Optimization of the hydrolytic-acidogenic anaerobic digestion stage (55 ºC) of sewage sludge: influence of pH and solid content

*Sergio Ponsá[1], Ivet Ferrer[2], Felícitas Vázquez[3] and Xavier Font[1]**

[1]Composting Research Group

Department of Chemical Engineering

Escola Tècnica Superior d'Enginyeria

Universitat Autònoma de Barcelona

Bellaterra (Cerdanyola del Vallès, 08193-Barcelona, Spain)3

[2]Environmental Engineering Division

Department of Hydraulic, Maritime and Environmental Engineering

Technical University of Catalonia

C/ Jordi Girona 1-3 (08034-Barcelona, Spain)

[3],Institute of Biotechnology and Biomedicine Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès, 08193-Barcelona, Spain)

*Corresponding author:

Xavier Font

Tel.: (34) 93 5814480; Fax: (34) 93 5812013; E-mail: xavier.font@uab.cat

Abstract:

In conventional single-stage anaerobic digestion processes, hydrolysis is regarded as the rate limiting step in the degradation of complex organic compounds, such as sewage sludge. Two-stage systems have been proposed to enhance this process. However, so far it is not clear which are the best conditions for a two-stage anaerobic digestion process of sewage sludge, in terms of temperature and hydraulic retention time of each stage. The aim of this work was to determine the optimal conditions for the hydrolytic-acidogenic stage treating real sludge with a high concentration of total solids (40-50 g L^{-1}) and volatile solids (25-30 g L^{-1}), named high concentration sludge. The variables considered for this first stage were: hydraulic retention time , (1 to 4 days) and temperature (55 and 65ºC). Maximum volatile fatty acids generation was obtained at 4 days and 3 days hydraulic retention time for 55ºC and 65ºC, respectively. Consequently, 4 days hydraulic retention time and temperature of 55ºC were set as the working conditions for the hydrolytic-acidogenic stage treating high concentration sludge . The results obtained when operating with high concentration sludge were compared with a low concentration sludge low concentration sludge consisting of 17-28 g L^{-1} Total Solids and 13-21 g L^{-1} Volatile Solids. The effect of decreasing the influent sludge pH, when working at the optimal conditions established, was also evaluated.

Keywords: Anaerobic digestion; hydrolysis-acidogenesis; thermophilic; two-stage process, volatile fatty acids; waste sludge.

Abbreviations

HRT: Hydraulic Retention Time (d^{-1})

TS: Total Solids ($g \cdot L^{-1}$)

VS: Volatile Solids ($g \cdot L^{-1}$)

HCS: High Concentration Sludge

LCS: Low Concentration Sludge

VFA: Volatile Fatty Acids

H-A: Hydrolytic-Acidogenic

WWTP: Wastewater Treatment Plants

CSTR: Continuously Stirred Tank Reactor

COD: Chemical Oxygen Demand ($g\ kg^{-1}$)

OLR: Organic Loading Rate ($g\ VS_{fed} \cdot (L_{reactor} \cdot day)^{-1}$)

PCO_2: CO_2 content in the biogas (%)

SBP: Specific Biogas Production (L biogas $(g\ VS_{fed})^{-1}$)

VSR: Volatile Solids Removal (%)

VFAP: Volatile Fatty Acid Production (g VFA produced$\cdot(g\ VS_{fed})^{-1}$)

1. Introduction

The residue generated during the primary, secondary and tertiary wastewater treatment is often called “sludge”. Anaerobic digestion is an appropriate technique for the treatment of sludge before its final disposal and it is employed worldwide as the oldest and most important process for sludge stabilization (Metcalf and Eddy, 1991; Mata-Alvarez *et al*., 2000).

The microbiology of anaerobic digestion is complicated, involving several bacterial groups forming a complex interdependent food web. However, four major steps can be distinguished. In the first hydrolysis step, both solubilisation of insoluble particulate matter and biological decomposition of organic polymers to monomers or dimmers take place. Acidogenesis and acetogenesis follow in the second and third step while in the fourth and final step, methane is produced by methanogenic population (Pavlostathis and Giraldo-Gomez, 1991).

