ELSEVIER

Process Biochemistry 41 (2006) 79–86

PROCESS BIOCHEMISTRY

www.elsevier.com/locate/procbio

Effect of solids retention time (SRT) on pilot scale anaerobic thermophilic sludge digestion

M.A. de la Rubia *, M. Perez, L.I. Romero, D. Sales

Department of Chemical Engineering, Food Technology and Environmental Technology, Faculty of Sea and Environmental Sciences, University of Cadiz, Campus Río San Pedro s/n, 11510 Puerto Real, Cadiz, Spain

Received 15 October 2004; received in revised form 31 January 2005; accepted 15 March 2005

Abstract

This paper describes anaerobic thermophilic sludge digestion (55 °C) in a continuously stirred tank reactor (CSTR) on a pilot-plant scale (150 L). The experimental protocol was defined to examine the effect of the increase in the organic loading rate on the efficiency of the digester and to report on its steady-state performance. The reactor was subjected to a programme of steady-state operation over a range of solids retention times (SRTs) of 75, 40, 27, 20 and 15 days and organic loading rates (OLR) in the range 0.4–2.2 kg VS/(m^3 day). The digester was fed with raw sludge (containing approximately 35 kg/m^3 volatile solids (VS)) once daily during the 75-day SRT period, twice daily during the 40-day SRT period and three times a day during the 27-, 20- and 15-day SRT periods. The reactor was initially operated with an organic loading rate of 0.4 kg VS/(m^3 day) and an SRT of 75 days. The volatile solids removal efficiency in the reactor was found to be 73%, while the volumetric methane production rate produced in the digester reached 0.02 m^3/(m^3 day). Over a 338-day operating period, an OLR of 2.2 kg VS/(m^3 day) was achieved with 49.1% VS removal efficiency in the pilot sludge digester, at which time the volumetric methane production rate content of biogas produced in the digester reached 0.4 m^3/(m^3 day). The chemical oxygen demand (COD) mass balance obtained indicated that COD used for methane generation increased when the SRT was decreased or when the influent organic loading rate was increased. This implies that the amount of COD used in the anabolism route decreased with SRT due the microbial population becoming adapted to new operational conditions and more COD being used to generate methane.

Keywords: Anaerobic digestion; Methanogenic bacteria; Municipal sludge; Solids retention time; Thermophilic; Volatile fatty acids

1. Introduction

Anaerobic digestion has been and continues to be one of the most widely used processes for the stabilisation of wastewater treatment plant sludge. Its potential advantages to other stabilisation processes include: the production of energy as methane (in excess of that required for process operation); a reduction of 30–50% of sludge volume requiring ultimate disposal; the protection of sludge generally free from objectionable odours when fully digested; a high rate of pathogen destruction, particularly with the thermophilic process. However, conventional anaerobic sludge digestion has two well-known problems that have limited its application: digester foaming and low efficiency in volatile solids reduction [1,2]. Several new processes have been reported for upgrading sludge digestion employing thermophilic anaerobic digestion (55 °C) as an important alternative to mesophilic anaerobic digestion (35 °C) [3–7]. In general, thermophilic anaerobic plants offer attractive advantages such as higher volatile solids destruction efficiency, higher biogas generation, less foaming and better dewaterability [8–10].

Volatile solids reduction is commonly used to measure the performance of anaerobic digestion processes. The volatile solids (VS) content is used as an indicator of the amount of organic matter contained in a sludge. Hence, the amount of VS destruction achieved in a sludge stabilisation process may be used to measure its effectiveness in stabilising the organic component of the sludge. The amount of VS reduction achieved depends on the type of sludge digested (primary, waste activated, trickling filter or a

* Corresponding author. Tel.: +34 956 016158; fax: +34 956 016038.
E-mail address: mariangeles.delarubia@uca.es (M.A. de la Rubia).

1359-5113/$ – see front matter
doi:10.1016/j.procbio.2005.03.073

mixture of these sludges), temperature and solids retention time (SRT). It is well known that the hydraulic (or solid) retention time of a digester is one of the most important factors for the control of anaerobic digestion systems. Although a tremendous amount of research has gone into the effect of SRT on anaerobic systems, sufficient information is still not available to clarify the effect of SRT on thermophilic reactor performance [3,6,11–14].

The stability of the system depends on the viable bacterial groups and SRT is a significant factor in selecting the predominant microbial species [11,15,16]. Understanding the functioning of anaerobic reactors requires quantitative information on microbial numbers, biomass and activities of the bacterial groups involved in the process. The measurement of biomass as volatile solids is a significant limitation in studies on the kinetics of the process, development, operation and monitoring of reactors. Direct count procedures by microscopy methods yield the highest estimates of members of micro-organisms and are occasionally used for indirect calculation of biomass. Epifluorescence microscopy is widely used for direct counting of bacteria, since it does not require culturing [17].

