteristics of the anaerobic supernatant

Parameter	Average $\pm$ standard deviation
pH	7.63 $\pm$ 0.4
TSS (mg/L)	323.3 $\pm$ 129.5
TVS (mg/L)	273.0 $\pm$ 129.8
COD (mg/L)	363.2 $\pm$ 94.0
SCOD (mg/L)	155.4 $\pm$ 86.5
$\text{NH}_4\text{-N}$ (mg/L)	472.1 $\pm$ 100.8
TKN (mg/L)	546.3 $\pm$ 42.2
$\text{PO}_4\text{-P}$ (mg/L)	18.9 $\pm$ 7.3
TP (mg/L)	25.8 $\pm$ 1.9
Alkalinity pH 4.3 (mg $\text{CaCO}_3\text{/L}$ )	1480 $\pm$ 309
Alkalinity pH 5.7 (mg $\text{CaCO}_3\text{/L}$ )	1955 $\pm$ 237

**Table 1b** Sewage sludge characteristics

Parameter	Average $\pm$ standard deviation
TS (mg/L)	39796 $\pm$ 3319
TVS (mg/L)	33420 $\pm$ 3526
TVS (%)	84
COD (mg/g TS)	914.2 $\pm$ 40.8
TKN (mg/g TS)	33.9 $\pm$ 7.9
TP (mg/g TS)	13.7 $\pm$ 2.1

### 3.2 Stripping CO<sub>2</sub> in the supernatant

The stripping of CO<sub>2</sub> was accomplished through the provision of air by suitable diffusers. The rate of CO<sub>2</sub> transfer to the gas state  $R_{CO_2}$  is given by the following equation (Lisitsin et al, 2008):

$$R_{CO_2} = K_L \alpha ([CO_2]_{bulk} - [CO_2]_{equil})$$

Where:

[CO<sub>2</sub>]<sub>bulk</sub>: CO<sub>2</sub> concentration in wastewater (mol)

[CO<sub>2</sub>]<sub>equil</sub>: gas-liquid CO<sub>2</sub> equilibrium concentration (mol)

$K_L$ : Overall mass transfer coefficient (m/s)

$\alpha$ : Interfacial area of the air bubbles (1/m)

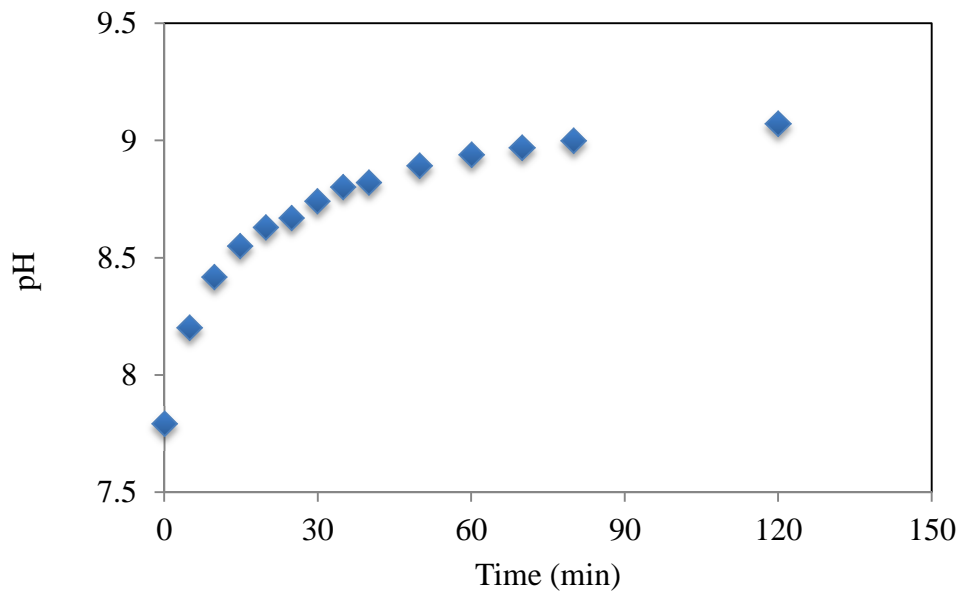
As the air comes into contact with water, the dissolved CO<sub>2</sub> within wastewater is transferred from the liquid to the gas phase. The driving force for this to occur is the concentration gradient between the CO<sub>2</sub> concentration in wastewater and the CO<sub>2</sub> equilibrium concentration. As seen in Fig. 1, the pH increased up to 9.1 after 2 h. The stripping of CO<sub>2</sub> resulted in a reduction of the total alkalinity by approximately 40% and of ammonia concentration by 20%.