In a conventional single-stage anaerobic digestion process, hydrolysis, acidogenesis, acetogenesis and methanogenesis all take place in the same reactor. To maintain a favourable environment for the mixed culture of microorganisms in such a reactor, volatile fatty acids (VFA) production and utilization must be balanced. At short retentions times, VFA production could exceed the utilization, leading to reactor failure (Harper and Pohland, 1986; Siegert and Banks, 2005). Because the metabolic characteristics and growth rates of the methanogenic and acetogenic bacteria are different, two-stage anaerobic processes to separate VFA and methane forming stages have been proposed to optimize each stage (Pohland and Ghosh 1971; Elefsiniotis and Oldham, 1994; Bhattacharya *et al*., 1996; Veeken and Hamelers, 1999; Shana *et al*., 2002; Oktem *et al*., 2006)

Hydrolysis is reported as the rate limiting step of anaerobic digestion of semi-solid wastes as sewage sludge, whereas methanogenesis is considered rate-limiting for fermentation of soluble substrates (Eliosov and Argaman, 1995; Gavala *et al*., 2003). Although, the specific operational conditions for acetogenic-methanogenic stages have been extensively studied, relevant literature on the acidogenic stage is still scarce. Few studies report optimal conditions for the hydrolytic stage, besides in all of them very low TS (5-10 g L^{-1}) and sometimes synthetic substrates are used (Shana *et al*., 2002; Yu *et al*., 2003). There is a lack of knowledge about operation conditions of acidogenic reactors fed with a real mixture of primary and secondary sludge with high TS and high VS concentrations. However, it is known that for a good performance of such reactors, methanogenic activity must be low. For this reason, short retention times are generally used in hydrolytic-acidogenic (H-A) reactors, since they favour the washing out of methanogenic microorganisms. Besides, neither high VFA concentrations nor high temperatures favour the growth of these microorganisms (Bhattacharya *et al*., 1996). Moreover both, temperature and HRT are important control parameters which can be manipulated causing considerable effects on the hydrolytic products (Elefsiniotis and Oldham, 1994, Metcalf and Eddy, 1995; Veeken and Hamelers, 1999; Ahn and Foster, 2000; Zábranská, 2000; Ahring *et al*., 2001). Additionally, a thermophilic range of temperatures is generally used in the acidogenic reactor operation since it results in a biochemical reactions rate acceleration and higher growth rate of microorganisms that means a higher hydrolytic rate (Pavlostathis and Giraldo-Gómez, 1991; Veeken and Hamelers, 1999; Lu, et al., 2007).

Many authors have also reported the influence of pH on the hydrolytic-acidogenic stage (Zoetemeyer *et al*., 1982; Joubert and Britz, 1986; Henry *et al*., 1987; Kisaalita *et al*., 1987; Yu *et al*., 2003; Massanet-Nicolau, J. *et al*., 2007), concluding that a slightly acid pH, close to 6, improves the working conditions for hydrolytic-acidogenic bacteria.

Sludge production in wastewater treatment plants is not constant, and neither are its characteristics, since daily precipitation, amongst others, directly affects the sludge solids content (De la Rubia, 2003). Moreover, in some cities, population varies in thousands of people depending on the season and the amount of wastewater treated in wastewater treatment plants (WWTP) fluctuates accordingly, which directly affects sludge characteristics in terms of solids content (De la Rubia, 2003).

The aim of this work is to investigate the thermophilic anaerobic hydrolysis of waste sludge of different solid content by providing optimal growth conditions to different anaerobic populations in a H-A reactor.

2. Materials and Methods

2.1 Acidogenic reactor

Experiments were carried out in a stainless steel continuously stirred tank reactor (CSTR) with a working volume of 4 L. The reactor was provided with a gas collection unit, an influent and effluent line and was operated continuously over a period of approximately 360 days. The reactor was mechanically mixed by stainless steel paddles on a central shaft operated at constant speed by an electric motor with a speed controller. The temperature was maintained constant at 55±1 or 65±1ºC, depending on the experiment, by using a heating tape (Entesis, 200 W, Spain) wrapped around the vessel and, a temperature digital controller (Osaka OR-31, Japan).

The reactor was kept under the same working conditions for a period corresponding to at least 3 HRT, ensuring steady-state conditions, and maintained for at least 3 more HRT for data collection. The reactor was operated in a semi-continuous way- A mixture of 25% primary and 75% secondary real sludges was fed to the reactor 3 times per day.

