Staged and Non-staged Anaerobic Filters: Microbial Activity Segregation, Hydrodynamic Behaviour and Performance

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Abstract: This work describes a comparative study of staged and non-staged anaerobic filters for treating a synthetic dairy waste under similar operating conditions. The effect of increasing the substrate concentration from 3 to 12 g COD dm^{-3} at a constant hydraulic residence time (HRT) of 2 days was evaluated with respect to overall reactor performance, biogas production, volatile fatty acids profiles along the height, methanogenic and acidogenic activity distribution, and hydrodynamic behaviour. The potential maximum specific methanogenic activity against acetate, hydrogen, propionate and butyrate and the lactose specific activity were determined for sludge sampled from three different points in each reactor, under two operating conditions (influent COD of 3 and 9 g COD dm^{-3}). Although all trophic groups involved in the anaerobic process were found throughout the reactors, it was possible to identify different specific sludges at different heights in both reactors. Performances of the two configurations were very similar under the operating conditions tested and the plug flow behaviour of the staged reactor was clearly reduced when the influent concentration increased from 3 to 9 g COD dm^{-3}. © 1998 Society of Chemical Industry

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Key words: Anaerobic filter; methanogenic activity; hydrodynamic studies; dairy waste

NOTATION

AFI	Non-staged digester
AFII	Staged digester
COD	Chemical oxygen demand
D	Longitudinal dispersion coefficient
HRT	Hydraulic retention time
J	Number of mixed tanks in the 'tanks in series model'
L	Length
MRT	Mean residence time
u	Upflow velocity
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
β	Fraction of dead volume of the plug flow zone in the compartmental flow model
$\tau a1, \tau a2$	Mean residence time in the first and second perfectly mixed zone in the compartmental flow model
τp	Mean residence time of the plug flow zone in the compartmental flow model

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1 INTRODUCTION

The operation of staged reactors is described in the literature from a number of viewpoints.[1–5] Removal of biogas from the early stages of substrate conversion was stated by Harper and Pohland[2] to be a factor in protection against toxic gaseous products (ammonia, sulphide, and air), thereby improving conditions for methanogenesis in the later stages. These workers concluded that the removal of biogas maintained low levels of hydrogen, particularly during pulse loading, so favouring acetic and propionic acid removal in the later stages.

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.[6] More recently Lettinga *et al.* stated that the type and sequence of stages should be optimised, according to each specific application.[7]

Enhanced stability of a thermophilic treatment system was achieved by Van Lier *et al.*[4] using a staged process in an Upflow Staged Sludge Bed (USSB). In the last compartment, a very low volatile fatty acid concentration and a low hydrogen partial pressure were obtained. These authors also reported that staged degradation led to a segregation of biomass in terms of its biological and physical properties.[8] Guiot *et al.*[5] described the operation of a full scale multiplate anaerobic digester with a large capacity for solid retention, and Grobicki and Stuckey[3] found that formate was an important intermediate in the anaerobic baffled reactor.

Although there is in the literature several references to staged systems, the application to fixed bed digesters is not vast. El-Shafie and Bloodgood[1] studied the degradation of a synthetic complex wastewater in a staged anaerobic filter with six compartments. Cheung *et al.*[9] operated and characterised a staged fixed bed process with three compartments and observed that methanogenic bacteria were predominant at the top. Comparison with non-staged systems was not done and the microbial studies were based on scanning electron microscopy.

In the anaerobic degradation process the organic matter is transformed in methane by a complex sequence of metabolic pathways where the microbial trophic groups are related by their substrate and product specificity. Biomass development and relative bacterial diversity is primarily governed by operating conditions (pH, temperature, waste composition, organic and hydraulic loading rate) which affect the physiological behaviour of bacteria and their physical properties, such as cell mass, size and density of aggregates.[10] However, reactor design and hydrodynamic behaviour may induce the accumulation of different intermediates, thus affecting the biological and physical properties of microbial aggregates.

The microbial consortia developed in anaerobic digesters can be characterised by several methods, such as direct enumeration, quantification of coenzyme F420 and direct, measurement of methanogenic activity based on methane production. Few of these methodologies are suitable for routine analysis. Typical practical problems associated with some of these techniques include the amount of VSS needed, lack of reliability and reproducibility and time-consumption.[11] In this work, the biomass developed in a staged upflow anaerobic filter is studied in terms of specific methanogenic activity against acetate, hydrogen, propionate and butyrate. The specific lactose activity is also measured. A control reactor, non-staged, is operated under the same operating conditions for comparative purposes.