A characteristic peculiarity of methanogens is their UV-induced blue-green autofluorescence which permits counting by autofluorescence microscopy [18]. However, this method is subjective: it only shows methanogens with a high content of F420 such as hydrogen-utilising methanogens; acetate-utilising methanogens belonging to the genus *Methanosaeta* cannot be counted at all and the genus *Methanosarcina* is found in clumps made up of many individual cells. Nevertheless, it is one frequently used method to count autofluorescent methanogens in anaerobic reactors [16,19,20].

The aims of this study were to investigate the influence of SRT on performance and treatment efficiency (based on chemical oxygen demand (COD) and volatile solids removal) of sludge digestion in a pilot digester which decomposes municipal sludge under thermophilic conditions (55 °C) and to obtain direct experimental evidence regarding the influence of SRT on the population levels of methanogenic anaerobic micro-organisms in the digester. This is the most important novelty of the data presented here.

2. Materials and methods

The pilot-plant scale continuously stirred tank reactor (CSTR) employed in this study had an operational volume of 150 L (Fig. 1). Temperature was maintained within the thermophilic range (55 ± 2 °C) by applying recirculation of temperature-controlled water through an internal coil. The reactor was started with thermophilic sludge.

Recycle flow was drawn at the bottom of the reactor and pumped through a variable speed centrifugal pump at the top of the reactor in order to maintain the mixed conditions into digester.

The reactor was fed with prethickened combined primary and secondary waste sludge from the Guadalete Waste Water Treatment Plant (Jerez de la Frontera, Spain).

The study was conducted over a period of 338 days. The experimental work consisted of the study of the dynamic changes provoked in the system by the increase in the organic loading rate (OLR) as the retention time was reduced from 75 to 15 days, passing through the stages of 40, 27 and 20 days.

The main characteristics of feed used are summarised in Table 1. Full-scale anaerobic sludge processes are generally affected by the variations of influent flow, leading to a high variability of the solid influent concentrations and other influent operation parameters (pH, COD, etc.).

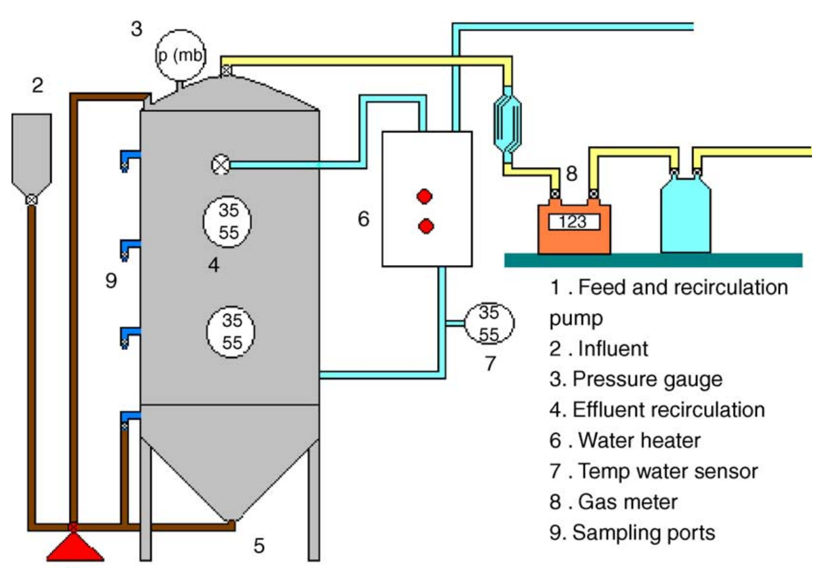

Fig. 1. Schematic diagram of pilot-plant digester used in this study.

Table 1
Main characteristics of raw sludge

Parameter	Mean value	Minimum value	Maximum value
COD (kg/m^3)	64	42	74
pH	6.2	5.8	6.4
Total solids (kg/m^3)	55	38	68
Volatile solids (kg/m^3)	35	27	51

The organic loading rate was 0.4 kg VS/(m^3 day) (0.8 kg COD/(m^3 day)) with an SRT of 75 days. Subsequently, the solids retention time was decreased, being maintained constant during each stage until reaching steady-state conditions. Attainment of the steady state was verified after an initial period by checking whether the constant effluent characteristic values (VS removal, COD removal and volumetric methane production rate) were the means of the last measurements in each stage. For operation at an SRT of 27, 20 and 15 days, the digester was operated for 3 SRTs before the steady state, but for an SRT operation of 75 and 40 days, the operation time to the steady state was 1 SRT, due the low organic loading rate applied. Also, this higher SRT is not interesting at full-scale.

