Staged and non-staged anaerobic filters: performance in relation to physical and biological characteristics of microbial aggregates

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Abstract: This work describes a comparative study of staged and non-staged anaerobic filters for treating a synthetic dairy waste. The effect of decreasing the hydraulic retention time from 2 days to 10 h at a constant substrate concentration of 9 g COD dm^{-3} by applying lateral feedings in the staged digester was evaluated with respect to overall reactor performance, in comparison with a conventional up-flow anaerobic filter. There was no advantage on the use of a multi-feed staged system under the operating conditions tested. The overall performance and the microbial activity segregation were similar for both configurations. The microbial aggregates present in both digesters, particularly in the top sections, changed significantly in biological, physical and morphological properties. The presence of aggregates larger than 4 mm equivalent diameter in those sections did not prevent a strong washout phenomenon. An effect of disintegration attributed to biogas accumulation and release was observed, when those large particles become smaller and their surface became rougher. Due to biomass accumulation, at the end of the trial period, only 40% of the total volume was occupied by the liquid phase.

Keywords: anaerobic filter; methanogenic activity; microbial aggregates; image analysis; effluent treatment

NOTATION

AFI	Non-staged digester
AFII	Staged digester
Bv	Applied organic loading rate
COD	Chemical oxygen demand
D	Longitudinal dispersion coefficient
HRT	Hydraulic retention time
L	Length
MRT	Mean residence time
Q	Influent flowrate
u	Superficial velocity
VS	Volatile solids

1 INTRODUCTION

Staged anaerobic digesters present a number of advantages over non-staged systems. When toxic gaseous compounds such as oxygen, sulfide or ammonia are present, there are better conditions for methanogenesis in staged systems. On the other hand, the removal of biogas in the early stages of degradation maintains low levels of hydrogen, particularly during pulse loading, so favouring acetic and propionic acid removal in the later stages.[1]

Lettinga[2] suggested that staged processes provide an optimal environment for degradation of intermediates such as propionate, which may be particularly useful for thermophilic processes, but also for mesophilic treatment. More recently Lettinga *et al* stated that the type and sequence of stages should be optimised, according to each specific application.[3]

Van Lier *et al*[4] reported that staged degradation in an upflow staged sludge bed (USSB) led to a segregation of biomass in terms of its biological and physical properties, which was beneficial for achieving low hydrogen partial pressure and low volatile fatty acid concentration in the last compartment.

Although not very popular when compared with sludge blanket reactors, anaerobic filters can be considered a good alternative for effluents that hinder sludge settleability such as effluents with a high lipids content.[5] There are some laboratory applications of anaerobic filters operating in a staged mode. El-Shafie

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and Bloodgood[6] studied the degradation of a synthetic complex wastewater in a staged anaerobic filter with six compartments and Cheung *et al*[7] operated and characterised a staged fixed bed process with three compartments. In a previous work Alves *et al*[8] compared a staged with a non-staged anaerobic filter and concluded that, under the operating conditions tested, there was no advantage in using the staged system. The physical properties of the microbial aggregates such as size and settling velocity varied along the height. Flocs located at the top grew with the increase in substrate concentration, growth that was in part attributed to biogas entrapment. A clear increasing trend of methanogenic activity with propionate as the substrate with the settling velocity was observed.

It is expected that in a single-feed staged system the first stage is highly loaded which can affect the subsequent stages due to the low prevailing pH. The biomass becomes too fluffy, flocs have an undefined size and do not settle.

In the present work the effect of reducing the hydraulic retention time was studied. As it was previously concluded that staging was not advantageous in this kind of digester, it was decided to apply lateral feedings at the inlet of the second and third stages of a three-stage anaerobic filter and to compare with a single-feed non-staged configuration. In addition to the overall and partial performances, the effect on biomass activity and microbial aggregates morphology was evaluated in both digesters. At the end of the operation tracer experiments were performed for both digesters.

2 MATERIALS AND METHODS

2.1 Experimental set-up and mode of operation

The two anaerobic filters have been described elsewhere.[8] In the non-staged configuration (AFI) the initial liquid volume was 14.2 dm^3 and in the staged configuration (AFII) it was 17.7 dm^3. Both digesters were cylindrical with a diameter of 1.47 dm and had an equal volume of support medium, which consisted of PVC Raschig rings 21 mm in size, with a specific surface area of 230 $m^2 m^{-3}$ and a porosity of 92.5%. In the staged reactor (AFII), a gas–solid separator was fitted to allow biogas release from each compartment.

The substrate was stored at 4 °C in order to minimise acidification. Several sampling ports allowed withdrawal of liquid and solid samples. The reactor temperature was kept constant at 35 ± 1 °C by a heating water jacket. Routine reactor performance was monitored by determining influent and effluent chemical oxygen demand (COD), influent flow rate, effluent volatile fatty acids (VFA) (including each stage of AFII), the rate of gas production and its methane content from each reactor (including each stage of AFII). The gas flow rate was measured daily and all the other routine analyses were made three to four times a week.