2 MATERIALS AND METHODS

2.1 Experimental set-up and operation mode

The two anaerobic filters (AFI and AFII) were constructed in Plexiglas and are presented schematically in Fig. 1. The initial liquid volume of each reactor was 14·2 and 17·7 dm³ for AFI and AFII, respectively. Both configurations 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. The seed sludge was obtained from a municipal sewage sludge digester. Both reactors were inoculated with equal
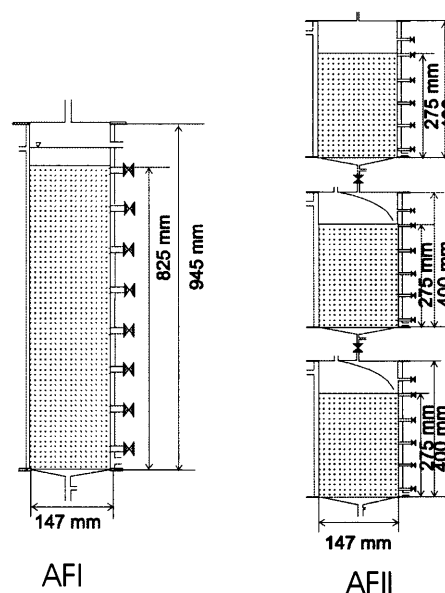

Fig. 1. Schematic configuration of AFI and AFII.

amounts of seed sludge (74·2 gVSS), but in AFII it was equally distributed among the three stages. 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 or four times a week. After attaining pseudo-steady state conditions, profiles of COD and volatile fatty acids along the height of each reactor were determined in duplicate. For influent concentrations of 3 and 9 g COD dm^{-3}, 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). Sludges were analysed for specific methanogenic activity against acetate, H_2/CO_2, propionate and butyrate. The specific rate of lactose consumption was also determined. Tracer experiments were conducted for each operating condition and the flow pattern was determined for both configurations.

2.2 Substrate

The substrate was made by dilution of skim milk with tap water and was supplemented with macro- and micronutrients which had the following composition. Macronutrients—$MgSO_4 \cdot 7H_2O$: 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—$FeCl_2 \cdot 6H_2O$: 2 g dm^{-3}; H_3BO_3: 0·05 g dm^{-3}; $ZnCl_2$: 0·05 g dm^{-3}; $CuCl_2 \cdot 2H_2O$: 0·038 g dm^{-3}; $MnCl_2 \cdot 4H_2O$: 0·5 g dm^{-3}; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: 0·05 g dm^{-3}; $AlCl_3 \cdot 6H_2O$: 0·09 g dm^{-3}; $CoCl_2 \cdot 6H_2O$: 2 g dm^{-3}; $NiCl_2 \cdot 6H_2O$: 0·092 g dm^{-3}; $Na_2SeO_3 \cdot 5H_2O$: 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.*[12] 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.[13] 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 using a Pye Unicam GCD gas chromatograph

(Cambridge, UK) using a column Chrompack Haysep Q (80–100 mesh). Nitrogen was used as carrier gas (30 cm^3 min^{-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.[11,14] 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) is 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 $\pm 202\cdot6$ kPa) over a range of -200 to $+200$ mV. The sensing element is connected to a digital panel module and the device is powered by a 9·0 V DC transformer. 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 the 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' from MATLAB (The Mathworks, USA) was used to perform calculations.

2.3.4 *Tracer experiments*
The residence time distribution (RTD) studies were performed by the stimulus–response technique[15] using lithium chloride as tracer. An automatic sampler (New Brunswick Scientific, Mx Biosampler, USA) was used to collect and refrigerate samples. Lithium was analysed by flame emission photometry (ATS, 200 MKI, Switzerland). The 'Optimisation Toolbox' from MATLAB (The Mathworks, Inc., USA) was used to obtain the optimal set of parameters by non-linear regression, using the Levenberg Marquardt method.[16]

3 RESULTS AND DISCUSSION

3.1 Reactor performance

Table 1 summarises the operating conditions and performance data of AFI and AFII. The overall performance of the two reactors was very similar over the

