Start-up and HRT Influence in Thermophilic and Mesophilic Anaerobic Digesters Seeded with Waste Activated Sludge

T. Benabdallah El-Hadj, J. Dosta, and J. Mata-Álvarez*
University of Barcelona, Department of Chemical Engineering,
Martí i Franquès, 1, 6th floor, 08028 Barcelona

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Since thermophilic anaerobic digestion represents an efficient alternative to mesophilic anaerobic digestion, multiple studies have been developed to compare their performance and viability. One of the problems related to thermophilic anaerobic digestion is the availability of an adequate seed to start-up the process.

The goal of this study is to evaluate the possibility of using waste activated sludge (WAS) as a seed for both mesophilic (35 °C) and thermophilic (55 °C) anaerobic digesters fed with a real sludge waste (primary and secondary sludge mixture) based on the gradual substitution of synthetic substrate by real feed. The obtained results show that mesophilic and thermophilic anaerobic digesters were rapidly stabilized within 60 and 85 days at a hydraulic retention time (HRT) of t = 35 and 30 d with 450 and 520 mL biogas per g COD_t added and 65 % and 72 % as methane content, respectively. Moreover, HRT was progressively reduced in order to assess the maximum organic load that can be treated in the thermophilic reactor. The minimum HRT reached was t = 8 d with a VS removal efficiency of 50.32 % and a biogas yield index of 440 mL biogas per g VS added (54 % as methane content).

Key words:
Anaerobic digestion, HRT, mesophilic, seed, start-up, synthetic substrate, thermophilic, WAS

Introduction

The increasing amount of sludge from wastewater treatment plants (WWTP) and the restrictive legislations on their management and final destination invite to enhance the actual treatment processes and/or to find a reliable alternative. In Europe, since new legislations have been approved, the sludge produced has increased more than 50.9 % from 1992 to 2005.[1] Today, the main destinations of this product are landfilling, incineration and soil fertilization. This last destination is the more prioritized option by the EU legislation due to its positive effect on nutrients recycling and organic material reconstitution in the soil. However, sludge reuse in agricultural soil must satisfy health-related conditions in order to avoid detest agents and harmful elements transference to the receptor medium. The most common processes used for sludge treatment are composting, lime stabilization and anaerobic digestion. This last treatment, also known as biomethanization, is the most widely used process for WWTP sludge stabilization because it is addressed to both energy recovery and environmental protection.

The anaerobic thermophilic digestion (55 °C) seems to be a feasible alternative to anaerobic mesophilic digestion (35 °C) in order to improve the sludge quality, to reduce vector attraction and to minimize the pathogenic load. *Alatiqi* et al.[2] reported that thermophilic anaerobic digestion was a good alternative to the mesophilic process, especially for the treatment of raw sewage sludge (RSS) with a high organic load (γ = 54 g L^{-1} COD_t) in warm climates.

However, one of the main problems of thermophilic digesters start-up is the availability of an appropriate seed, since a limited number of WWTP operate in this range of temperature. According to *Rimkus* et al.,[3] the anaerobic mesophilic inoculum acclimation to thermophilic temperature range can be achieved in approximately 43 weeks. *Wu* et al.[4] reported that waste activated sludge (WAS) is a good alternative to mesophilic anaerobic sludge since it contains a considerable amount of methanogenic bacteria. *Kim* and *Speece*[5] studied the possibility of converting WAS to an anaerobic inoculum using synthetic substrate (acetate and propionate) with an acceptable methanogenic activity without previous acclimation to the temperature range.

The aim of this research is to evaluate the possibility of using WAS as a seed for mesophilic (35 °C) and thermophilic (55 °C) anaerobic digesters fed with a real raw sewage sludge (RSS) from the Barcelona Metropolitan Area ((mixture of primary (75 % of TS) and secondary (25 %) sludge)), and to

* Author to whom correspondence should be addressed.
Tel: +34-93-4021305, Fax: +34-93-4021291, E-mail: jmata@ub.edu

compare their efficiency at different hydraulic retention time (HRT) conditions.