Steady-state conditions are not often reached in full-scale anaerobic digestion sludge processes because most plants are subjected to disturbances working due to the variations of influent flow.

A small amount of sodium carbonate was added at a concentration of 2N to maintain the digestion at the optimum pH for anaerobic thermophilic digestion (0.5 L when the pH dropped below 7.3) [21].

The progress of the digestion was determined by monitoring COD and VS reduction (COD_r and VS_r), gas production and gas composition, pH, alkalinity and total and individual volatile fatty acids (VFA) levels. COD and volatile solids reduction are commonly used to measure the performance of anaerobic digestion processes.

The volume of gas produced in the reactor was directly measured daily with a mass flow-sensor, while gas composition (methane and carbon dioxide) was analysed by gas chromatography separation (Shimadzu GC-14 B) using a method previously described [22]. The concentration of VFA in sludge was determined by gas chromatography (Shimadzu GC-17 A) using a method previously described [22]. These parameters were analysed twice per week. Analyses of total solids (TS), volatile solids and chemical oxygen demand were performed daily according to standard methods [23].

The methanogenic population was determined by autofluorescence microscopy [17,18,24].

3. Results and discussion

Fig. 2 shows the temporal evolution of the digestion process: (a) the organic loading and removal rates, OLR and ORR, respectively (as kg VS/(m^3 day)); (b) the organic removal efficiency (as a percentage of initial VS); (c) volumetric biogas and volumetric methane production rate (m^3/(m^3 digester day)).

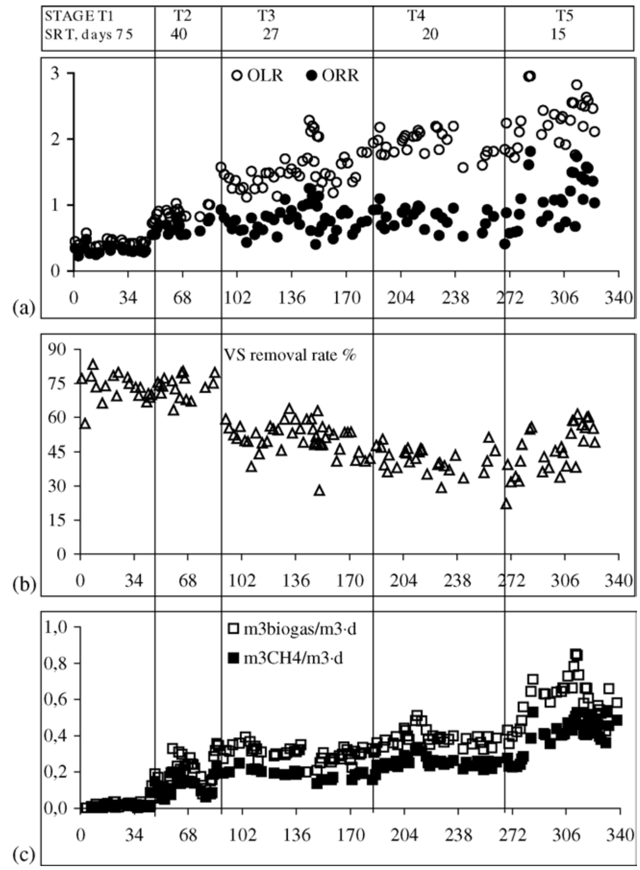

Fig. 2. Variations of the characteristic parameters of the anaerobic process: (a) organic loading and removal rate, OLR and ORR (as kg VS/(m^3 day)); (b) organic removal efficiency (as a percentage of initial VS); (c) volumetric methane and biogas production rate (m^3/(m^3 digester day)).

An important variable for effluent quality is the volatile solids concentration. Fig. 2a shows the changes of OLR and ORR, as kg VS/(m^3 day), at different SRTs. During the initial period of operation, including SRTs between 75 and 40 days, both parameters have almost identical values, indicating that, in these conditions, the experimental digester provides good volatile solids reduction. Subsequently, for SRT lower than 20 days, the differences between the OLR and ORR values are higher. The VS removal efficiency decreased gradually with the increase of SRT, as can be observed in Fig. 2b.

The initial removal efficiencies were quite high (73% VS removal at 75-day SRT). The percentage of VS removed decreasing from 73 to 49% as the OLR increased from 0.4 to 2.2 kg VS/(m^3 day), which was further illustrated by the increase in volumetric gas production rate and methane yield. The COD removal (as %) decreased with decreasing SRT (or increasing OLR), varying from 72 to 35.2% when SRT varied from 75 to 15 days.