TABLE 1
Operating Conditions and Performance Data of AFI and AFII

	Time (days)	HRT (± 0.01) days	Influent COD ($mg\ dm^{-3}$)	Effluent total COD ($mg\ dm^{-3}$)	Organic loading rate ($kg\ COD\ m^{-3}\ day^{-1}$)	Soluble COD Efficiency (%)	CH_4 (%)	Methane yield $m^3\ CH_4\ kg\ COD\ removed^{-1}$
AFI	343–430	1·93	2948 ± 61	320 ± 129	1·53 ± 0·03	97·9 ± 0·3	65·3 ± 1·0	0·38 ± 0·03
	430–495	1·93	5831 ± 287	572 ± 173	3·02 ± 0·15	97·9 ± 0·6	64·8 ± 0·6	0·39 ± 0·04
	495–596	1·93	8837 ± 411	973 ± 217	4·58 ± 0·21	98·2 ± 0·4	62·3 ± 1·3	0·38 ± 0·03
	596–628	1·93	12 143 ± 402	2369 ± 254	6·29 ± 0·21	98·2 ± 0·2	61·1 ± 0·4	0·35 ± 0·02
AFII	343–430	2·05	2952 ± 60	178 ± 42	1·44 ± 0·03	98·0 ± 0·3	67·0 ± 6·9	0·34 ± 0·10
	430–495	2·05	5831 ± 287	204 ± 47	2·84 ± 0·14	98·8 ± 0·3	64·4 ± 5·0	0·35 ± 0·04
	495–596	2·05	8837 ± 411	1410 ± 407	4·31 ± 0·20	97·9 ± 0·7	61·0 ± 3·2	0·33 ± 0·03
	596–628	2·05	12 143 ± 402	4506 ± 1677	5·92 ± 0·20	98·3 ± 0·5	58·4 ± 1·3	0·32 ± 0·02

trial period. In AFI the methane yield was higher than in AFII and the effluent volatile solids in AFII, measured by the total effluent COD, increased more than in AFI. This could suggest a shift of COD removal towards biomass production in the staged digester. However the values are not significantly different, considering the associated 95% confidence intervals.

In the staged digester the contribution of each stage to the biogas production changed along the operation. Initially the first stage produced 73% of the total biogas, the second stage 20% and the third stage only 7%. At the end of the trial period the two first stages produced similar volumes of biogas, but the contribution of the third stage was only 11% (Fig. 2).

For influent concentrations of 3 and 9 g COD dm^{-3}, the soluble COD and the COD of volatile fatty acids decreased sharply at the inlet of each reactor and the evolution through the reactors was identical for the two operating conditions (Fig. 3).

However, as can be seen in Fig. 4, the increase in the influent COD altered the aspect of VFA evolution. At an influent COD of 3 g dm^{-3}, a net decrease of acetic and propionic acids was measured at the bottom of AFI

and in the first stage of AFII. In the upper sections, practically no VFA were detected in either reactor. This may be explained by the relatively low load applied and by the hydrodynamic behaviour of each configuration. As discussed later, AFI was found to be well mixed in 79% of its volume, but in the same conditions AFII was less well mixed. At an influent COD of 9 g dm^{-3}, the bottom of AFI and the first stage of AFII became more acidogenic with a detectable production of acetate and propionate.

Lactate was degraded very quickly in all situations and formate, which was considered by Grobicky and Stuckey[3] to be an important intermediate formed in the initial compartments of the anaerobic baffled reactor, disappeared close to the inlet of the reactors. This fact may be attributed to the lower loading rates applied and to the lower number of stages in AFII, compared with the baffled reactor studied by these authors.

3.2 Characterisation of microbial aggregates: activity and physical properties

Table 2 gives the average volatile solids determined after collection of a representative volume of entrapped solids, and shows the effect of influent concentration on the entrapped VS accumulated in the reactors. For influent concentration of 3 g COD dm^{-3}, the ash content was slightly lower in the bottom section, but in the later sections differences were not very significant. At an influent COD of 9 g dm^{-3} the ash content increased towards the middle and top sections in both configurations. pH values were higher in the upper sections, which could explain the presence of a higher content of inorganic solids at the top. Higher values of VS at the top of AFI were observed when compared with AFII but biomass growth was, on average, lower in AFI than in AFII. This reinforces the hypothesis of a shift in COD removal toward biomass production in the staged digester. However, it should be emphasised that these values represent only entrapped biomass. Other experiments using the same support media and
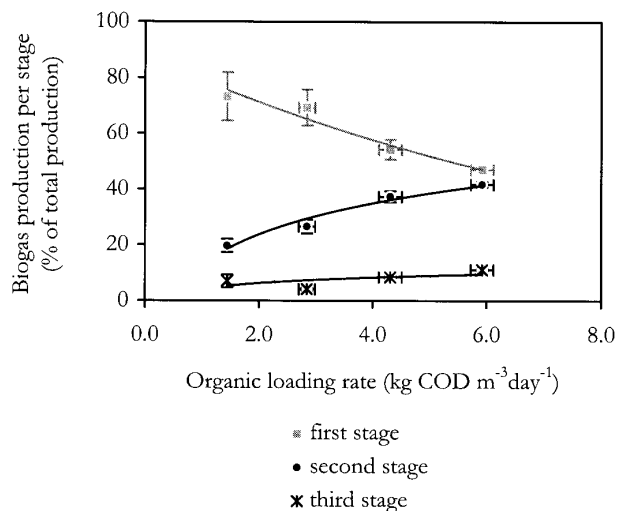