After an initial operation period of 495 days described elsewhere,[8] the substrate concentration was increased from 9 to 12 g COD dm^{-3} during a short period (between days 596 and 628), but returned to 9 g COD dm^{-3} thereafter. In the staged digester (AFII), the HRT was decreased from 2 days to 10 h by applying lateral feedings at the inlet of the second (double feed-DF) and third stages (triple feed-TF). After achieving the complete feed distribution among the three stages, the overall flow rate was increased, achieving an hydraulic retention time of 10 h and an organic loading rate of 20 kg COD m^{-3} d^{-1}. The non-staged digester (AFI) was operated under identical operating conditions of substrate concentration and HRT.

For the organic loading rate of 4.5 kg COD m^{-3} d^{-1} (single feed) and 13 kg COD m^{-3} d^{-1}, for double and triple feed in AFII, and single feed in AFI, the biomass was withdrawn from three points in each reactor – first sampling port of each stage of AFII and equivalent points in AFI, located at 5, 32 and 65 cm from the bottom. Figure 1 represents the operating conditions of both digesters at the moment of sludge sampling for microbial characterisation.

Sludges were analysed for specific methanogenic activity with acetate, H_2/CO_2, propionate, and butyrate as substrates. The specific lactose consumption rate was also determined as well as the settling velocity, size and a number of morphological parameters such as the fractal dimension and compactness.

2.2 Substrate

The substrate was made by dilution of skim milk with tap water and was supplemented with macro- and micronutrients as follows. Macronutrients – $MgSO_4.7H_2O$: 30.2 g dm^{-3}; KH_2PO_4: 28.3 g dm^{-3}; KCl: 45 g dm^{-3}; 0.6 cm^3 of this solution was added per gram of COD fed. Micronutrients – $FeCl_2.6H_2O$: 2 g dm^{-3}; H_3BO_3: 0.05 g dm^{-3}; $ZnCl_2$: 0.05 g dm^{-3}; $CuCl_2.2H_2O$: 0.038 g dm^{-3}; $MnCl_2.4H_2O$: 0.5 g dm^{-3}; $(NH_4)_6Mo_7O_{24}.4H_2O$: 0.05 g dm^{-3}; $AlCl_3.6H_2O$: 0.09 g dm^{-3}; $CoCl_2.6H_2O$: 2 g dm^{-3}; $NiCl_2.6H_2O$: 0.092 g dm^{-3}; $Na_2SeO_3.5H_2O$: 0.164 g dm^{-3}; EDTA: 1 g dm^{-3}; resazurin: 0.2 g dm^{-3}; HCl 37%: 1 $cm^3 dm^{-3}$. The composition of this solution was based on the work of Zehnder *et al.*[9] Micronutrients were supplemented to the influent feed by addition of 1 $cm^3 dm^{-3}$ of feed.

2.3 Analytical methods

2.3.1 Routine analysis

Chemical oxygen demand (COD), volatile and total solids (VS and TS) were determined by standard methods.[10] Volatile fatty acids (VFA) were determined by HPLC (Jasco, Japan) using a Chrompack column (300 × 6.5 mm) set at 40 °C and a mobile phase of 5 mM H_2SO_4 at 0.7 $cm^3 min^{-1}$. The absorption at 210 nm was measured with a spectrophotometer (Jasco, 870 UV). The methane content of the biogas was measured by use of a Pye Unicam GCD gas chromatograph (Cambridge, England) fitted with a