### 3.3 Temperature

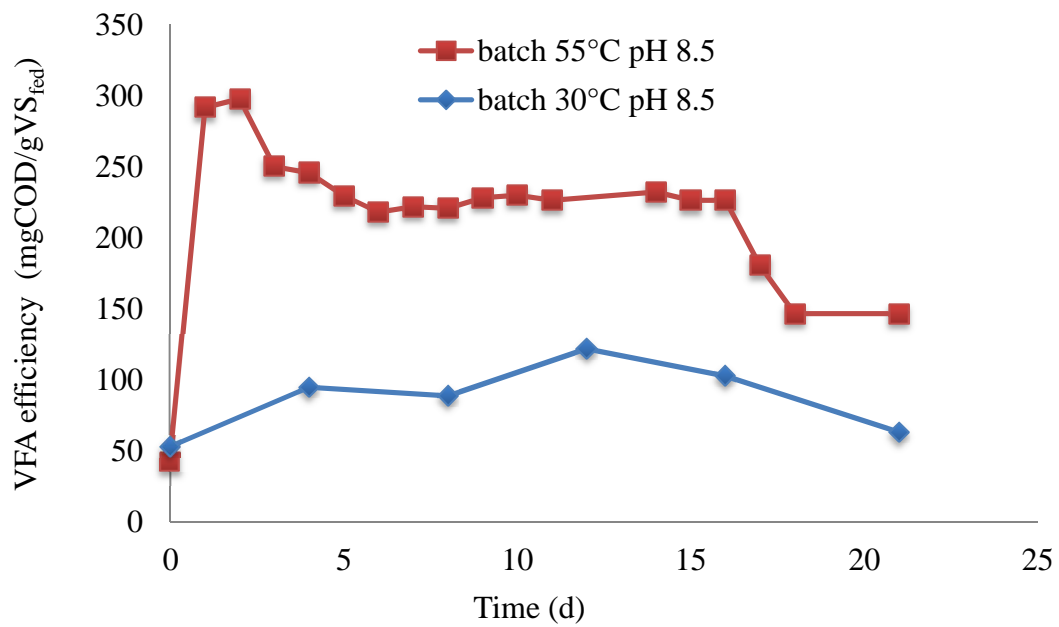
Fig. 2 shows the effect of temperature on the production of VFAs. The fermentation tests were conducted for 15 days in mesophilic and thermophilic conditions (i.e. 30 and 55°C). In order to keep a constant pH (8.3–8.8), during the experimental period some amount of pre-aerated supernatant was added. The maximum VFA efficiency was 298 mg COD/g VS<sub>fed</sub> after only 2 days of fermentation at 55°C. In the case of mesophilic (i.e. 30°C) fermentation, the maximum VFA efficiency was only 122 mg COD/g VS<sub>fed</sub> and occurred 12 days after the initiation of the fermentation process. The thermophilic environment improved the hydrolysis of the organic matter, since the maximum VFA efficiency was 2.5 times higher compared to the respective one obtained under

mesophilic conditions. Furthermore, the time required to reach the maximum VFA concentration was much shorter during the thermophilic temperatures. Moreover, the pH at thermophilic environment was much more stable; after the first two days of the fermentation process, the pH was stable within 8.5–8.8 and thus, pH adjustment with the supernatant was not required. On the contrary, under mesophilic conditions it was difficult to maintain stable pH (at 8.5) with the addition of pre-aerated supernatant, and thus continuous dosing was required.