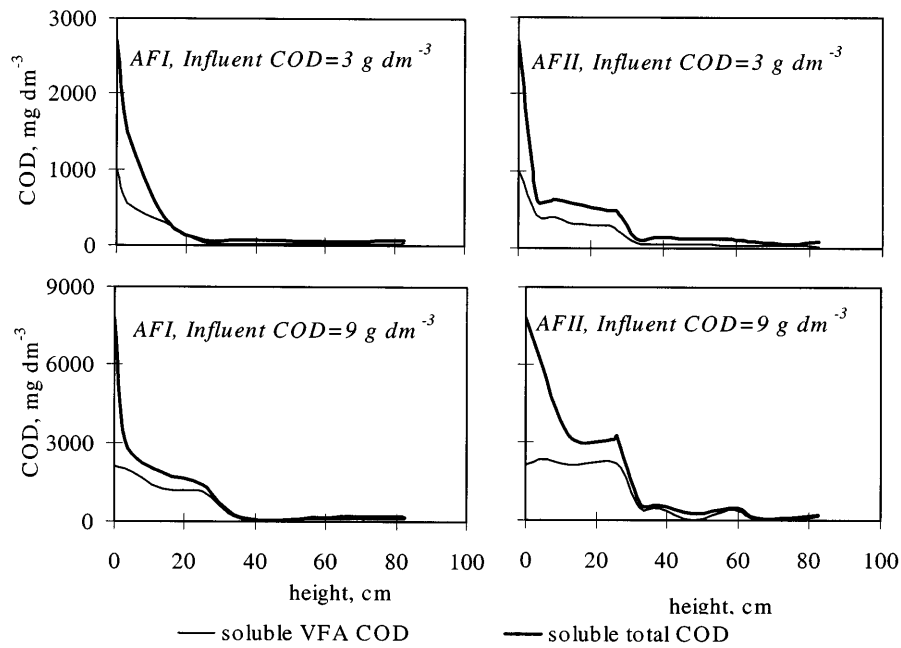**Fig. 2.** Contribution of each stage of AFII for the biogas production.

Fig. 3. Soluble COD and VFA-COD along the height of reactors AFI and AFII for influent COD of 3 and 9 g dm^{-3}.

substrate, but an influent COD of 12 g dm^{-3}, have demonstrated that the adhered biomass reached 40% of the total biomass retained in reactors of the configuration under study.[17]

The specific methanogenic activity against direct methanogenic substrates (acetate and H_2/CO_2) and the specific acidogenic activity against lactose are shown in Table 3 for the two operating conditions tested. At an influent COD of 3 g COD dm^{-3}, both methanogenic activities were higher for AFII, except in the upper

section where mixing effects might make the access of substrate in AFI higher than in AFII. The acetoclastic activity was maximal at the middle sections and the hydrogenophilic activity increased towards the top.

After the increase in the influent concentration, the specific acetoclastic activity became more stratified than the hydrogenophilic activity. This effect was more pronounced in the staged reactor. As suggested for staged reactors by Van Lier *et al.*[4] and by Guiot *et al.*,[5] the dilution of methanogenic bacteria with acidifying
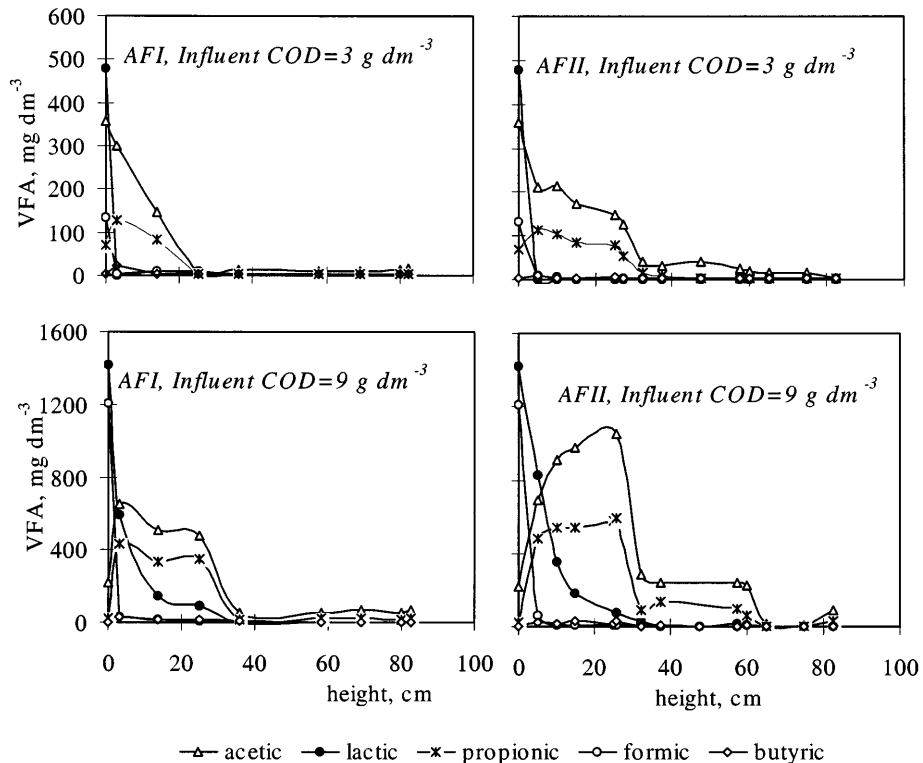

Fig. 4. VFA concentration along the height of reactors AFI and AFII for influent COD of 3 and 9 g dm^{-3}.