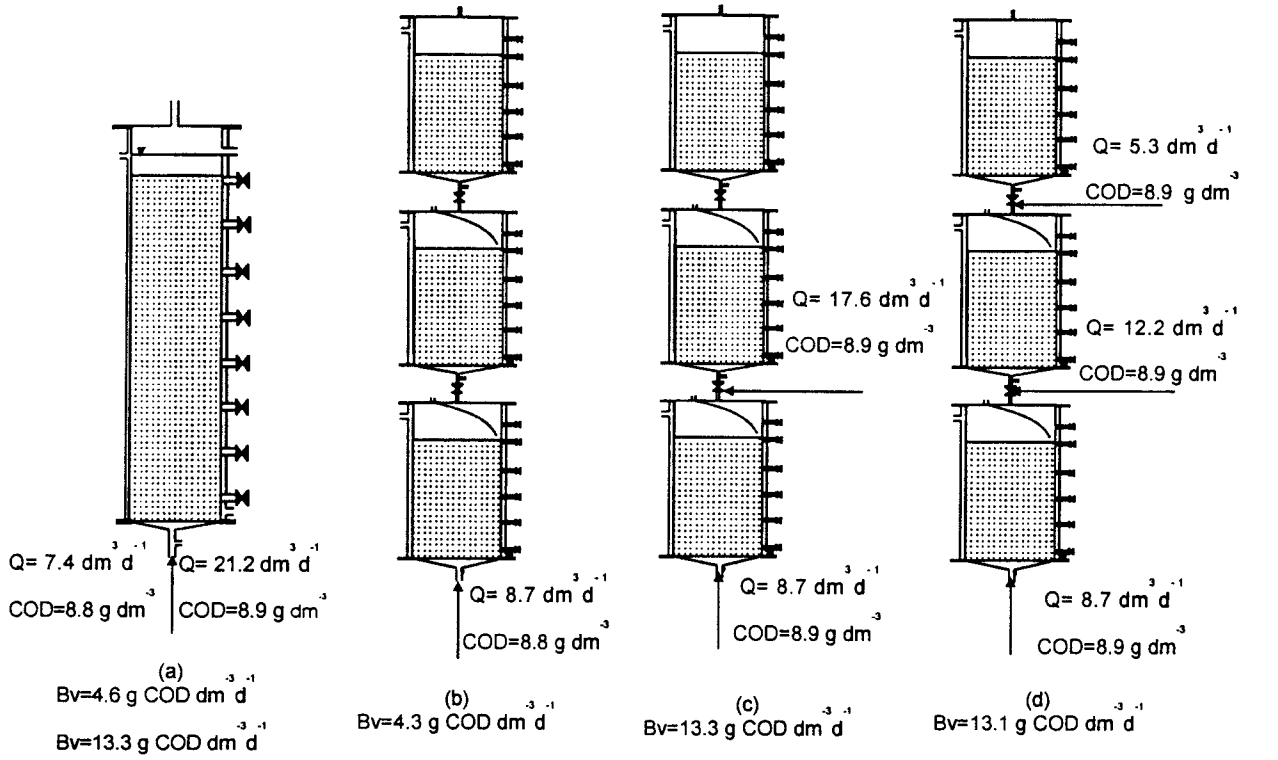

Figure 1. Operating conditions at the moments of biomass withdrawal. (a) Non-staged digester – AFI; (b) single feed (SF) staged digester – AFII; (c) double feed (DF) – AFII; (d) triple feed (TF) – AFII.

Chrompack Haysep Q (80–100 mesh) column. Nitrogen was used as carrier gas ($30\,cm^3min^{-1}$) and the temperatures of injection port, column and flame ionisation detector were 120, 40 and 130 °C, respectively.

2.3.2 *Activity measurements*

Methanogenic activity was determined using a pressure transducer technique.[11,12] The pressure increase developed in sealed vials fed with non-gaseous substrates, or pressure decrease in vials previously pressurised with gaseous substrates (hydrogen and carbon dioxide – H_2/CO_2 80:20 v/v) was monitored. Strict anaerobic conditions were maintained. The hand-held pressure transducer used was able to measure a pressure increase or decrease of two atmospheres (0 to ±202.6 kPa) over a range of −200 to +200 mV. The sensing element was connected to a digital panel module. All tests were performed in triplicate. The lactose activity tests were performed by measuring the specific consumption rate of lactose in closed vials. Lactose was analysed by a Boehringer–Mannheim® kit.

2.3.3 *Physical characterisation of microbial aggregates*

The settling velocity was determined by measuring the average settling time of 50 aggregates in a water column. The size of the microbial aggregates was measured by the projected area using an image analysis system. The images were acquired with a video camera Sony AVC-D5CE CCD (Japan) adapted to an Olympus SZ40 (Tokyo, Japan) binocular lens. The 'Image Processing Toolbox' for Matlab (The Mathworks, Inc, USA) was used to perform calculations.

The box counting algorithm was used for the determination of the fractal dimension.[13] It is known that the number of L-sized boxes to cover a fractal surface is L^{-D}, where D is the fractal dimension. The number of L-sized boxes containing part of an object is referred as $N(L)$. If the object to be analysed is fractal then $N(L)$ must be proportional to L^{-D} (eqn. (1)):

$$N(L) = K \times L^{-D} \tag{1}$$

Compactness is defined as the ratio between the area of an object and the area of a circle with the same perimeter[14] (eqn. (2)):

$$\text{Compactness} = \frac{4\pi\text{Area}}{\text{Perimeter}^2} \tag{2}$$

2.3.4 *Tracer experiments*

The residence time distribution (RTD) studies were performed at the end of the trial period, by the stimulus–response technique[15] using lithium cloride as tracer. Tracer was injected as a pulse function at the inlet of AFI and separately at each influent line of AFII. An automatic sampler (New Brunswick Scientific, Mx Biosampler) was used to collect and refrigerate samples. Lithium was analysed by flame emission photometry (ATS, 200 MKI, Switzerland). The 'Optimisation Toolbox' for Matlab (The Mathworks, Inc, USA) was used to obtain the optimal set of

Table 1. Operating conditions and performance data of AFI. Average values of at least 10 days of pseudo steady state conditions ± interval with 95% confidence