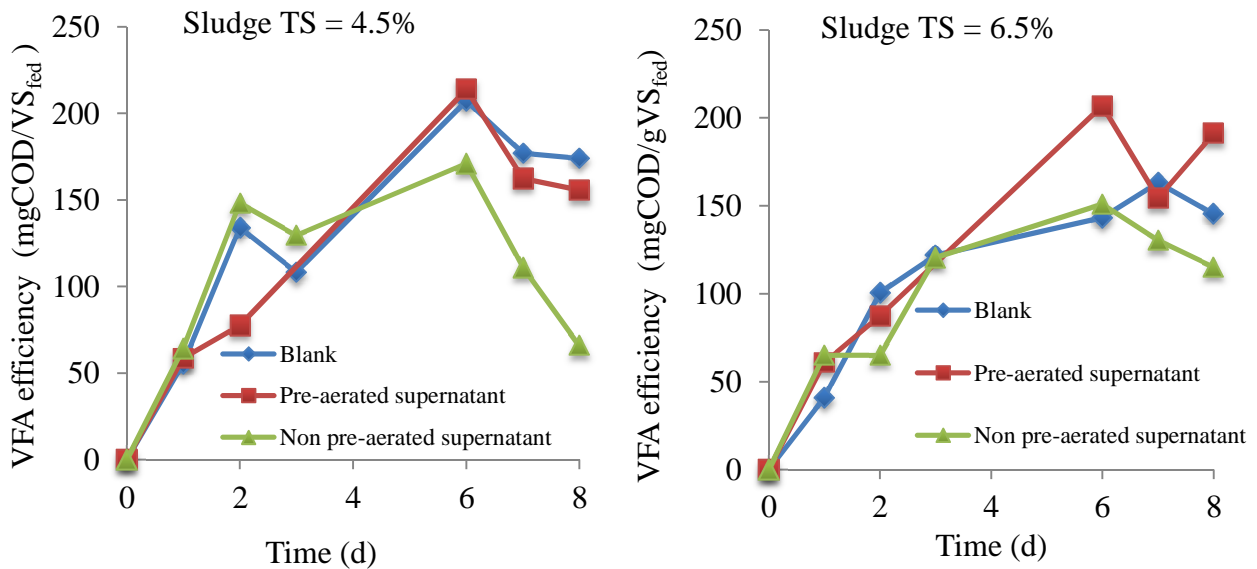
The results of other studies have also shown that the production of VFAs increases with temperature and the sludge hydrolysis is higher at elevated temperatures (Mahmoud et al., 2004). Feng et al. (2009) found that more soluble protein and carbohydrate were produced at higher temperature during WAS fermentation, while the hydrolysis of WAS at pH 10 could be improved by increasing the temperature. Zhang et al. (2010) found that the optimum pH for SCFAs production was temperature dependent; the maximum SCFAs yields were obtained in the following order: thermophilic, pH 8 > mesophilic, pH 9 > ambient, pH 10 > ambient, uncontrolled pH. This shows that the SCFAs yield is both pH and temperature dependent. The best combination of pH and temperature is required in order to maximize the SCFAs yield. Mahmoud et al. (2004) also reported that primary sludge hydrolysis at 35°C was significantly higher than that at 25°C. Feng et al. (2009) observed that for the same SRT (= 12 d) and pH (= 10) the increase in temperature from 10°C to 35°C resulted in an increase of the soluble protein concentration from 312.7 to 3050 mg COD/L and the soluble carbohydrate concentration from 80.7 to 418.9 mg COD/L. Finally, in our work, we saw that both the rate and the maximum yield of VFAs were significantly higher when thermophilic conditions were employed rather than mesophilic.



**Figure 1** Effect of CO<sub>2</sub> stripping on the supernatant pH



**Figure 2** Impact of temperature on the efficiency of the fermentation process



**Figure 3** Effect of initial solids concentration in sludge and solution type on VFA efficiency

### 3.4 Total solids concentration and type of solution for pH adjustment

Anaerobic supernatant of sewage sludge was used to adjust the pH in the fermentation process of sewage sludge. Fig. 3 shows the effect of the initial TS concentration (4.5% and 6.5%) and the type of anaerobic supernatant used (i.e. no supernatant-blank, pre-aerated, non-aerated) for pH adjustment on the production of VFAs. The highest VFA efficiency in almost all cases was achieved 6 days after the initiation of the fermentation process. The highest VFA concentration was obtained for the sludge having the highest initial TS concentration (i.e. 6.5%). Specifically, for the 'blank' sludge the highest VFA was 12.6 g COD/L (day 7) and for sludge in which pre-aerated anaerobic supernatant was dosed the maximum VFA was 12.0 g COD/L (day 6). The use of pre-aerated anaerobic supernatant seems to produce a higher VFA content than the use of non aerated anaerobic supernatant. However, the addition of the anaerobic supernatant could not maintain the pH at the desired levels. The pH was almost the same in all batch

configurations 6 days after the initiation of the experiments.

Table 2 presents the concentrations of SCFAs that were contained in the fermented liquid at the 6<sup>th</sup> day of the fermentation process. The type of anaerobic supernatant used for pH adjustment and the solids concentration of sewage sludge affected the proportions of the SCFAs to the total VFAs and the efficiency of the fermentation process. Higher proportion of propionic acid and butyric acid were obtained in the 'blank' and 'without any aeration' anaerobic supernatant. On the other hand, the proportion of propionic acid and butyric acid was higher in the experiments that were performed with higher concentration of solids.