TABLE 2
Volatile Solids Distribution Along the Height for AFI and AFII

Influent COD ($g\ dm^{-3}$):		Entrapped VS ($g\ dm^{-3}$)		% VS/TS	
		3	9	3	9
AFI	Top	$0\cdot66 \pm 0\cdot01$	$5\cdot22 \pm 0\cdot56$	73·5	59·5
	Middle	$2\cdot95 \pm 0\cdot09$	$9\cdot71 \pm 0\cdot19$	73·7	53·6
	Bottom	$5\cdot02 \pm 0\cdot60$	$17\cdot10 \pm 1\cdot04$	76·9	82·7
	Average	$2\cdot88 \pm 0\cdot61$	$10\cdot7 \pm 1\cdot20$		
AFII	Third stage	$0\cdot21 \pm 0\cdot11$	$3\cdot81 \pm 0\cdot04$	74·4	58·7
	Second stage	$2\cdot38 \pm 0\cdot26$	$17\cdot5 \pm 0\cdot04$	70·2	48·9
	First stage	$5\cdot92 \pm 0\cdot45$	$16\cdot10 \pm 1\cdot48$	77·9	73·0
	Average	$2\cdot84 \pm 0\cdot53$	$12\cdot5 \pm 1\cdot48$		

biomass can explain the low value of acetoclastic activity in the first stage of AFII. The acidogenic activity, measured by the activity against lactose, was mainly located at the bottom of each reactor, for an influent COD of 3 g dm^{-3}. For influent COD of 9 g dm^{-3} it was extended to all sections, though the COD and VFA profiles along the height suggest that all the substrate was acidified at the inlet of the third stage of AFII. Higher stratification of the acidogenic activity was observed in AFII than in AFI. The hypothesis of methanogenic activity dilution with acidifying biomass was not observed in the latter sections which were dominated by the growth of methanogenic acetoclastic and hydrogenophilic bacteria. Similarly, the hydrogenophilic activity in AFII's bottom section remained unchanged.

The two genera of acetate-utilising bacteria, *Methanosaeta* (formerly *Methanothrix*) and *Methanosarcina*, differ with respect to substrate affinity, maximum growth rate and substrate specificity. *Methanosarcina* is able to utilise several substrates, including hydrogen and acetate, whereas *Methanosaeta* can only degrade acetate. Harper and Pohland[18] suggested that the multiple substrate utilisation ability of *Methanosarcina* (Hydrogen Oxidising Acetotroph or HOA) confers a

higher survivability on these species. They also commented on the inhibitory effect of hydrogen on acetate degradation by HOA species and on the preference of HOA species for hydrogen over acetate, as confirmed by thermodynamic considerations. These facts suggest that in the first stage of AFII, with an influent COD of 9 g COD dm^{-3}, *Methanosarcina* should be the predominant acetate degrader exhibiting a low acetoclastic activity. On the other hand, in the first stage, the pH values were 6·4–6·6, which are beneath the optimal pH for growth of *Methanosaeta*.

The specific methanogenic activity against propionate and butyrate is shown in Table 4. Since these are indirect methanogenic substrates, a valid measurement of the maximum specific methanogenic activity against these acids can only be obtained when the acetoclastic and hydrogenophilic activities are not rate-limiting.[19] A comparison of Tables 3 and 4 suggests that this situation prevailed for all samples, with the single exception of biomass removed from the bottom section of AFII at an influent COD concentration of 9 g dm^{-3} where the acetoclastic activity was lower than the measured propionate utilising activity.