2.2 Sewage sludge and inoculum characteristics

Low Concentration Sludge (LCS) and High Concentration Sludge (HCS) were obtained from two different municipal wastewater treatment plants near Barcelona (Spain) and stored at 4ºC. Working ranges for TS and VS content in LCS were 17-28 g L^{-1} and 13-21 g L^{-1} respectively, whereas for HCS, TS content was 40-50 g L^{-1} and VS content was 25-30 g L^{-1}. A mixture 1:3 primary to secondary sludge was used in all experiments. Inoculum was obtained from a single-stage laboratory anaerobic digester which had been working for over 1 year with LCS. Main characteristics of inoculum are shown in Table 1.

2.3 Efficiency parameters

Optimal conditions for the H-A process were determined by comparing process performance at 4 different HRT (1, 2, 3 and 4 days) and 2 temperatures within the thermophilic rante (55 and 65 ºC). Influent acidification was also considered as an improving option for the H-A reactor performance.

Efficiency parameters considered were CO_2 content in biogas, specific biogas production (SBP), VS removal and VFAproduction (VFAP). The latter was considered as the key parameter since it is the main acid-stage product reflecting the organic matter that has been hydrolyzed. VFA generation was expressed as g $VFA_{produced}(g\ VS_{fed})^{-1}$

2.4 Analytical methods

TS and VS content, total COD, total alkalinity, were determined according to Standard Methods (APHA, 1999). VFA were determined by gas chromatography (Perkin Elmer Autosystem XL Gas Cromatograph) with a flame ionization detector and a capillary column (J&W Scientific High Resolutions Gas Cromatography Column, 30 m, inner diameter 025 mm, film 0.25µm) and oven, injector and detector temperatures of 130ºC, 250ºC and 260ºC respectively. Biogas composition was also determined by gas chromatography (Perkin Elmer Autosystem XL Gas Cromatograph) with a stainless silica column (HAYESEP D 3m, 1/8" inner diameter and oven, injector and detector temperatures of 70ºC, 150ºC and 180ºC respectively.

Statistical significance of values of different parameters obtained during each experimental condition was carried out by means of F-test (variance analysis) and Student's t-test (mean analysis) using a 5% of significance level.

3. Results and discussion

3.1 Hydrolytic specialization stage

Since the inoculum used for these experiments came from a single stage digester, the transition from a methanogenic to a hydrolytic reactor (specialization stage) was considered of particular importance for the development of the hydrolytic process.

Initially a 10 days HRT was used since it was the operating HRT of the inoculum source reactor. In order to wash out the methanogens, consequently obtaining a specialized acidogenic bacteria population, HRT was progressively reduced until a sharp decreasein SBP and a rise in VFA generation were reached (Figure 1, HRT 5-4 days).

During the acid-stage bacteria specialization, HRT was reduced progressively from 10 to 4 days using LCS as feeding material. The main feeding characteristics during this experimental length are presented in Table 1. When reducing the HRT a drop in the biogas production and VS removal would be expected provided that hydrolysis is enhanced because the methanogenic population became growth limited. On the other hand, an increase in the PCO_2 in biogas and VFA accumulation would also be expected since they are the main acid-stage products.

Evolution of specialisation parameters in the H-A reactor during bacteria specialization is shown in Figure 1. From this Figure it can be observed that VS removal followed a decreasing pattern when reducing the HRT with values from 30% and 38%, as expected.