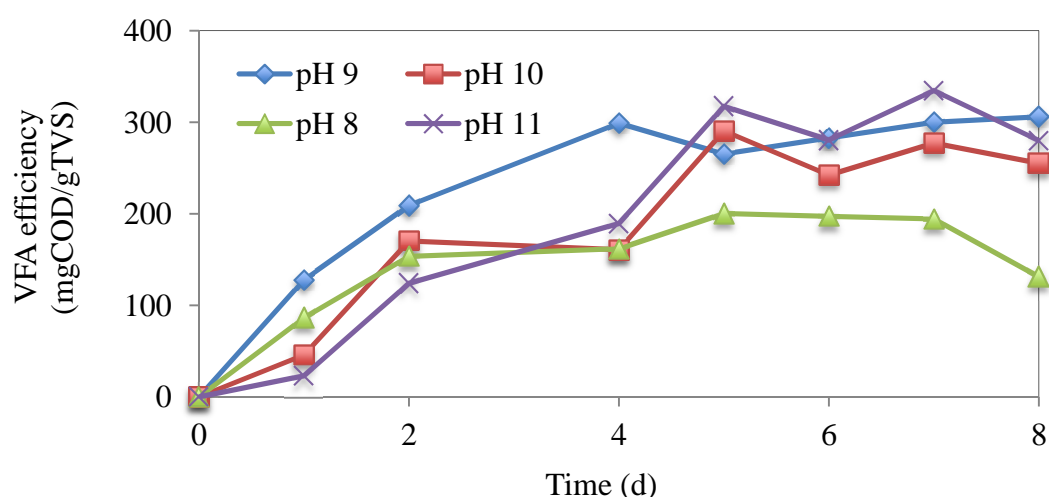
The highest ratio of VFA/NH<sub>4</sub>-N was obtained in the 'blank' experiment (= 17 g COD/g N). This work demonstrated that high TS concentration (6.5%) and a retention time of 6 days were the most favourable conditions for fermentation. The use of pre-aerated anaerobic supernatant to control the fermentation pH did not significantly enhance the process efficiency.



**Table 2** SCFAs concentration in the fermented liquid produced at the 6<sup>th</sup> day of the fermentation process under different operating conditions

Acid <sup>a</sup>	TS: 4.5%			TS: 6.5%		
	Blank	Pre-aerated supernatant	Non-aerated supernatant	Blank	Pre-aerated supernatant	Non-aerated supernatant
Hac (mg COD/L)	2595	4011	2143	2989	5291	5291
Hbut (mg COD/L)	1706	1515	1223	2074	2316	1975
Hpr (mg COD/L)	3403	1861	3034	4046	4046	4013
VFA (mg COD/L)	9547	8597	8084	11409	11955	11219

<sup>a</sup>Hac: acetic acid; Hbut: butyric acid; Hpr: propionic acid

**Figure 4** Effect of pH on the production of VFAs in the fermentation process

### 3.5 Effect of pH

The effect of pH (8–11) on VFA production was also investigated by the daily control of the pH using caustic soda at the temperature of 37°C. Fig. 4 shows the variation in the VFA efficiency with time. The maximum VFA efficiency was obtained for a residence time of 6–7 days. The increase in pH from 8 to 9 resulted in enhanced maximum VFA efficiency (i.e. from  $\approx 200$  mg COD/g VS<sub>fed</sub> to 300 mg COD/g VS<sub>fed</sub>). The maximum VFA efficiency obtained in the reactor for pH 8 was similar to

that obtained with the use of sludge in which pre-aerated supernatant was used to initially adjust the pH at 8.5 (i.e. 200 mg COD/g VS<sub>fed</sub>).

### CONCLUSIONS

The application of alkaline fermentation for the treatment of primary and activated sludge can provide the external carbon source that is required to treat the anaerobic supernatant and remove nutrients at low cost. This work showed that VFA production in the fermenta-

tion liquid is favoured at higher TS concentration (6.5%) and at a retention time of 6–7 days when operating at mesophilic temperatures, reaching up to 12 g COD/L. Thermophilic temperatures (55°C) could further increase VFA production and decrease the required residence time to only 2 days. The use of pre-aerated supernatant to initially adjust the pH (around 8.5) did not enhance VFA production compared to that obtained with the continuous adjustment of the pH at 8 using caustic soda.

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