At an influent concentration of 3 g COD dm^{-3} there was no stratification of propionate activity in both

TABLE 3
Specific Acetoclastic and Hydrogenophilic Activity and Specific Acidogenic Activity on Lactose

Influent COD ($g\ dm^{-3}$):		Specific methanogenic activity against ($cm^3\ CH_4$ at STP $g^{-1}\ VS\ day^{-1}$)				Specific lactose activity ($mmoles\ lactose\ g^{-1}\ VS\ day^{-1}$)	
		Acetate		H_2/CO_2		3	9
		3	9	3	9		
AFI	Top	$102\cdot4 \pm 6\cdot2$	$281\cdot1 \pm 5\cdot8$	1020 ± 113	$1085\cdot1 \pm 34\cdot1$	n.d.	$9\cdot9 \pm 1\cdot5$
	Middle	$127\cdot5 \pm 4\cdot8$	$258\cdot6 \pm 41\cdot1$	$676\cdot9 \pm 8\cdot2$	$819\cdot7 \pm 40\cdot3$	$0\cdot7 \pm 0\cdot1$	$11\cdot0 \pm 1\cdot6$
	Bottom	$83\cdot0 \pm 9\cdot8$	$171\cdot0 \pm 16\cdot4$	$624\cdot4 \pm 167\cdot6$	$805\cdot6 \pm 35\cdot5$	$5\cdot2 \pm 0\cdot8$	$11\cdot6 \pm 1\cdot7$
AFII	Third stage	$89\cdot8 \pm 12\cdot3$	$255\cdot6 \pm 13\cdot9$	$933\cdot5 \pm 159\cdot4$	1030 ± 108	n.d·	$7\cdot0 \pm 1\cdot1$
	Second stage	$172\cdot5 \pm 7\cdot5$	$232\cdot9 \pm 19\cdot7$	$907\cdot3 \pm 65\cdot3$	$961\cdot8 \pm 26\cdot0$	$0\cdot6 \pm 0\cdot1$	$7\cdot8 \pm 1\cdot2$
	First stage	$143\cdot5 \pm 11\cdot6$	$31\cdot6 \pm 10\cdot9$	$819\cdot7 \pm 95\cdot3$	$796\cdot3 \pm 123\cdot1$	$8\cdot0 \pm 1\cdot2$	$11\cdot5 \pm 1\cdot7$

TABLE 4
Specific Methanogenic Activity Against Propionate and Butyrate

Influent COD ($g\ dm^{-3}$):		Specific methanogenic activity against ($cm^3\ CH_4$ at STP g^{-1} VS day^{-1})			
		Propionate		*Butyrate*	
		3	9	3	9
AFI	Top	n.d.	85·0 ± 28·1	n.d.	6·2 ± 5·1
	Middle	48·8 ± 3·1	151·6 ± 5·4	81·9 ± 3·04	34·9 ± 5·4
	Bottom	58·3 ± 8·8	55·4 ± 18·0	94·8 ± 10·7	1·0 ± 2·0
AFII	Third stage	n.d.	93·4 ± 9·4	n.d.	14·6 ± 2·2
	Second stage	100·5 ± 1·7	146·6 ± 23·2	64·3 ± 6·7	13·7 ± 8·1
	First stage	96·2 ± 13·5	37·0 ± 1·7	58·3 ± 1·7	1·5 ± 5·6

n.d., not determined.

digesters, but, at an influent COD of 9 g dm^{-3}, a definite maximum was observed in the middle sections, indicating that syntrophic bacteria were predominantly located in these sections. The specific methanogenic activity against butyrate was clearly reduced with the increase in influent concentration. For the non-staged reactor (AFI) and influent concentration of 9 g dm^{-3}, a net maximum in the middle section was observed. The comparison between the spatial distribution of activity in both configurations (Tables 3 and 4) led to the conclusion that, for the lowest influent concentration tested, no clear stratification of activity was observed. With the exception of the methanogenic activity against butyrate, the staged digesters promoted a more active microbial population. With the increase in substrate concentration, the methanogenic activity against propionate became maximal at the middle sections and acetoclastic and hydrogenophilic activities were predominantly located at the top in both digesters. An evident stratification was observed for the acetoclastic activity in the staged digester.

Guiot *et al.*[10] found, in the multiplate anaerobic reactor treating cheese whey effluent, values of specific methanogenic activity between 45 and 270 cm^3 CH$_4$ g^{-1} VSS day^{-1} and between 560 and 1260 cm^3 CH$_4$ g^{-1} VSS day^{-1} for acetoclastic and hydrogenophilic activity respectively. For methanogenic activity against propionate they found values between 29 and 98 cm^3 CH$_4$ g^{-1} VSS day^{-1}. These values are in the same range of those obtained in the present work.

Table 5 gives the physical properties of the microbial aggregates determined for the same operating conditions. At an influent COD of 3 g COD dm^{-3}, the size of the microbial flocs was almost uniform over all the sections, but the increase in influent COD induced their growth especially at the top sections. In the staged digester the size of the aggregates located at the top increased 2·4-fold. By comparing size and settling velocity, it is possible to conclude that the biggest aggregates are less dense than the aggregates present in the middle sections, which are smaller but have a higher settling velocity. A possible explanation is the internal accumulation of biogas in the particles located at the top.