Time (days)	HRT (±0.01) (days)	Influent COD ($g\,dm^{-3}$)	Effluent total COD ($g\,dm^{-3}$)	Organic loading rate ($kg\ COD\ m^{-3}\ d^{-1}$)	Soluble COD efficiency (%)	CH_4 (%)	Biogas ($dm^3\,dm^{-3}\ d^{-1}$)
495–596	1.92	8.8±0.4	1.0±0.2	4.6±0.2	98.2±0.4	62.3±1.3	2.6±0.1
596–628	1.92	12.1±0.4	2.4±0.2	6.3±0.2	98.2±0.3	61.0±0.4	3.5±0.1
628–670	1.92	8.9±0.1	1.6±0.4	4.6±0.1	98.0±0.6	62.0±0.6	2.7±0.1
670–693	1.49	8.9±0.1	1.8±0.2	6.0±0.1	96.1±0.7	61.2±1.2	3.5±0.1
693–725	1.20	8.9±0.1	2.5±0.4	7.4±0.1	97.2±0.2	59.3±1.7	4.2±0.3
725–741	1.00	8.9±0.1	5.1±2.2	8.9±0.1	95.7±1.5	56.7±0.1	4.1±0.1
741–764	0.82	8.9±0.1	2.2±0.4	10.8±0.2	94.8±0.8	60.7±1.2	5.4±0.2
764–862	0.67	8.9±0.1	6.1±2.0	13.3±0.2	88.7±1.6	60.2±0.9	6.2±0.2
862–881	0.51	8.9±0.1	5.3±1.5	17.4±0.3	67.4±5.9	57.1±1.4	7.8±0.3
881–908	0.43	8.9±0.1	6.7±0.9	20.6±0.3	67.9±9.1	54.8±0.8	9.8±0.2

parameters by non-linear regression, using the Levenberg–Marquardt method.[16]

3 RESULTS

3.1 Reactor performance

Table 1 summarises the operating conditions and performance data of AFI and Table 2 presents the operating conditions and performance data of AFII, including the partial performance of each stage.

The influent COD was initially increased from 9 to $12\,g\,dm^{-3}$, but, in AFII, the first stage became highly loaded and consequently the pH at the exit of the first stage decreased to below 6.5. The removal efficiency of the first stage achieved a value as low as 47% and the methane content of the biogas decreased to below 50%. The specific methanogenic acetoclastic activity decreased, achieving a value of $32\,cm^3CH_{4(STP)}$ $(g\ VS)^{-1}d^{-1}$, which was low, when compared with other values obtained elsewhere.[8] A threshold pH value of 6.5 at the exit of the first stage was considered to be the minimum required to keep the normal operation of the second stage, and below this value it was considered that the operation of the second stage could be adversely affected. Then, the influent COD was reset at $9\,g\,dm^{-3}$ and the first stage was operated with a constant loading rate of 13 kg COD $m^{-3}d^{-1}$ between days 628 and 862. Average COD removal efficiency was 61% and $4\,dm^3dm^{-3}d^{-1}$ of biogas were produced with a methane content of 61% (Table 2).

The HRT was decreased by applying lateral feeding at the inlet of the second and the third stages of AFII. When the flow rate of the lateral feeding introduced at the inlet of the second stage was increased from 2.9 to $12.1\,dm^3d^{-1}$, no significant pH decrease was observed. The HRT of the second stage was consequently decreased from 0.68 to 0.28 days, keeping an average COD removal of 80%. However, when a further increase from 12.1 to $17.6\,dm^3d^{-1}$ was applied, although the pH remained at a stable value above the threshold value of 6.5, the removal efficiency of the second stage decreased significantly from 80 to 54%. Then the HRT of the second stage was reset at 0.28 days and the extra influent flow ($5.3\,dm^3d^{-1}$ – Fig 1) was introduced at the inlet of the third stage, determining a complete feed distribution on day 809 (Table 2). This change induced an increase from 57.5 to 65.4% in the COD removal efficiency of the third stage.

After the complete feed distribution through the three stages according to the criteria of maximum applied loading on each stage, based on either pH or removal efficiency decrease, the removal capacity of each stage was calculated, on day 862. The second stage had the highest COD removal capacity, 14.5 kg $COD_{removed}$ $m^{-3}d^{-1}$, in the third stage the removal capacity was 10 kg $COD_{removed}$ $m^{-3}d^{-1}$ and in the first one it was 8 kg $COD_{removed}$ $m^{-3}d^{-1}$. The non-staged configuration removed 11.7 kg $COD_{removed}$ $m^{-3}d^{-1}$. On days 862 and 881 all the influent flow rates were increased by 31 and 55% respectively. The applied loading rate in the overall reactors and in the first stage of AFII increased consequently from 13 to 17 and 20 kg COD $m^{-3}d^{-1}$. However in the second stage, the increase was from 22 to 30 and 36 kg COD $m^{-3}d^{-1}$ and in the third stage it was from 15 to 25 and 31 kg COD $m^{-3}d^{-1}$, respectively. This effect was due to the increase in the applied organic load directly in the stage and indirectly, from the decrease in performance of the previous stage. However no adverse effect was observed in the operation of the staged system. In fact, the overall performance of the two reactors was very similar over the trial period, which is evident in Fig 2 where the removal efficiency is plotted against the hydraulic retention time for both digesters.