Materials and methods

Experimental set-up

Two completely mixed and jacketed anaerobic digesters ($V = 5$ L) were used in this study. Each one was seeded with $V = 3.5$ L of waste activated sludge (WAS) from a municipal WWTP of the Barcelona Metropolitan Area. The operating temperature was controlled by means of two heating systems (Haake DC 40) at 55 $T =$ °C and 35 °C for the thermophilic and mesophilic reactors, respectively. Both digesters were equipped with a pH probe (Crison pH28) and fed by peristaltic pumps (Multiflex) regulated by a PLC (Siemens Logo 230RC-DM8 230R). The gas produced in the system was measured by a displacement device equipped with a photocell counter to record their total volume.[6]

Analytical methods

Total chemical oxygen demand (COD_t), soluble chemical oxygen demand (COD_s), total solids (TS), volatile solids (VS), pH, alkalinity and ammonium concentration (NH_4^+–N) were analyzed according to the Standard methods.[7] Volatile fatty acids (VFA) and gas composition were analyzed by gas chromatography equipped with a flame ionization detector (FID)[8] and a thermal conductivity detector (TCD),[9] respectively.

Substrate and inoculum

The WAS used as seed for both reactors was pre-concentrated to achieve a mass concentration up to 14.6 g L^{-1} VS. Table 1 shows the main characteristics of WAS and the real raw sewage sludge (RSS) used during the experimental period. This RSS was composed by a mixture of primary (75 % on TS basis) and secondary (25 %) sludge from a WWTP of the Barcelona Metropolitan Area. The RSS was maintained in a freezer for a long period and the feed tank was maintained at $T = 4$ °C.

Start-up procedure

The start-up procedure was divided into two steps. In the first step, acetate was used as sole substrate to promote anaerobic action of the WAS in both digesters as reported by *Ahn* et al.[10] Once anaerobic activity was developed, acetate was substituted by glucose, since this carbon source is more complicated and it can incite other microbial group activation and proliferation.[11] In the second step, the synthetic feed was changed gradually substituting the amount of glucose by an equivalent amount of COD_s from the RSS substrate.[12,13] As stated in Table 2, this step was divided into four periods. The first period (A) corresponded to the feeding with a mixture of 75 % of COD_s from glucose and 25 % of COD_s from RSS. Subsequently, periods B, C and D (corresponding to feeding with 50 % : 50 %, 25 % : 75 % and 0 % : 100 % of COD_s from glucose: COD_s from RSS, respectively) were run.

HRT reduction procedure

After the seed acclimation to anaerobic conditions fed with a mixture of primary and secondary sludge (start-up procedure), the steady HRT reached was 35 and 30 d in the mesophilic and thermophilic digester, respectively. The procedure

Table 1 – *Waste activated sludge (WAS) and raw sewage sludge (RSS) characterization (average values along experimental periods)*

	TS γ/g L^{-1}	VS γ/g L^{-1}	VSS %	COD_t γ/g L^{-1}	COD_s γ/g L^{-1}
Pre-concentrated WAS	29.75 ± 1.49	14.60 ± 0.70	66 ± 3.3	17.12 ± 0.86	4.90 ± 0.29
RSS	40.28 ± 1.26	29.95 ± 0.89	74 ± 5.7	51.00 ± 3.13	15.00 ± 1.8

Table 2 – *Synthetic and real RSS feed mixture composition in the second step of start-up*

	Thermophilic				Mesophilic			
	a	b	c	d	a	b	c	d
Total COD_s, γ/g L_r^{-1} COD	0.535	0.535	0.535	0.535	0.437	0.437	0.437	0.437
Glucose, % of COD_s	75	50	25	0	75	50	25	0
RSS added COD_t, γ/g L_r^{-1} d^{-1} COD	0.450	0.890	1.334	1.700	0.328	0.728	1.093	1.457
COD_t of Glucose + RSS added, γ/g L_r^{-1} d^{-1} COD	0.850	1.156	1.468	1.700	0.691	0.947	1.202	1.457

selected for HRT reduction for both digesters was the successive increasing of the organic load by ~14 % of the initial VS reactor content.[3] Steady-state conditions for every studied HRT were achieved approximately after a time equivalent to three times HRT.