The higher particle density in the middle sections can, in part, explain the maximum methanogenic activity against propionate observed in those points. In fact, it was suggested by Schink and Thauer[20] that syntrophic associations are favoured by a close physical association

TABLE 5
Size and Settling Velocity of Microbial Aggregates

Influent COD ($g\ dm^{-3}$)		*Size* (μm)		*Settling velocity* ($m\ h^{-1}$)	
		3	9	3	9
AFI	Top	1306 ± 61	2033 ± 97	20·5 ± 3·1	27·5 ± 4·2
	Middle	1177 ± 60	1518 ± 110	17·9 ± 2·2	36·6 ± 6·2
	Bottom	1580 ± 79	—[a]	24·5 ± 3·2	—[a]
AFII	Third stage	951 ± 56	2248 ± 215	19·9 ± 2·0	27·7 ± 3·5
	Second stage	1520 ± 68	2100 ± 111	26·4 ± 3·7	59·8 ± 10·4
	First stage	1421 ± 66	—[a]	29·0 ± 4·4	—[a]

[a] The aggregates were too fluffy, had an undefined size and did not settle.
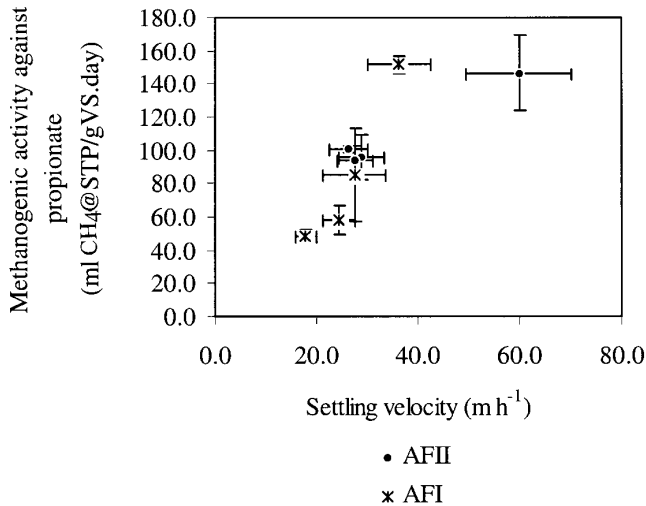
Fig. 5. Methanogenic activity against propionate versus settling velocity of microbial aggregates.

3.3 Tracer experiments

Tracer experiments were performed for influent concentrations of 3 and 9 g COD dm^{-3}, in order to evaluate the effect of increasing gas production and biomass in the flow pattern for both reactors. Table 6 represents the results obtained with the application of the 'dispersion model' proposed by Levenspiel.[15] This is a simple model with a single parameter (Dispersion Number = D/uL) and predicts flow patterns from ideal plug flow (dispersion number = 0) to ideal completely mixed flow (dispersion number = infinite). A value of 0·2 is defined as a large degree of dispersion and 0·02 as an intermediate degree of dispersion.

Although the obtained parameters represent optimal values for each experiment, it can be observed from Table 6 that, in some situations, the correlation coefficient is low, indicating that the dispersion model is not suitable to predict flow in such cases. The multi-parameter model proposed by Young and Young[21] fits well to the experimental data obtained for AFI, for influent concentrations of 3 g COD dm^{-3} (a) and 9 g COD dm^{-3} (b) (Fig. 6).

of acetogenic and hydrogenotrophic bacteria. The increasing trend between the methanogenic activity against propionate and the settling velocity, measured for the microbial aggregates present in all the sections of both configurations is presented in Fig. 5.

TABLE 6

Results of Tracer Experiments. Application of the Dispersion Model

	Influent COD = 3 g dm^{-3}			*Influent COD = 9 g dm^{-3}*		
	D/uL (correlation coefficient, R^2)	*Recovered tracer (%)*	*MRT (h)*	*D/uL (correlation coefficient, R^2)*	*Recovered tracer (%)*	*MRT (h)*
AFI	0·82 (0·596)	73·1	35·4	0·92 (0·858)	100·6	38·5
AFII	0·15 (0·848)	93·8	55·0	0·59 (0·975)	109·3	42·4