The design operating temperature establishes the minimum SRT required to achieve a given amount of volatile solids destruction. Under mesophilic conditions and full-scale, a volatile solids reduction of 40% is an acceptable performance value in the sludge digestion process [25].

Fig. 2c shows the temporal evolution of volumetric methane and biogas rate production. At SRT of 75 days, the values of both parameters are the same as a consequence of CO_2 being used for the formation of CO_3^{2-}/CO_3H^-. Subsequently, the methane production rate increased and the percentage of CH_4 in the gas produced decreased with time (with decreasing SRT) until reaching 65%.

Organic matter removal (expressed as VS_r or COD_r) included two processes: conversion of organic matter to methane and synthesis of new microbial biomass.

Fig. 3 shows the effect of SRT on methane production rate (Fig. 3a) and the effect of OLR, expressed as kg COD/(m^3 day) and kg VS/(m^3 day), on volumetric methane production rate (Fig. 3b and c). The daily production of gases and their composition were monitored in all stages of the thermophilic digester. The gas composition varied from 60 to 65% methane when the SRT was less than 27 days. As would be expected, the volumetric methane production rate increased with decreasing SRT. Volumetric methane production rate averaged 0.02 and 0.40 m^3/(m^3 day) at SRTs of 75 and 15 days, respectively. As can be seen in Fig. 3a, the results obtained show a linear relationship between the volumetric methane production rate and solids retention time [26].

Fig. 3b and c shows a linear relationship between the volumetric methane production rate and OLR, expressed as kg COD/(m^3 day) and kg VS/(m^3 day).

Methane yield, expressed as m^3 CH_4/(kg COD_r), increased with the increase of SRT (days), as can be observed in Table 2. However, the calculated values are inferior to the stoichiometric theoretical value of 0.35 m^3 CH_4/(kg COD) removal (1 kg of COD is equivalent to 0.35 m^3 of methane at STP conditions). This indicates that a high amount of COD removal is used in the synthesis of new micro-organisms for the anabolism route. Last valued, 0.29 m^3CH_4/(kg $COD)_r$ is near to the theoretical value.

The influence of OLR on methane yield is shown in Fig. 3b and c. Volumetric methane production rate (as m^3/(m^3 day)) may be expressed as a linear function of OLR (as kg COD/(m^3 day) and kg VS/(m^3 day)). Over the range of OLR imposed, the methane yield (as m^3 of methane produced per gram of COD and VS applied) was inversely proportional to the SRT applied. This observation is in agreement with the findings of Kiyohara et al. [27] operating on thermophilic sludge from a municipal sludge treatment plant at laboratory scale. This is due to the fact that anaerobic thermophilic digestion of sludge supports the organic loading applied; therefore, the microbial population can metabolise without promoting organic overload or washout in the system. Fang et al. [28] report the same relationship between the volumetric methane production rate (m^3/(m^3 day)) and COD loading rate (kg COD/(m^3 day)). This observation is also in agreement with that reported by Pérez et al. [29–31] operating with vinasses at thermophilic conditions.