From day 764 onwards, an increasing washout of biomass was observed in both configurations. Volatile suspended solids ranged from 1.7 to $3.6\,g\,dm^{-3}$ and from 1.3 to $3.9\,g\,dm^{-3}$ for AFI and AFII, respectively. The maximum values, 3.6 and $3.9\,g\,dm^{-3}$ for AFI and AFII respectively, were obtained for the period between days 764 and 862.

3.2 Characterisation of microbial aggregates: acidogenic, syntrophic and methanogenic activity

The increase in organic loading rate from 4.5 to 13 kg COD $m^{-3}d^{-1}$, between days 495 and 862, induced significant changes in the microbial activities of the consortia established at different heights of both digesters (Table 3).

Table 2. Operating conditions and performance data of AFII. Average values of at least 10 days of pseudo steady state conditions ± interval with 95% confidence

Feed	Time (days)	HRT (±0.01) (days)	Influent COD ($g\,dm^{-3}$)	Effluent total COD ($g\,dm^{-3}$)	Organic loading rate ($kg\ COD\ m^{-3}\ d^{-1}$)	Soluble COD efficiency (%)	CH_4 (%)	Biogas ($dm^3\,dm^{-3}\ d^{-1}$)
First stage	495–596	0.68	8.8±0.4		13.0±0.6	62.1±3.1	55.2±1.4	3.7±0.2
	596–628	0.68	12.1±0.4		17.9±0.6	47.7±5.0	47.7±1.0	4.7±0.1
	628–862	0.68	8.9±1.4		13.1±0.2	61.3±2.3	60.6±1.9	4.0±0.1
	862–881	0.52	8.9±1.4		17.4±0.3	61.9±9.2	58.0±1.8	4.8±0.6
	881–908	0.44	8.9±1.4		20.2±0.6	64.0±8.6	53.4±6.8	4.7±0.7
Second stage	495–596	0.68	3.3±0.2		4.8±0.4	80.8±3.2	67.6±2.6	2.6±0.1
	596–628	0.68	6.2±0.5		9.1±0.8	81.4±2.0	67.0±0.9	4.1±0.1
	628–670	0.68	4.4±0.3		6.6±0.5	77.5±4.4	65.8±1.0	3.1±0.1
	670–693	0.51	4.7±0.3		9.2±0.7	75.6±5.1	62.7±1.4	4.4±0.1
	693–725	0.40	5.7±0.3		14.5±0.7	84.1±1.4	60.0±2.3	5.8±0.3
	725–741	0.33	6.3±0.5		19.3±1.4	83.3±1.6	55.8±1.3	8.7±0.5
	741–764	0.28	6.4±0.3		22.9±1.1	80.5±2.1	59.1±0.9	8.5±0.5
	764–809	0.22	6.7±0.2		30.6±1.0	53.8±4.7	56.6±2.6	5.9±0.1
	809–862	0.28	6.2±0.4		22.0±1.3	66.1±4.3	57.3±1.3	5.1±0.2
	862–881	0.21	6.2±0.2		29.7±1.1	50.0±10.0	56.6±0.7	4.1±0.1
	881–908	0.18	6.5±0.5		36.2±3.4	46.3±7.4	53.5±1.8	7.6±0.4
Third stage	495–596	0.68	0.6±0.1		0.9±0.2	70.1±8.1	69.0±1.5	0.6±0.1
	596–628	0.68	1.1±0.1		1.7±0.2	82.8±4.0	71.4±0.7	1.1±0.1
	628–670	0.68	1.0±0.2		1.5±0.3	73.4±5.9	69.5±0.9	1.0±0.1
	670–693	0.51	1.0±0.2		2.1±0.4	62.3±2.9	68.3±1.0	1.1±0.2
	693–725	0.40	1.0±0.1		2.4±0.3	58.6±9.4	63.5±2.6	1.1±0.1
	725–741	0.33	1.1±0.1		3.3±0.3	60.8±1.6	62.2±0.5	2.2±0.1
	741–764	0.28	1.3±0.1		4.5±0.5	70.0±2.8	65.2±0.6	3.4±0.2
	764–809	0.22	3.0±0.3		13.3±1.2	57.5±6.1	64.2±2.3	7.2±0.5
	809–862	0.22	3.3±0.2		14.6±1.1	65.4±5.7	61.5±1.5	7.7±0.6
	862–881	0.17	4.2±0.5		24.6±2.8	56.2±9.6	60.1±2.9	nd
	881–908	0.15	4.6±0.4		30.6±3.0	40.6±6.6	52.8±3.7	nd
Overall SF	495–596	2.05	8.8±0.4	1.4±0.4	4.3±0.2	97.9±0.7	61.0±3.2	2.3±0.1
	596–628	2.05	12.1±0.4	4.5±1.7	5.9±0.2	98.3±0.5	58.4±1.3	3.3±0.1
	628–670	2.05	8.9±1.4	1.4±0.7	4.3±0.1	97.0±0.9	60.6±1.9	2.7±0.1
DF	670–693	1.53	8.9±1.4	2.5±0.6	5.8±0.1	95.1±0.9	59.9±1.5	3.2±0.1
	693–725	1.19	8.9±1.4	2.4±0.5	7.5±0.1	95.5±1.4	58.3±3.1	3.6±0.1
	725–741	1.00	8.9±1.4	2.5±0.7	8.9±0.1	95.3±0.4	56.4±2.4	5.0±0.1
	741–764	0.85	8.9±1.4	2.9±1.6	10.5±0.2	95.8±0.4	59.2±2.9	5.3±0.2
	764–809	0.67	8.9±1.4	6.8±3.1	13.3±0.2	84.8±2.7	59.3±2.8	5.6±0.2
TF	809–862	0.68	8.9±1.4	5.9±2.9	13.1±0.2	87.1±1.8	58.5±3.3	5.6±0.2
	862–881	0.52	8.9±1.4	3.7±1.0	17.1±0.3	78.0±5.6	58.0±1.8	nd
	881–908	0.44	8.9±1.4	5.2±0.4	20.2±0.6	69.3±4.8	53.4±6.8	nd