As shown in Figure 1, SBP followed an increasing tendency with decreasing HRT from 10 to 5 days. However, when HRT was reduced from 5 to 4 days, a significant drop in the SBPfrom 0.140 to 0.059 $L_{biogas}(g\ VS_{fed})^{-1}$ was registered. The reason for this profile may be explained as follows. Assuming that, at 10 days HRT (OLR of 2.1 g VS_{fed} $(L_{reactor}d)^{-1}$), anaerobic bacteria were able to rapidly degrade easily biodegradable organic matter, obtaining the corresponding biogas. In a similar manner, at 7 and 5 days HRT (OLR of 3.0 and 4.2 g VS_{fed} $(L_{reactor}\cdot d)^{-1}$, respectively) anaerobic microorganisms were still able to degrade the easily biodegradable organic matter that was fed to the system, and consequently when more VS were fed, more biogas was produced. Since VSR rate at 10, 7 and 5 days HRT were 0.81, 0.92 and 1.2 g $VS_{removed}\cdot(L_{reactor}\cdot d)^{-1}$ respectively, SBP increased accordingly. The critical step was the HRT reduction from 5 to 4 days, when a considerable drop in the SBP was observed. As a portion of the VS fed may be readily degradable organic matter, when reducing HRT (increasing OLR) the VFAP coming from this readily degradable organic matter may increase accordingly. At these working conditions, methanogenesis should become the limiting stage (Zahller *et al*. 2007). Therefore, it could mean that at these conditions, methanogenic population became growth limited and were being washed-out of the system.

PCO_2 increased when reducing the HRT because methanogenic microorganisms which are methane producers were being washed-out of the digester. Nevertheless a certain percentage of methane was always detected in the biogas, due to the continuous re-inoculation with methanogenic microoganisms contained in the raw sludge fed.

VFA generation followed an increasing tendency with decreasing the HRT. Variation was from 0.004 g $VFA_{produced}(g\ VS_{fed})^{-1}$ at 10 days HRT to 0.017-0.019 g $VFA_{produced}(g\ VS_{fed})^{-1}$ at 5 and 4 days HRT respectively. This may indicate that biochemical equilibrium between anaerobic populations was disrupted and became unbalanced as HRT decreased.

The main goal of the specialization stage was to verify the biological reactions imbalance that suggested that methanogens were limited (inhibited or washed-out of the system). To our knowledge no references on methanogens inhibition by VFA in sewage sludge anaerobic digestion processes have been reported. However, some authors have reported data to recognize anaerobic digester destabilization and methanogen's growth limitation using VFA as indicators treating different wastes such as synthetic media (Marchaim and Krause, 1993; Hanaki, 1994; Wu *et al*., 1995) (Kugelman and Chin, 1971; wastewaters (Boardman *et al*., 1995); swine wastes (Hill and Holmbert, 1998). VFA are easily lab measurable parameters which can give essential process information. Comparing limit VFA concentrations reported in the literature (taking into account that they were not obtained for sludge anaerobic digestion) with those obtained in the present work, it can be observed that acetic acid concentration (maximum value of 0.5 g

L^{-1}) was always below the failure concentration limit of 0.8 g L^{-1} (Hill and Holmbert, 1998). However, the propionic to acetic acid ratio (maximum value of 4.5 at 4 days HRT) was always above the limit of 1.4, indicative of the one-stage anaerobic digester destabilization (Hill and Holmbert, 1998). Effluent concentration of iso-butiric and iso-valeric acids were always above the limits proposed by Wu (1995) and Boardman (1995) (0.005 g L^{-1} and 0.015 g L^{-1}, respectively), and reached maximum values of 0.6 g L^{-1}, also indicating one-stage anaerobic digester destabilization.

Hill and Holmert (1988) established that methane yields below 0.25 g CH_4(g VS_{fed})$^{-1}$ were indicative of anaerobic digester instability. In this work, during the specialization period, methane yield never exceeded 0.14 g CH_4(g VS_{fed})$^{-1}$, thus indicating that methanogenic population were limited at all HRT.

Methane production is affected by physic–chemical conditions in the digester. Bacterial activity may be inhibited by either reaction substrates or products when they are present in extreme concentrations. For example, high VFA concentrations in the system cause the inhibition of methanogenesis. Under conditions of organic overloading, and in presence of inhibitors, methanogenic activity cannot remove hydrogen and volatile organic acids as quickly as they are produced. The result is the accumulation of acids, the depletion of buffering capacity and the depression of pH to levels that also inhibit the hydrolysis/acidogenesis stage. It has also been shown that even when process pH is optimal, accumulation of VFAs may contribute to reduce the rate of hydrolysis of organic solids, or even to inhibit it at extremely high levels (>10 g L^{-1}) (Palmisano and Barlaz, 1971). In addition, Kugelman and Chin (1971) established that methanogens were inhibited at acetic acid concentrations of 6 g L^{-1}, whereas Hanaki (1994) established 3.2 g L^{-1} as the propionic acid concentration limit for methanogenic inhibition. Siegert and Banks (2005) established that anaerobic digestion was evidently inhibited when VFA concentration was over 6 g L^{-1}. However, theses considerations have to be taken into account when operating in the same working conditions (same substrate, alkalinity, pH and temperature) otherwise the limits established can vary moderately. During the specialization stage, acetic acid, propionic acid ant total VFA concentrations never exceeded 0.73 g L^{-1}, 1.25 g L^{-1} and 2.41 g L^{-1} respectively. Therefore, it could be stated that the unique reason for methanogens limitation was the wash-out of the system and not the VFA inhibition.