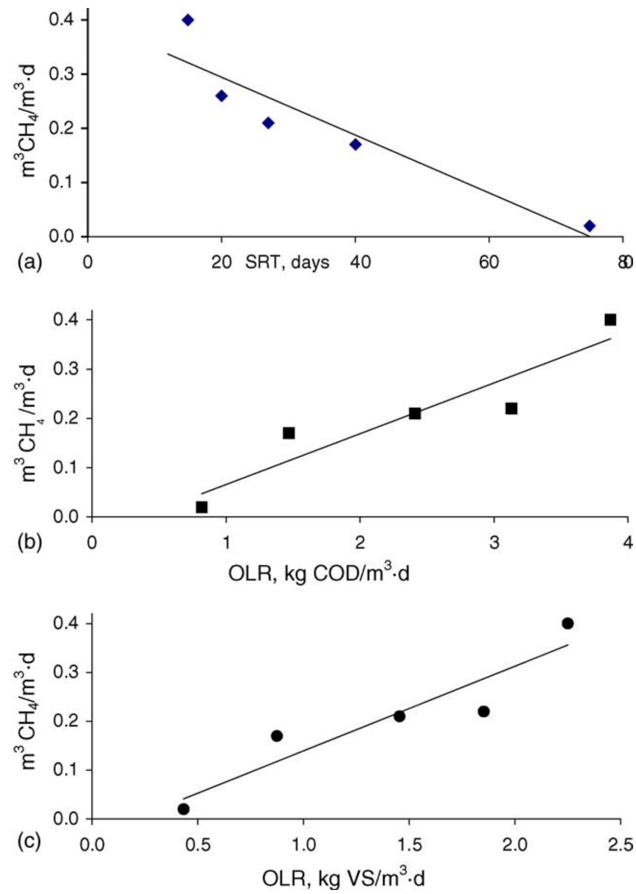

Fig. 3. (a) SRT vs. volumetric methane production rate (m^3/(m^3 day)). (b) Effect of OLR, expressed as kg COD/(m^3 day), on volumetric methane production rate (m^3/(m^3 day)). (c) Effect of OLR, expressed as kg VS/(m^3 day), on volumetric methane production rate (m^3/(m^3 day)).

Table 2
Performance parameters data using in COD mass balance

SRT (days)	COD_0 (kg/m^3)	COD_e (kg/m^3)	COD_r (%)	OLR (kg COD/(m^3 day))	ORR (kg COD/(m^3 day))	CH_4 (m^3/(m^3 day))	CH_4 (m^3/kg CODr)
75	61.5	17.2	72.0	0.8	0.6	0.02	0.03
40	58.8	16.2	72.5	1.5	1.1	0.17	0.16
27	65.2	42.4	34.9	2.4	0.8	0.21	0.25
20	62.9	44.2	29.3	3.1	0.9	0.26	0.28
15	58.1	37.6	35.2	3.9	1.4	0.40	0.29

Table 3
Concentration of individual and total volatile fatty acids at different SRT (days)

SRT (days)	Acetic (mg/L)	Propionic (mg/L)	*n*-Butyric (mg/L)	*n*-Valeric (mg/L)	*n*-Caproic (mg/L)	Total VFA (mg acetic/L)
75	2758	1486	274	241	17	5435
40	1817	1858	318	264	11	5045
27	1758	1941	400	468	67	5662
20	1770	2181	449	597	98	6322
15	2362	2145	450	527	47	6655

The individual VFA levels (acetic, propionic, *n*-butyric, *n*-valeric and *n*-caproic) and total VFA, as mg/L in the effluent of the reactor, from each operation reactor stage is shown in Table 3. As can be seen, the total VFA increased when the SRT decreased.

Acetic acids concentration decreased slowly with a decrease in SRT, except the last SRT studied (15 days). The levels of propionate also increased with SRT and were usually greater than those of acetic acids. However, the process showed stable operation during all studied stages. Fukuzaki et al. [32] reported that the inhibition of propionate on its own degradation to methane was dependent on pH; at pH 7.6, propionate had no inhibition effect up to approximately 3500 mg/L. Thus, the accumulation of residual acetate and propionate in the effluent was probably not due to the inhibition of propionate, but to the high loading rate of sludge, exceeding the methanogenic activity of the biomass.

n-Butyric concentrations increased linearly during the entire stable process in the range between 274 and 450 mg/L. Fang et al. [33] reported that degradation of butyrate to acetate was not a rate-limiting step of the anaerobic thermophilic process.

One of the major criticisms of the use of thermophilic digestion is that the final effluents contain higher concentrations of volatile fatty acids than those from a mesophilic digester. However, the stable performance of the digester was observed in all stages of this study.

The high values of total VFA in thermophilic processes are also reported by Dinsdale et al. [21]. They operated continuous thermophilic studies on coffee waste over long periods, achieving stable digestion at a variety of loading rates. However, they did find that some studies began to have increasing levels of volatile fatty acids after a certain time of operation. Notwithstanding, Kiyohara et al. [27] report that the thermophilic process has an advantage in treating raw sludge under high loading rates compared with the mesophilic process. The activity of thermophilic bacteria was higher than that of mesophilic bacteria [6], though thermophilic bacteria tend to remove propionic acid more slowly than mesophilic bacteria [3,27].

The pH in the reactor was kept at a constant level of 7.9–7.4 throughout the study by adding sodium carbonate (500 mL, 2N sodium carbonate) when the pH dropped below 7.3 (only 15-day SRT period). Bicarbonate alkalinity was maintained above 12,500–14,000 mg $CaCO_3$/L. The high alkalinity level indicates that the bacterial groups are in equilibrium.

The acidity/alkalinity relationship decreased with SRT until stabilisation was reached at constant values in the range 0.25–0.30 mg acetic/(mg calcium carbonate) (very high for operation at thermophilic conditions). Therefore, thermophilic digestion of municipal sludge could be stabilised at 3.8 kg COD/(m^3 day) (15 days) with the addition of sodium carbonate, Na_2CO_3.