Results and discussion

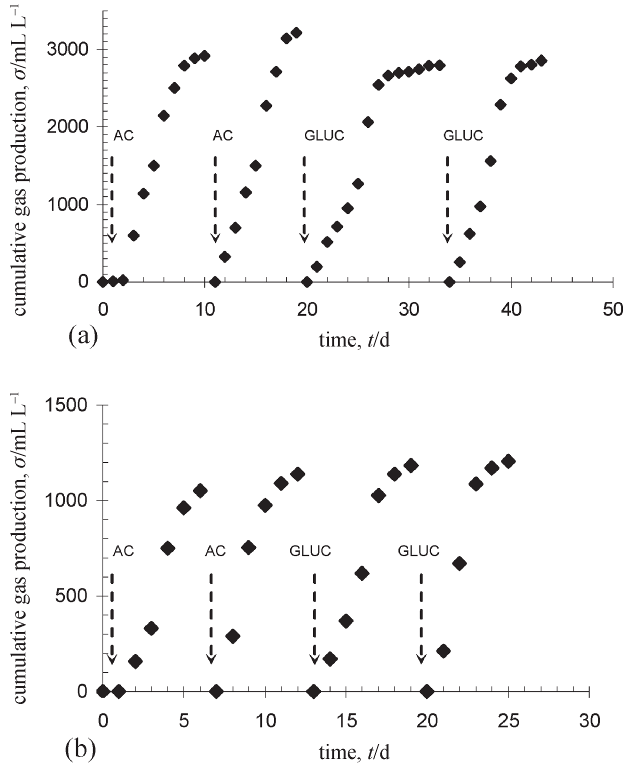
Start-up experience

The performance of both digesters was examined by means of biogas production, methane content in the biogas, pH, COD_t, COD_s and VFA levels at each step. A previous batch of assays were carried out (not shown here) in order to assess the maximum initial concentration of synthetic feed (acetate and glucose) for both mesophilic and thermophilic reactors. The achieved COD_f/inoculum VS ratio was lower than that reported by *Kim* and *Speece*,[5] since higher COD_f/inoculum VS values would had represented the external addition of alkalinity to control the pH.

First step. Acetate was initially added to develop the methanogenic activity of WAS and subsequently it was substituted by glucose. When acetate was changed by glucose as substrate, neither major alteration in biogas production nor in digester stability was observed. For the mesophilic digester, the maximum initial concentration of organic substrate was the same for both synthetic feed (γ = 2.187 g L^{-1} COD). However, for the thermophilic digester, the maximum initial mass concentration was γ = 5.425 g L^{-1} COD for acetate and γ = 4.800 g L^{-1} COD for glucose.