3.2 Experiments with low TS and VS concentration sludge (LCS).

Once the results indicated that the prevalent population in the reactor was hydrolytic bacteria, optimal working conditions (in terms of HRT) for the H-A reactor were sought.

VFA generation, SBP, PCO_2 and VSR for the operational conditions assayed with LCS are shown in Figure 1. As it can be seen, VFAP further increased while decreasing HRT, whereas VSR decreased as HRT decreased. Maximum VFAP of 0.059 g $VFA_{produced}\cdot(g\ VS_{fed})^{-1}$, was obtained at 1 day HRT. This value was statistically different from the results corresponding to all the other HRT assayed. Once hydrolytic conditions have been reached, HRT < 4 days, the maximum VSR of 36% was obtained at 4 days HRT, being statistically equal to that obtained at 3 days HRT. PCO_2 was close to 55% in all the experiments with a slight decreasing tendency when increasing HRT, reaching a minimum value of 57% at 1 day HRT. SBP followed a decreasing tendency when decreasing HRT reaching the maximum, value of 0.140 l biogas · $(g\ VS_{fed})^{-1}$ working at 4 days of HRT.

Statistical analysis of obtained results is shown in Table 2. According to these analyses, best working conditions (those which allow maximum hydrolysis of organic matter, thus maximum VFA generation) correspond to 1 day HRT.

Furthermore, influent acidification in the H-A reactor was carried out to assess its effect on organic matter solubilisation when working at 1 day HRT since it has been shown (Yu *et al.*, 2003; Massanet-Nicolau, J. *et al.*, 2007) that low pH values in H-A stage improve hydrolysis. The results for influent acidification operation are shown and compared with normal influent operation in Table 3. Neither VFAP nor VSR were improved with influent acidification. Therefore, this option was discarded for H-A reactor operation. VFA concentration never exceeded inhibition levels during the length of this experimental stage.

3.3 Experiments with high TS and VS concentration sludge (HCS).

In order to acclimatize anaerobic microorganisms to HCS, the HRT of the H-A reactor was set to 4 days and HCS was fed for more than 1 month, until control parameters were stabilized. The main feeding characteristics during this experimental length are presented in Table 4.

The results obtained when working with HCS at 4, 3, 2, and 1 day HRT are shown in Figure 2. A maximum value for VFA production of 0.108 g $VFA_{produced}(g\ VS_{fed})^{-1}$ is observed when working at 4 days HRT, being this value statistically different from all the others (Table 5). A significant decrease in VFAP is observed when HRT is reduced from 4 to 3 days, probably due to the wash-out of part of the hydrolytic consortium, and it remains almost constant in subsequent HRT reductions. VSR is within the range of 20-25% during the whole experiment, with no significant variations.

PCO_2 fluctuated between 59% and 75%. However, the only statistical differences were found between 4 and 3 days HRT.

Table 5 shows the statistical analysis of these results. Since maximum VFAP was obtained at 4 days HRT and this result is statistically different from all the other results obtained at the other HRT assayed, it can be established that the best working conditions, in terms of organic matter solubilisation, were found at 4 days HRT.

These results differ from those obtained for LCS possibly due to an increasing of organic loading rate, from 5.3 g $VS_{fed}(L_{reactor}d)^{-1}$ for LCS at 4 days HRT to 7.8 g $VS_{fed}(L_{reactor}d)^{-1}$ for HCS at 4 days HRT). Another possible explanation may be the inhibition exerted by the VFA produced. It should be noticed that OLR for optimal working conditions for LCS was 13 g $VS_{fed}(L_{reactor}d)^{-1}$ whereas for HCS was 7.8 g $VS_{fed}(L_{reactor}d)^{-1}$. At these optimal conditions VFAP rate for LCS was 3.06 g $VFA_{produced}(L_{reactor}d)^{-1}$ and 3.34 g $VFA_{produced}(L_{reactor}d)^{-1}$ for HCS and the VSR in terms of $VS_{removed}(L_{reactor}d)^{-1}$ were 3.22 for LCS and 1.96 for HCS.