Microbial populations in anaerobic digestion have been investigated, with the finding that SRT was a significant factor in selecting the predominant microbial species [15,16]. One of the objectives of the present study was to obtain direct experimental evidence for the influence of SRT on the population levels of methanogenic anaerobic microorganism in the digester.

Table 4 shows the evolution of methanogenic biomass concentration at different SRT (days). The bacterial counts were realized at the end of each period [17,18,24,34] when the microbial population is adapted to the new organic loading rate conditions.

The methanogenic population increased with the organic loading rate, since more organic matter may support a larger population. This is in agreement with the results showed by Zhang and Noike [11]. The increase in population size is especially notable from 1.9 kg VS/(m^3 day) (SRT: 20 days) to 2.2 kg VS/(m^3 day) (SRT: 15 days) with 5.30×10^9 and 6.75×10^9 cells/mL, respectively.

The methanogens biomass increased when SRT decreases. The decrease of RT (which meant an increase

Table 4
Methanogenic bacteria concentrations (cells/mL) and microbial increment

SRT (days)	Methanogenic bacteria (cells/mL)	Microbial increase
12.44	3.81E+09	3.81E+09
22.09	4.06E+09	2.50E+08
36.89	4.50E+09	4.40E+08
55.79	5.30E+09	8.00E+08
77.63	6.75E+09	1.45E+09

Table 5
Transformed COD factor for each volatile fatty acid

Acid	CH_3	CH_2	COOH	Molecular mass	Ratio	COD (mg/mg)
Acetic	1	0	1	60	1.000	1.067
Propionic	1	1	1	74	1.233	1.514
Butyric	1	2	1	88	1.467	1.818
Valeric	1	3	1	102	1.700	2.039
Caproic	1	4	1	116	1.933	2.207

Table 6
Summary of a mass balance data (referred to COD)

SRT (days)	COD_0 (g/day)	DQO_e (g/day)	Consumed COD			Non-consumed COD	
			COD_r (g/day)	COD_{CH_4} (g/day)	$COD_{biomass}$ (g/day)	COD_{acids} (g/day)	DQO_{recalc} (g/day)
75	123.1	34.4	88.6	8.6	80.1	12.4	22.0
40	220.5	60.6	159.9	72.9	87.0	22.1	38.5
27	362.2	235.7	126.4	90.0	36.4	36.9	198.8
20	469.3	331.8	137.5	111.4	26.1	55.9	276.0
15	580.9	376.3	204.6	171.4	33.1	77.6	298.7

of COD in this study) made it possible for hydrolytic bacteria with shorter doubling times to increase rapidly, which resulted in a faster acid production. Also, although the population levels of H_2-utilising methanogens increased with a decrease in retention time, these were 100-fold lower than hydrolytic and acetogenic bacteria [11]. Therefore, the rates of interspecies hydrogen transfer were poor causing the inhibition of the conversion of caproic, valeric, butyric and propionic acids to acetic acid. The accumulation of total VFA is due to VFA > C4.

4. COD mass balance and energy requirements

COD is a parameter used to represent the evolution of organic matter during the digestion process. Using the experimental data obtained, the following COD balance mass is presented. The main equations of the COD balance are:

$$COD_0 = COD_r + COD_e$$

$$COD_r = COD_{CH_4} + COD_{biomass}$$

$$COD_e = COD_{acids} + COD_{recalc}$$

Steady-state condition is accepted. All calculations are realized accepting a useful digester volume of 150 L.

COD removal (COD_r) can be calculated as a difference between feeding COD (COD_0) and the effluent COD_e (non-used in the process). Feeding COD is used to generate methane by the catabolic route (COD_{CH_4}) and to produce biomass by the anabolism route ($COD_{biomass}$); COD_{CH_4} is quantified accepting the stoichiometric coefficient: 0.35 m^3 CH_4/(kg COD); COD non-used, (COD_e), is calculated by the addition of volatile fatty acids COD in effluent (COD_{acids}) and the recalcitrant COD (COD_{recalc}); volatile fatty acids COD, COD_{acids}, is referred to the COD due the volatile acids present in effluent. This value is calculated considering the amount of each acid and its transformed COD factor, as is presented in Table 5; COD_{recalc} is the COD associated to non-biodegradable mass in the digester in the operation conditions selected. It is calculated as a difference between COD_e and COD_{acids}.

Table 2 shows the experimental data used in the COD balance.

Table 6 shows the results obtained of the COD balance. The main conclusions obtained indicate that COD used for methane generation increased when SRT decreased or when the influent organic loading rate increased. This implies that the amount of COD used in the anabolism route decreased with SRT due the microbial population being adapted at new operational conditions and more COD being used to generate methane (see Table 4).