nd – not determined

SF: single feed; DF: doublet feed; TF; triple feed.

For the single-feed non-staged configuration a clear enhancement of acetoclastic methanogenic activity was observed throughout all the sections with a relative increase of 53, 31 and 46% in the bottom, middle and top sections, respectively. Stratification was recorded with 64 and 57% more acetoclastic activity at the top section than at the bottom section for *Bv* of 4.5 and 13 kg COD $m^{-3}d^{-1}$, respectively (Table 3). However hydrogenotrophic activity remained much more homogeneous throughout all the sections, with a maximum difference of 35% between the bottom and the top for *Bv*=4.5 kg COD $m^{-3}d^{-1}$. The maximum methanogenic activity with propionate as substrate was clearly displaced from the middle to the top section and a clear increase of methanogenic activity with butyrate as substrate was also observed at the top. The acidogenic activity with lactose as substrate was not improved in the top section, but a clear increase of 42% was detected in the bottom section.

The biomass of the staged digester was characterised for the same operating conditions (*Bv*=4.5 and 13 kg COD $m^{-3}d^{-1}$), but the effect of changing from double to triple feed was also investigated (Table 3). As stated before for the single-feed staged digester,[8] the microbial activity segregation was similar to that observed for the non-staged configuration. The degree of stratification was recorded with 84 and 47% more acetoclastic activity at the third stage than at the first one, for *Bv*=13 kg COD $m^{-3}d^{-1}$, double and triple

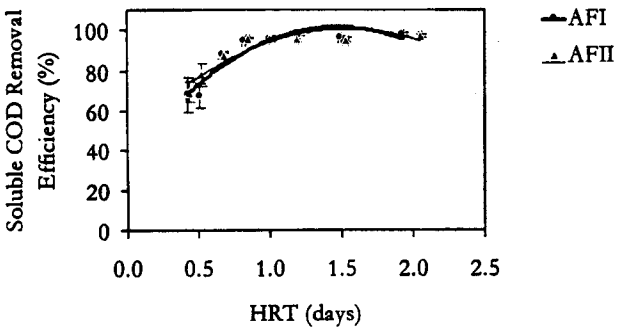

Figure 2. Effect of HRT on the removal efficiency in AFI and AFII. Error bars represent interval with 95% of confidence.

feed, respectively. It was interesting to verify a 10-fold improvement of acetoclastic activity in the first stage between days 495 and 862, without any change in the applied conditions in that stage (Table 3). Only a transitory increase in influent concentration from 9 to 12 g COD dm^{-3} was applied between days 596 and 628, but this was returned to the previous value of 9 g COD dm^{-3} after day 628 until day 862. This increase may be attributed to the natural adaptation of sludge with the time and reflects well the non-stationary behaviour of microbial consortia under pseudo-steady state conditions observed for the routine parameters. This was also observed for the hydrogenophilic and syntrophic activities in the first stage and can justify the good response of this stage to the further increase in the organic loading rate from 13 to 17 and to 20 kg COD $m^{-3}d^{-1}$, on days 862 and 881, respectively.

3.3 Characterisation of microbial aggregates: physical and morphological properties

When the organic loading rate increased from 4.5 to 13 kg COD $m^{-3}d^{-1}$, a clear growth of the microbial aggregates present at the top section of both configuration was observed (Table 4). The equivalent diameter of aggregates present in AFI and AFII increased by 90 and 68%, respectively. Consequently the settling velocity increased, achieving 91 m h^{-1} and 109 m h^{-1} respectively for AFI and AFII under double feed conditions. The tentative quantification of aggregate morphology revealed that the increase in size and settling velocity was connected with an increase in compactness, also traduced by a significant increase of fractal dimension, corresponding to a less rough surface (Table 4).