To compare similar OLR for the two solid contents assayed, results from HRT of 2 days for LCS and 4 days for HCS were used. Under these working conditions, OLR was 7.0 and 7.8 g $VS_{fed}(L_{reactor}d)^{-1}$ respectively. Corresponding VFAP rates were 1.06 g $VFA(L_{reactor}d)^{-1}$ for LCS and 2 days HRT and, 3.34 g $VFA(L_{reactor}d)^{-1}$ for HCS and 4 days HRT. These results indicate that the higher is the concentration in the feed, the higher the VFAP rate should be.

Influent acidification was also assessed with HCS. The results and the corresponding comparison are presented in Table 3.As it can be observed, neither VFA production nor VS removal increased at lower influent pH, thus influent acidification was also discarded as an alternative to improve the process. At the different HRT assayed, differences in VFAP were statistically significant while no significant differences were found for VSR. These results suggest that under the experimental conditions assayed, preacidification had a negative effect since it resulted in a 35% reduction in VFAP.

Some authors have reported that high temperatures improve the hydrolytic reactions kinetics (Gavala et al., 2003, Kuo and Cheng, 2007). In order to further improve the H-A performance, a working temperature of 65 ºC, was set up in the reactor and the results obtained are showed in Figure 3 and Table 6. VSR followed an increasing tendency, reaching the maximum and statistically different value of 33% when working at a 4 day HRT, meanwhile PCO_2 fluctuated between 62% and 70%. The maximum VFAP of 0.097 g $VFA_{produced}\cdot(g\ VS_{fed})^{-1}$ was registered at 3 days HRT, but it is not statistically different from the production obtained at 4 days HRT (0.091 g $VFA_{produced}\cdot(g\ VS_{fed})^{-1}$) and from the one obtained at 4 days and 55ºC (0.108 g $VFA_{produced}\cdot(g\ VS_{fed})^{-1}$).

Some facts have to be taken into account to establish the optimal working conditions in terms of HRT and temperature. It has been proved that a higher temperature improves (or at least statistically maintains) organic matter solubilisation during hydrolysis, expressed as VFA produced. However, working at higher temperatures conveys higher economical costs. In order to balance out the cost of higher temperature, a shorter HRT should be established. An economical analysis may be required to assess whether smaller reactor volume due to 1 day HRT reduction could compensate the energy expenses of 10ºC temperature increase

As a general discussion, if the results of this work were to be applied in a treatment plant, where the solid concentration at the entrance of the H-A reactor could be modified, for a given VS concentration, LCS process would allow treating the same amount of VS but in half the process time than HCS process, since the OLR (13 g $VS_{fed}(L_{reactor}\cdot day)^{-1}$) for the former is almost twice the OLR for the latter (7.8 g

$VS_{fed}(L_{reactor}\cdot day)^{-1}$. However, these solids would be hydrolysed to a lesser extend, as demonstrated by the corresponding VFAP 0.56 (g VFA produced·(g $VS_{fed})^{-1}$) and 0.108 (g VFA produced·(g $VS_{fed})^{-1}$) for LCS and HCS, respectively, in spite VFAP rate being similar in both processes. If less hydrolysed solids were to be obtained in the H-A reactor, this probably would mean that longer times would be needed in the subsequent anaerobic digestion to meet the required VS removal. In consequence, an economic analysis of the process would necessary comprise both stages, the H-A and the anaerobic digestion.