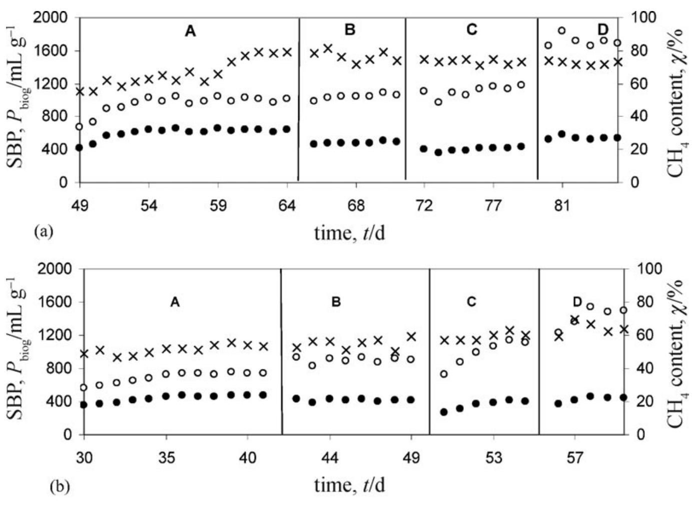
Fig. 1 illustrates the accumulated biogas production in the thermophilic and mesophilic reactor. As observed in this figure, the biogas production capacity of the thermophilic reactor was clearly higher for both synthetic substrates. The biogas production profile was almost the same, but the high amount of feed loaded to the thermophilic digester had a noticeable impact on the amount of biogas produced. At the fourth feeding with synthetic substrate, a biogas production of P_{biog} = 594.64 and 550.47 mL biogas per g COD_t was measured with a methane content of 64.0 % and 61.2 % for the thermophilic and mesophilic digester, respectively. These results indicate that WAS has significant capacity to degrade acetate and glucose under anaerobic conditions at an acceptable removal rate and methane content in the biogas produced.

Second step. Table 2 shows the operational conditions of this second period, where the feed was changed gradually substituting the quantity of



F i g . 1 – *Biogas production within the synthetic substrate feeding step in (a) thermophilic and (b) mesophilic digester; AC: acetate feeding; GLUC: glucose feeding*

glucose added by an equivalent amount of COD_s from the RSS substrate. In Fig. 2 it can be appreciated the evolution of biogas production per unit of COD added and its methane content during this treatment. At the end of period A, a sensitive increase was observed in biogas production that exceeded P_{biog} = 630 and 470 mL biogas per g COD_t in the thermophilic and mesophilic digester, respec-



F i g . 2 – *Biogas production per mass unit of organic matter and methane composition in the (a) thermophilic and (b) mesophilic digester within RSS substitution feed step;* ● *mL biogas per g COD_t;* ○ *mL biogas per g COD_s;* × *methane content (%)*

tively. Therefore, the biogas production efficiency was highly improved under thermophilic conditions. These results indicate that WAS has a higher initial yield of biogas production when it is used as an anaerobic digestion seed, especially in the thermophilic temperature range, and can biodegrade a complex feed such as RSS.

At period B, the thermophilic and the mesophilic digesters produced, approximately, P_{biog} = 490 mL biogas per g COD_t and 410 mL biogas per g COD_t, respectively. This clearly indicates that WAS, at both temperatures, was able to biodegrade a complex feed in the absence of an important amount of easily biodegradable organic matter. On the other hand, the biogas production per unit of soluble COD added was higher than in the previous stage, since the substitution of synthetic substrate by RSS was done on a soluble COD basis and, consequently, the total COD added to the system was increased.

During period C, the biogas production per unit of total COD added in the mesophilic and thermophilic digester were P_{biog} = 400 mL biogas per g COD_t and 430 mL biogas per g COD_t, respectively. When the digesters were fed exclusively with RSS (period D), they were rapidly stabilized (5–7 d). The average biogas production was 480 mL biogas per g COD_t with 65 % of CH_4 and 525 mL g^{-1} COD_t with 72 % CH_4 under mesophilic and thermophilic conditions, respectively. The difference between both reactors, in terms of the aforementioned values, demonstrates the higher efficiency of the thermophilic digester inoculated with WAS in relation to the mesophilic digester when a real RSS is fed.

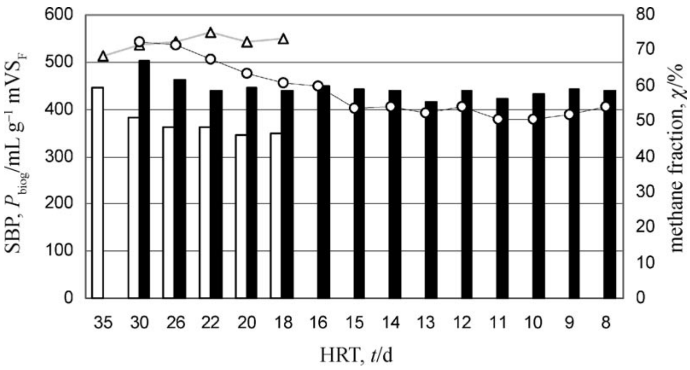
A statistical study (F-test (variance analysis) and Student's t-test (mean analysis), both at $P < 5$ % level of probability) was performed in order to assure the difference in SBP and methane content at every step of the gradual substitution of synthetic feed by real substrate.