The energy requirements can be calculated considering the data published by Rimkus et al. [9] in a full-scale thermophilic digester. The total energy consumed by the digester (8900 m^3) was monitored. The total energy consumed by the digester is used to heat the feed sludge to the operating temperature and to make up for heat loss from digester. At similar operational conditions, gas consumption in the digester is 6740 m^3 biogas/day (or 0.76 m^3 biogas/(m^3 digester day)). Therefore, the pilot digester is energy self-sufficient in all operational stages (Fig. 2). In all stages, amounts higher than 0.11 m^3 biogas/day were produced.

5. Conclusions

It was confirmed experimentally that the thermophilic sludge digester can achieve >50% VS and 42% COD reduction at a VS and COD loading rate of 2.2 kg VS/(m^3 day) and 3.9 kg COD/(m^3 day), respectively, treating raw sludge from a municipal treatment plant under steady-state conditions (SRT: 15 days).

Higher degradation efficiency is associated with increased gas production and improvement in the energy balance of the process. The greatest efficiency of the volumetric methane production rate was 0.40 m^3/(m^3 day) for OLR of 2.2 kg VS/(m^3 day) (0.29 m^3 CH_4/(kg $(COD)_r$)).

High values of total and individual VFA levels in the effluent were obtained in all stages of the process, although the digester showed stable operation.

The retention time has a considerable effect on the population levels of methanogens and on the composition of fermentative products (VFA).

The COD mass balance indicates that COD used for methane generation increased when SRT decreased or when the influent organic loading rate increased. This implies that the amount of COD used in the anabolism route decreased with SRT due the microbial population being adapted under new operational conditions and more COD being used to generate methane. The pilot digester is energy self-sufficient at all operational stages.

Acknowledgements

This research was supported by a grant from the Comisión Internacional de Ciencia y Tecnología (CICYT) of the Spanish Government (PETRI, ref. 95-0208-OP; PPQ2001-4032 Ministerio de Ciencia y Tecnología, Madrid, Spain).

The authors wish to thank the Guadalete Wastewater Treatment Plant of Jerez de la Frontera, Cadiz, Spain (Aguas de Jerez Empresa Municipal S.A. and AQUALIA), for their helpful cooperation.

Appendix A

COD	chemical oxygen demand
COD_0	feeding COD
COD_{acids}	COD related to volatile fatty acids in effluent
$COD_{biomass}$	COD related to biomass
COD_{CH_4}	COD related to methane
COD_e	effluent COD
COD_r	removal COD
COD_{recalc}	non-biodegradable COD
OLR	organic loading rate
ORR	organic removal rate
SRT	solids retention time
VFA	volatile fatty acids
VS	volatile solids

References

[1] Li YY, Noike T. Upgrading of anaerobic digestion of waste activated sludge by thermal pretreatment. Water Sci Technol 1992;26(3–4):857–66.

[2] Halalsheh M, Koppes J, den Elzen J, Zeeman G, Fayyad M, Lettinga G. Effect of SRT and temperature on biological conversions and the related scum forming potential. In: Proceedings of the 9th world congress on anaerobic digestion; 2001. p. 505–10.

[3] Zabranska J, Dohanyos M, Jenicek P, Kutil J. Thermophilic process and enhancement of excess activated sludge degradability—two ways of intensification of sludge treatment in Prague central wastewater treatment plant. Water Sci Technol 2000;41(9):265–72.

[4] Ahn JH, Forster CF. A comparison of mesophilic and thermophilic anaerobic upflow filters. Biores Technol 2000;73:201–5.

[5] Kim M, Ahn YH, Speece RE. Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. Water Res 2002;36:4369–85.

[6] Moen G, Stensel HD, Lepistö R, Ferguson J. Effect of solids retention time on the performance of thermophilic and mesophilic digestion. Water Environ Res 2003;75(6):539–48.

[7] Gavala HN, Yenal U, Skiadas IV, Westermann P, Ahring BK. Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. Water Res 2003;37:4561–72.

[8] Garber W, Ohara G, Colbaugh J, Raksit G. Thermophilic digestion at the Hyperion Treatment Plant. J Water Pollut Control Fed 1975;47(5):950–61.

[9] Rimkus R, Ryan J, Cook E. Full scale thermophilic digestion at the west–southwest sewage treatment works, Chicago, Illinois. J Water Pollut Control Fed 1982;54(11):1447–57.

[10] Peddie CC, Tailford J, Hoffman D. Thermophilic anaerobic sludge digestion taking a new look at an old process. In: Proceeding of the 10th annual residuals biosolids management conference. Ed. Water Environment Federation Alexandria; 1996. p. 39–46.