When a triple feed was applied to the staged system, significant decreases of size, compactness and solidity were recorded for the aggregates present in the third stage. The projected area became much more irregular with a significant decrease of the fractal dimension from 1.86 to 1.81. However, the settling velocity increased, achieving a value as high as 130 m h^{-1}.

It was interesting that when large, morphologically compact and smooth-surface microbial aggregates were present in the top sections of both digesters, the washout effect was critical for both digesters (Tables 1 and 2). The possibility of biogas release during the washout process was considered. In fact it could be that biomass present at the digester had different characteristics from the biomass at the exit of the digesters. However, when a sample of the washed out biomass from AFII was analysed, an average equivalent diameter of 3.9 mm was measured, the settling velocity was 156 m h^{-1}, the acetoclastic and hydrogenotrophic activities were 400.9 and 1100.6 cm^3CH_4 at STP (g VS)$^{-1}d^{-1}$, respectively and the fractal dimension and compactness were 1.86 and 0.62,

Table 3. Specific methanogenic activity and specific acidogenic activity of biomass from AFI and AFII ± interval with 95% confidence

			Specific methanogenic activity (cm^3 CH_4 at STP g^{-1} VS d^{-1})				
	Bv (kg COD m^{-3} d^{-1})	*HRT (days)*	*Acetate as substrate*	*H_2/CO_2 as substrate*	*Propionate as substrate*	*Butyrate as substrate*	*Specific lactose activity (mmoles lactose g^{-1} VS d^{-1})*
AFI, bottom	4.5	2.0	171±16	805±35	55±18	0	12±2
	13	0.67	261±27	895±78	68±12	55±7	17±3
AFI, middle	4.5	2.0	259±41	820±40	152±5	35±5	11±2
	13	0.67	338±39	722±111	59±6	21±3	13±3
AFI, top	4.5	2.0	281±6	1085±34	85±28	6±5	10±1
	13	0.67	410±24	863±13	107±3	49±6	10±1
AFII, first stage	4.5 SF	2.0	32±11	796±123	37±2	0	12±2
	13 DF	0.67	324±43	941±82	75±8	37±9	11±2
	13 TF	0.67	324±43	941±82	75±8	37±9	11±2
AFII, second stage	4.5 SF	2.0	233±20	962±26	147±23	14±8	8±1
	13 DF	0.67	500±6	1093±35	65±14	18±9	22±2
	13 TF	0.67	325±19	770±43	46±8	18±9	26±4
AFII, third stage	4.5 SF	2.0	256±14	796±123	93±1	15±2	7±1
	13 DF	0.67	596±14	1020±30	190±7	38±4	7±1
	13 TF	0.67	475±40	951±42	144±4	30±5	11±2

SF: single feed; DF: double feed; TF: triple feed.

Table 4. Physical and morphological properties of microbial aggregates present in AFI and AFII at different heights ± interval with 95% confidence

	Bv (kg COD m^{-3} d^{-1})	HRT (days)	Size (mm)	Settling velocity (m h^{-1})	Fractal dimension	Compactness
AFI, bottom	4.5	2.0	–	–	–	–
	13	0.67	1.7±0.1	20±4	1.81±0.01	0.58±0.02
AFI, middle	4.5	2.0	1.5±0.1	37±6	1.82±0.02	0.60±0.03
	13	0.67	1.2±0.1	27±9	1.82±0.01	0.61±0.02
AFI, top	4.5	2.0	2.0±0.1	28±4	1.79±0.02	0.58±0.03
	13	0.67	3.8±0.2	91±13	1.86±0.01	0.69±0.02
AFII, first stage	4.5 SF	2.0	–	–	–	–
	13 DF	0.67	1.5±0.1	26±4	1.82±0.01	0.61±0.02
	13 TF	0.67	1.5±0.1	26±4	1.82±0.01	0.61±0.02
AFII, second stage	4.5 SF	2.0	2.1±0.1	60±10	1.81±0.01	0.58±0.02
	13 DF	0.67	1.2±0.1	53±14	1.81±0.01	0.58±0.02
	13 TF	0.67	1.8±0.1	50±12	1.82±0.01	0.59±0.02
AFII, third stage	4.5 SF	2.0	2.2±0.2	28±4	1.79±0.02	0.43±0.03
	13 DF	0.67	3.7±0.2	109±16	1.86±0.01	0.61±0.02
	13 TF	0.67	1.4±0.1	130±17	1.81±0.01	0.56±0.02

respectively. These values are in close agreement with those measured in the top sections of both digesters and revealed the washout of a highly active granular-like biomass. The strong washout effect detected between days 764 and 862 was reduced after this period even with a further increase in the applied flow rates on both digesters (Table 2).