The comparison between SBP and methane content at each period and the following one showed the adaptation of the inoculum to the real substrate. However, there were some exceptions where several obtained parameters did not differ significantly (t-test value $P > 5$ %) from one period to another (the SBP under mesophilic conditions between Periods A and B, and the methane content from period C to D in the thermophilic digester).

Therefore, it is concluded that WAS represents an appropriate seed to develop the anaerobic digestion process for the treatment of a real RSS and, taking into account the short time necessary to start-up the process and the efficiencies reached, it represents a very good alternative to other studied inoculums.[2,3,12,14]

HRT reduction

A gradual reduction of the HRT for both anaerobic digesters was carried out following the procedure described by *Rimkus* et al.[3] Fig. 3 shows the specific biogas production (SBP) profile for both systems at every studied HRT. As it can be appreciated, at same selected HRTs (30–18 d), the SBP was enhanced under thermophilic conditions with respect to mesophilic conditions (P value of t-test was $4 \cdot 10^{-4} < 0.05$ probability level considered), which is in concordance with *van Lier* et al.,[15,16] who experienced an improvement of the anaerobic activity at high temperatures. On the other hand, the SBP slightly decreased with HRT reduction and it was maintained within the range of P_{biog} = 400–450 mL biogas per g VS_f (thermophilic digestion) and P_{biog} = 300–350 L per g VS_f (mesophilic digestion). The assessed biogas yield values were similar or higher than other reported values.[3,17,18]



F i g . 3 – *Biogas production per organic matter unit added and methane content in both digesters at HRT reduction period; □ mesophilic specific biogas production; ■ thermophilic specific biogas production; Δ methane biogas content in the mesophilic digester; ○ methane biogas content in the thermophilic digester*

The mesophilic digester was stopped after several HRTs were tested (from 35 to 18 d), since thermophilic conditions clearly improved the anaerobic digestion efficiency. Therefore, thermophilic digestion performance was evaluated until achieving the minimum feasible HRT.

Fig. 4 shows the VS removal (VS_r) efficiency for all the tested HRTs, where it is stated that VS_r efficiency decreased sensibly at short HRTs in the mesophilic digester. However, at thermophilic conditions, HRT decrease did not clearly influence VS_r efficiency. Only the first HRT reduction (from 30 to 26 d) provided a significant decrease of VS removal. Moreover, it was observed that in both mesophilic and thermophilic digesters the VS reduction was carried out at the same efficiency range values (50–55 %) without significant difference, recording higher t-test P values with respect to the considered probability level (0.05).

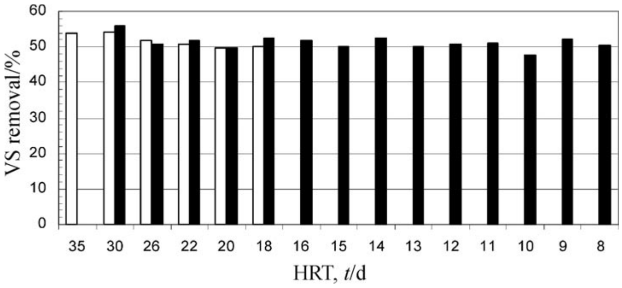


Fig. 4 – *Evolution of volatile solids removal (%) at each HRT;* □ *mesophilic digester;* ■ *thermophilic digester*