4. Conclusions

The results obtained in this work can be used to establish the best working conditions for the Hydrolytic-Acidogenic stage when treating sewage sludge of different solid content. These results indicated that maximum Volatile Fatty Acids Production for Low Concentration Sludge was obtained at 1 day Hydraulic Retention Time and an Organic Loading Rate of 13.0 g $VS_{fed}(L_{reactor}day)^{-1}$, whereas for High Concentration Sludge maximum Volatile Fatty Acids Production was obtained at 4 days Hydraulic Retention Time and an Organic Loading Rate of 7.8 g $VS_{fed}(L_{reactor}day)^{-1}$. Moreover, influent acidification did not increase Volatile Fatty Acids Production with either Low Concentration Sludge or High Concentration Sludge sewage sludge feeding while a higher operating temperature of 65ºC did not improve Volatile Fatty Acids Production when treating High Concentration Sludge.

The findings of this study can be used to optimise the Hydrolytic-Acidogenic stage when treating sewage sludge of different solid content. However, in order to assess their impact on the overall process, particularly on the process economics, both stages, the hydrolysis-acidogenic and the anaerobic digestion stage, should be considered.

Acknowledgements

The authors wish to thank the financial support provided by the Spanish Ministry of Science and Technology and FEDER (REN2002-00926/TECNO).

References

Ahn, J.H. and Forster, C.F. (2000) A comparison of mesophilic and termophilic anaerobic upflow filters. Bioresource Technology 73, 201-205.

Ahring, B.K., Mladenovska, Z., Iranpour, R. and Westrman, P. (2001) State of art and future perspectives of thermophilic anaerobic digestion. Proc. 9th World Congress on Anaerobic Digestion I., Antwerpen, Belgium. 455-460.

Bhattacharya, S.K., Madura, R.L., Walling D.A. and Farrell, J. B. (1996) Volatile solids reduction in two-phase and conventional anaerobic sludge digestion. Water Research 30(5), 1041-1048.

Boardman, G., Tisinger, J. and Gallagher, D. (1995) Treatment of clam processing wastewater by means of upflow anaerobic sludge blanket technology. Water Research 29(6), 1483-1490.

Climent, M., Ferrer, I., Baeza M.M., Artola, A., Vázquez, F. and Font, X. (2007) Effects of thermal and mechanical pretreatments of secondary sludge on biogás production under thermophilic conditions. Chemical Engineering Journal 133, 335-342.

De la Rubia, M.A. (2003) Puesta en marcha y optimización de la degradación anaerobia termofílica de lodos de EDAR. Tesis de Doctorado. Serv. Publicaciones de la Universidad de Cádiz. Cadiz, Spain.

Elefsiniotis P. and Oldham W.K. (1994) Effect of HRT on acidogenic digestion of primary sludge. Environmental Engineering 120(3), 645-660

Eliosov, B., Argaman, Y. (1995) Hydrolysis of particulate organics in activated sludge system. Water Research 29(1), 155–163.

Gavala, H.N., Yenal, U., Skiadas, I.V., Westermann, P. and Ahring, B.K. (2003) Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. Water Research 37, 4561-4572.

Hanaki K. (1994) Selective use of microorganisms in anaerobic treatment processes by application of immovilization. Water Research 28(4), 993-996.

Harper, S.R. and Pohland, F.G. (1986) Recent developments in hydrogen management during anaerobic biological waste water treatment. Biotechnology Bioengineering 28, 585–602.

Henry, M.P., Sajjad, A. and Ghosh, S. (1987) The effects of environmental factors on acid-phase digestion of sewage sludge. In: Proceedings 42nd Purdue Industrial Waste Congress; pp.727-737.

Hill, D.T. and Holmbert, R.D. (1988) Long chain volatile fatty acid relationship in anaerobic digestion of swine waste. Biological Wastes 23,195-214

Joubert, W.A. and Britz, T.J. (1986) The effect of pH and temperature manipulation on metabolite composition during acidogenesis in a hybrid anaerobic digester. Applied Microbiology Biotechnology 24, 253-258.

Kisaalita, W.S., Pinder, K.L. and Lo, K.V. (1987) Acidogenic fermentation of lactose. Biotechnology Bioengineering 33, 623-630

Kugelman, I.J. and Chin, K.K. (1971) Toxicity synergism and antagonism in anaerobic waste treatment processes. Anaerobic biological treatment processes. Advances in Chemistry Series, 105. American Chemical Society. Washington D.C

Kuo, W-C. and Cheng, K-Y. (2007) Use of respirometer in evaluation of process and toxicity of thermophilic anaerobic digestion for treating kitchen waste. Bioresource Technology 98(9), 1805-1811.