4 DISCUSSION

It is difficult to state that in anaerobic filters biomass properties changed due to a particular effect and not due to an adaptation effect. As was reported by Hanaki *et al*,[17] steady state conditions do not prevail for biomass. This fact should be borne in mind in all the present discussion. After comparing, in a previous work, a staged and a non-staged anaerobic filter under single feed conditions and after concluding that the degree of biological stratification was similar for both configurations,[8] in this work the staged digester was operated in a multifeed mode. When compared with the non-staged configuration, the degree of microbial activity stratification was not significantly diminished by the application of a multiple feeding regime in the staged digester (Table 3). This can be explained by the flux of intermediates throughout the reactor, which is determined by the hydrodynamic behaviour. A tracer experiment performed at the end of the trial period revealed the strong effect of short-circuiting and dead zones in both configurations. Near 60% of the total volume was other than liquid phase and it could be observed that liquid flow was only allowed by small channels opened by the increasing pressure of biogas production. In the non-staged digester a large degree of longitudinal dispersion was detected (Table 5). This fact can partially explain the similar behaviour between both configurations. The non-acidified substrate could access internally to the upper sections of the non-staged system, in a similar way as it was imposed externally by adding multiple feedings in the staged digester.

The acetoclastic activity was stratified (increasing towards the top) in both digesters even when a triple feeding regime was applied in the staged digester. A maximum rate of 596 cm^3CH_4 at STP (g VS)$^{-1}d^{-1}$ compares well with those found by Guiot *et al*[18] and by Van Lier *et al*[4] for granular sludge present in staged systems. However the hydrogenotrophic activity showed a relative insensitivity with changes in operating conditions. This fact was previously detected when the effect of increasing substrate concentration was evaluated.[8] It was also interesting to observe that the acetoclastic activity of the second and third stages of AFII was higher than the observed for the top of the single-feed non-staged configuration. The syntrophic activity on propionate and butyrate were maximum in the third stage and decreased slightly when the triple

	D/uL	Recovered tracer (%)	MRT (h)	HRT (h)
AFI	24.4	93.1	3.81	10.2
AFII				
First feed line	2.43	96.0	9.4	18.5
Second feed line	4.35	127.0	3.2	8.0
Third feed line	3.10	70.0	1.4	3.6

Table 5. Results of tracer experiments in AFI and in each feeding of AFII: Application of the dispersion model

D – Longitudinal dispersion coefficient; *u*-superficial velocity; *L*-length; MRT-mean residence time.

feed was applied. The same decrease was observed for the hydrogenotrophic activity, which probably is related to the increase of acidogenic activity. In fact, as expected, the potential maximum lactose degradation rate was also improved in the latter stages due to the introduction of fresh substrate on that sections.

The detection of the maximum methanogenic activity with propionate as substrate at the top of both digesters was expected. Van Lier *et al*[4] established that propionate oxidation was stimulated at the top of the upflow staged sludge bed (USSB) reactor, due to more favourable conditions of intermediates, such as hydrogen, affecting the Gibbs energy of propionate oxidation. Harper and Pohland[1] also concluded that the removal of biogas from the early stages of substrate degradation induced a more suitable environment for propionate and acetate degradation in the latter stages.

The physical and morphological properties of the microbial aggregates were measured and a stratification of morphological properties was observed. The morphological parameters measured (fractal dimension and compactness) allowed the quantification of surface morphology, which is usually hindered by the deformable nature and handling problems of microbial flocs. Fractal dimension was previously used to differentiate flocs from granules[19] and to describe biofilm surfaces.[20–22] Compactness is related to the spherical shape of the aggregates and has also been used to describe biofilm morphology.[22] Between days 764 and 809, at the top of both configurations, the accumulation of large, and morphologically compact microbial aggregates with a smooth surface was noted. Simultaneously severe washout of a highly active granular-like biomass was observed. The presence of hollow aggregates at that moment was a possible explanation for the washout phenomenon. Another possible explanation was the high superficial biogas velocity that could induce washout by attachment of bubbles to the microbial aggregate surface. When a triple feed was introduced into the staged digester, the previously-formed large microbial aggregates decreased significantly in size, became highly irregular, but kept values of settling velocity higher than 100 m h^{-1} (Table 4). The growth of acidifying biomass on the surface of the aggregates could explain the increasing surface irregularity, but not the decrease in size. This was probably due to disintegration and release of entrapped biogas with a consequent increase in aggregate density (not determined), in a similar phenomenon already described by Yoda and Nishimura[23] for granular biomass. Although it was not measured for the non-staged configuration, it could be observed by visual inspection that after severe washout of large aggregates, they also became much smaller in this reactor. This occurrence was attributed to the overall operating conditions applied, more than to the conditions at the microenvironment around the microbial aggregates. This suggests non-stationary behaviour of physical and morphological properties of microbial flocs, where growth and disintegration may occur sequentially in a slow dynamic process.

5 CONCLUSIONS

There was no advantage to the use of a multi-feed staged system under the operating conditions tested. The overall performance and the microbial activity segregation were similar for both configurations. The microbial aggregates present in both digesters, particularly in the top sections changed significantly with respect to microbial, physical and morphological properties. The presence of large aggregates on that section did not prevent a strong washout phenomena between days 764 and 809. After this occurrence, particles become smaller and with a rougher surface, which was attributed to disintegration due to biogas accumulation and release. At the end of the trial period only 40% of the total volume was accessible to liquid phase.

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