[11] Zhang TC, Noike T. Influence of retention time on reactor performance and bacterial trophic populations in anaerobic digestion processes. Water Res 1994;28(1):27–36.

[12] Maharajá I, Elefsiniotis P. The role of HRT and low temperature on the acid-phase anaerobic digestion of municipal and industrial wastewaters. Biores Technol 2000;76(3):191–7.

[13] Miron Y, Zeeman G, Van Lier JB, Lettinga G. The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems. Water Res 2000;34(5):1705–13.

[14] Banerjee A, Elefsiniotis P, Tuhtar D. The effect of addition of potato-processing wastewater on the acidogenesis of primary sludge under varied hydraulic retention time and temperature. J Biotechnol 1999;72:203–12.

[15] Li YY, Noike T. Metabolic characteristics and distribution of fatty acid-utilizing bacteria in anaerobic digester. In: Proceedings of 23rd annual conference of the Japan Society on Water Pollution Research; 1999. p. 445–6.

[16] Solera R, Romero LI, Sales D. Analysis of the methane production in thermophilic anaerobic reactors: use of autofluorescence microscopy. Biotechnol Lett 2001;23:1889–92.

[17] Solera R, Romero LI, Sales D. Determination of the microbial population in thermophilic anaerobic reactor: comparative analysis by different counting methods. Anaerobe 2001;7:79–86.

[18] Doddema HJ, Vogels GD. Improved identification of methanogenic bacteria by fluorescence microscopy. Appl Environ Microbiol 1978;36(5):752–4.

[19] Anderson GK, Kasapgil B, Ince O. Microbiological study of two-stage anaerobic digestion during start-up. Water Res 1997;28(11):2383–92.

[20] Ince BK, Ince O. Changes to bacterial community makeup in a two-phase anaerobic system. J Chem Technol Biotechnol 2000;75(6):500–8.

[21] Dinsdale RM, Hawkes FR, Hawkes DL. The mesophilic and thermophilic anaerobic digestion of coffee waste containing coffee grounds. Water Res 1996;30(2):371–7.

[22] De la Rubia MA, Pérez M, Romero LI, Sales D. Anaerobic mesophilic and thermophilic municipal sludge digestión. Chem Biochem Eng Q 2002;16(3):119–24.

[23] APHA. AWWA. WPCF. Standard methods for the examination of water and wastewater, 17th ed., Washington, DC, USA; 1989.

[24] Jain MK, Zeikus G, Bhatnagar L. Methanogens. In: Levett PN, editor. Anaerobic microbiology A practical approach, New York: Oxford University Press; 1991. p. 223–45.

[25] De la Rubia MA, Pérez M, Martínez A, Andrades JA, Romero LI, Sales D. Puesta en marcha y operación de un digestor anaerobio mesofílico de lodos. Tecnología del Agua 2002;220:53–7.

[26] Sánchez E, Borja R, Weiland P, Travieso L, Martín A. Effect of temperature and pH on the kinetics of methane production, organic nitrogen and phosphorus removal in batch anaerobic digestion process of cattle manure. Biopr Eng 2000;22:247–52.

[27] Kiyohara Y, Miyahara T, Mizuni O, Noike T, Ono K. A comparative study of thermophilic and mesophilic sludge digestion. J Chartered Inst Water Environ Manage 2000;14(2):150–4.

[28] Fang HHP, Li YY, Chui HK. UASB treatment of wastewater with concentrated mixed VFA. J Environ Eng 1995;121(2):153–60.

[29] Pérez M, Romero LI, Sales D. Steady state anaerobic thermophilic degradation of distillery wastewater in fluidized bed bioreactors. Biotechnol Prog 1997;13(1):33–8.

[30] Pérez M, Romero LI, Sales D. Comparative performance on high rate anaerobic thermophilic technologies treating industrial wastewater. Water Res 1998;32(3):559–64.

[31] Pérez M, Romero LI, Sales D. Organic matter degradation kinetics in an anaerobic thermophilic fluidised-bed bioreactor. Anaerobe 2001;7:25–35.
[32] Fukuzaki S, Nishio N, Shobayashi M, Nagai S. Inhibition of fermentation of propionate to methane by hydrogen, acetate and propionate. Appl Environ Microbiol 1990;56(3):719–23.
[33] Fang HHP, Li YY, Chui HK. Performance and sludge characteristics of UASB process treating propionate-rich wastewater. Water Res 1995;29(3):895–8.
[34] ASM. Growth measurement. Manual of methods for general bacteriology, vol. 11. 1991. p. 182–3.