Lu, J., Gavala, H.N., Skiadas, I.V., Mladenovska, Z. and Ahring B.K. (2007) Improving anaerobic sewage sludge digestion by implementation of a hyper-thermophilic prehydrolysis step. Journal of Environmental Management, doi:10.1016/j.jenvman.2007.04.020

Marchaim, U. and Krause, C. (1993) Propionic to acetic ratios in overloaded anaerobic digestion. Bioresource Technology 43, 195.

Massanet-Nicolau, J., Dinsdale, R. and Guwy A. (2008) Hydrogen production from sewage sludge using mixed microflora inoculum: Effect of pH and enzymatic pre-treatment. Bioresource Technology, doi:10.1016/j.biortech.2007.12.012

Mata-Alvarez, J., Macé, S., Llabrés, P. (2000) Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresource Technology 74 (1), 3–16.

Metcalf and Eddy Inc. Biological Unit processes. (1991) In: Clark B.J., Morriss J.M., editors. Wastewater Engineering; treatment, disposal and reuse, pp. 359-444. McGraw-Hill International Editions, New York .

Oktem, Y. A., Ince, O., Donnelly, T., Sallis, P., and Ince, B. K. (2006) Determination of optimum operating conditions of an acidification reactor treating a chemical synthesis-based pharmaceutical wastewater. Process Biochemistry 41 (11), 2258-2263.

Palmisano A.C. and Barlaz, M.A. (1971) Microbiology of solid waste. In: R.H. Vreeland, Editor, The microbiology of extreme and unusual environments, pp. 72-104 CRC Press, London. .

Pavlostathis, S.G. and Giraldo-Gómez, E. (1991) Kinetic of anaerobic treatment: a critical review. Critical reviews in environmental Control 21(5), 411-490.

Pholand, F.G. and Ghosh, S. (1971) Developments in anaerobic stabilization of organic wastes, the two-phase concept. Environmental Technology 1, 225-266.

Shana A., Asaadi M., Morchoisne T. and Marsh P. (2002) Acid phase digestion pre-treatment and its impact on conventional mesophilic anaerobic digestion. I&D. Thames Water, Spencer House. Reading RG2 0JN

Siegert, I. and Banks C. (2005) The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. Process Biochemistry 40(11), 3412-3418.

Veeken, A. andHameleres, B. (1999) Effect of temperatura on hydrolisis rates of selects biowastes components. Bioresource Technology, 29, 249-254.

Wu, W., Jai, M., Thiele, J. and Zeikus, G. (1995) Effect of storage on the performance of methanogenic granules. Water Research 29(6), 1445-1452.

Yu, H.-Q., Zheng, X.-J., Hu, Z.-H. and Gu, G.-W. (2003) High-rate anaerobic hydrolysis and acidogenesis of sewage sludge in a modified upflow reactor. Water Science and Technology 48(4), 69-75.

Zábranská, J., Stepová, J. Wachtl, R., Jenicek, P. and Dohanyos, M. (2000) The activity of anaerobic biomass in thermophilic and mesophilic digesters at different loading rates. Water Science and Techonology 42(9), 49-56.

Zahller, J.D., Bucher, R.H., Ferguson, J.F., Stensel, H.D. (2007) Performance and stability of two-stage anaerobic digestion. Water Environment Research 79(5), 488-497.

Zoetemeyer, R.J., van den Heuvel, J.C. and Cohen, A. (1982) pH influence on acidogenic dissimilation of glucose in an anaerobic digestor. Water Research 16, 303-311.

Figure Legends

Figure 1: Hydrolytic-acidogenic anaerobic digestion stage of LCD at 55 ºC. Volatile solid (VS) removal, CO_2 content in biogas, volatile fatty acid (VFA) generation and biogas production (SB) as function of HRT.

Figure 2: Hydrolytic-acidogenic anaerobic digestion stage of HCS at 55 ºC. Volatile solid (VS) removal, CO_2 content in biogas, volatile fatty acid (VFA) generation and biogas production (SB) as function of HRT.

Figure 3: Hydrolytic-acidogenic anaerobic digestion stage of HCS at 65 ºC. Volatile solid (VS) removal, CO_2 content in biogas, volatile fatty acid (VFA) generation and biogas production (SB) as function of HRT.