On the other hand, the biogas methane content oscillated between 70 and 75% at the HRTs studied in the mesophilic digester (see Fig. 3). In this case, the methane content increase could be explained by the fact that the methanogenic consortium adaptation was enhanced during the lag time. However, the thermophilic biogas methane content was lower than that obtained under mesophilic conditions and decreased with HRT reduction. This descent can be divided into two phases: in the first (30–15 d HRT), the methane biogas content decreased progressively and in the second phase (15–8 d HRT) it slightly fluctuated between 50–54 % CH_4. From these results, it seems that HRT reduction in the thermophilic digester affected the methanogenic activity drastically, and at low HRT ($t < 15$ d) the thermophilic methanogenesis was adapted to the organic load increasing regime. This experimental data is in concordance with the volatile fatty acids (VFA) mass concentration profile during the HRT reduction experience (shown in Fig. 5). As it is shown in this figure, the VFA accumulation inside the reactor increased until the limit inhibitory concentration was reached.

At HRT 7 d, the biogas production reached 149 mL g^{-1} VS_f with 23 % as methane content, due to an inhibition of the methanogenic activity caused by a pH decrease and a high total VFA (sum of C_2, C_3, C_4, C_5, C_6 and C_7) mass concentration (particularly acetic acid). From these results, it can be concluded that a mass concentration of $\gamma = 1250$ mg L^{-1} VFA represents the inhibitory limit for the studied thermophilic digestion. However, this reactor failure was overcome after the external addition of alkalinity and returning to an HRT of $t = 8$ d (see Fig. 5).

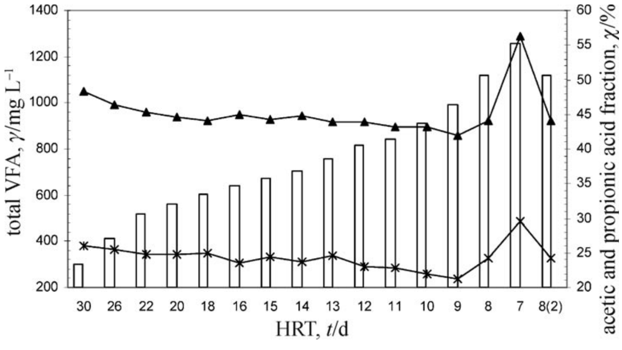


Fig. 5 – *Total VFAs amount and Acetic and Propionic acids content in the thermophilic digester effluent;* □ *total VFA;* ▲ *acetic acid;* ✱ *propionic acid*

Conclusions

The results obtained in this study show that WAS can be used as inoculum to anaerobic digestion in both mesophilic and thermophilic conditions. Furthermore, the start-up of the anaerobic digestion based on the gradual substitution of synthetic substrate by real substrate in anaerobic reactors seeded with WAS is very fast when compared with other seeds commonly used to develop this biological process.

The anaerobic digestion of a real raw sewage sludge (mixture of primary and secondary sewage sludge) provided good removal efficiencies for both mesophilic and thermophilic digesters. However, at same selected HRTs (from 30 to18 d), the treatment was clearly improved under thermophilic conditions in terms of specific biogas production.

Moreover, the HRT effect on thermophilic anaerobic efficiency was studied in order to assess the maximum organic load that can be treated in the thermophilic reactor. A minimum HRT of 8 d was found, since volatile fatty acids accumulation and pH decrease inhibited the process when working at a lower HRT.

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Abbreviations and Symbols

COD – Chemical Oxygen Demand
COD_s – Soluble COD
COD_t – Total COD
HRT – Hydraulic Retention Time
RSS – Raw Sewage Sludge
SBP – Specific Biogas Production
VFA – Volatile Fatty Acids
VS – Volatile Solids
m_{VS} – mass of VS, g
VS_r – VS removal (%)
WAS – Waste Activated Sludge
WWTP – Wastewater Treatment Plant
m – mass, g

γ – mass concentration, g L^{-1}

V – volume reactor, L

t – time, d

P_{biog} – production of biogas, mL of biogas per g COD or VS

σ – volume concentration, mL L^{-1}

x – mole fraction

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