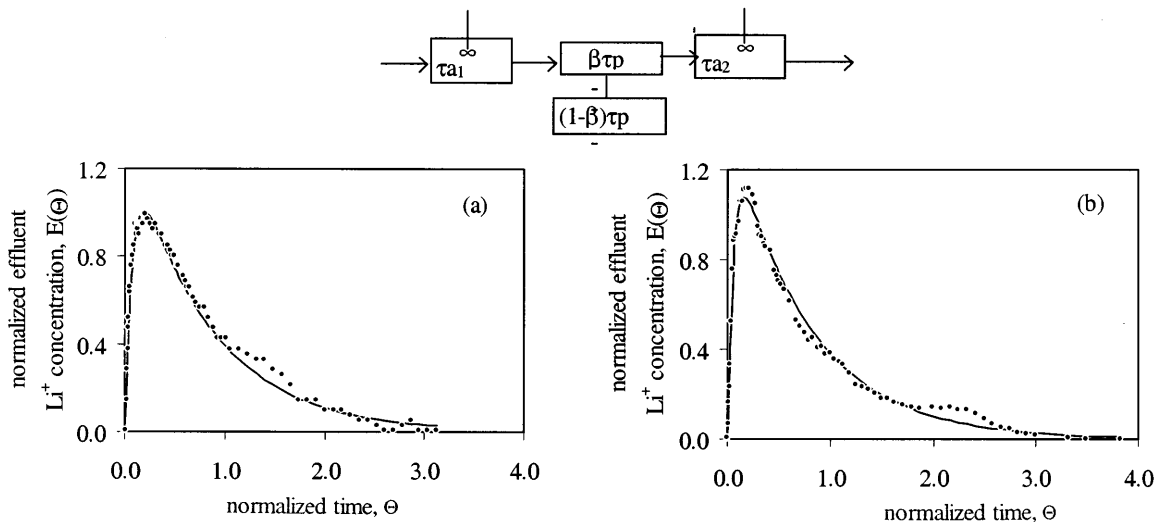D—longitudinal dispersion coefficient, u—superficial velocity, L—length; MRT—mean residence time.

Fig. 6. Application of the model proposed by Young and Young[21] to the residence time distribution experiment for AFI. (a) Influent COD = 3 g dm^{-3}. (b) Influent COD = 9 g dm^{-3}.
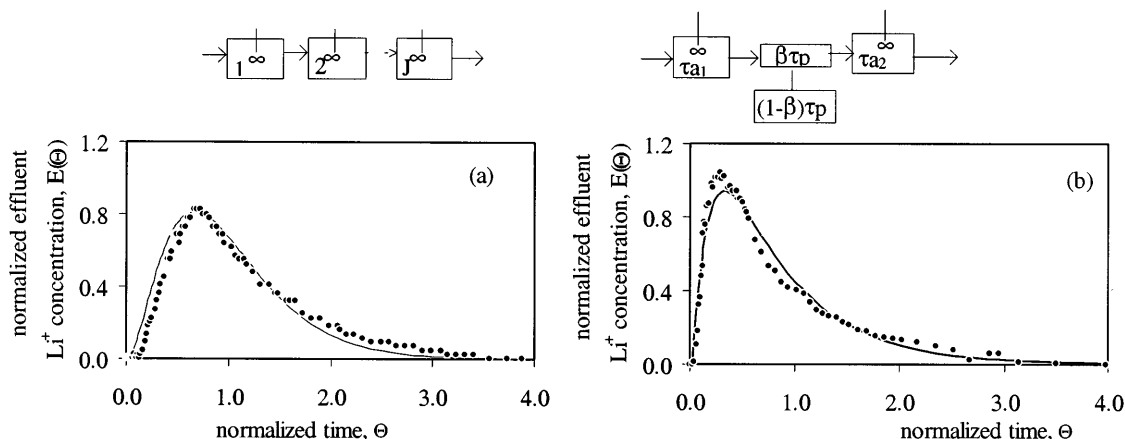
Fig. 7. Application of the 'tanks in series' model and the model proposed by Young and Young[21] to the residence time distribution experiment for AFII. (a) Influent COD $= 3$ g dm^{-3}. (b) Influent COD $= 9$ g dm^{-3}.

This model predicts the existence of a completely mixed inlet zone followed by a plug flow volume in parallel with a dead-zone and finally a second completely mixed zone. For AFII and with an influent COD of 3 g dm^{-3}, a series of three completely mixed volumes described well the flow pattern in this reactor, whereas for influent COD of 9 g dm^{-3}, the model of Young and Young[21] was applied with good agreement to the experimental data (Fig. 7).

Table 7 gives the results of the application of the above mentioned models for both configurations. The application of the Young and Young's model[21] to AFI revealed that the mixed inlet represented 6% of the total volume, the plug flow was 15% of the total volume (where there is detectable substrate depletion—Figs 3 and 4) and the remaining 79% of the volume was completely mixed. The dead volume was 14·9% of the total volume or 96·8% of the plug flow volume. As observed with the dispersion model (Table 6), no effect of increasing biogas and biomass was detected on the model parameters. This may be explained by the impossibility

of distinction between hydraulic and biological dead space with this technique of stimulus–response. It is possible that a shift from hydraulic to biological dead volume had occurred with the increase in influent concentration. Comparison of Fig. 7(a) and (b) suggests that the increase in influent COD increased the amount of dispersion in the staged digester. This was also clear from the application of the dispersion model (Table 6). Furthermore at an influent COD of 9 g dm^{-3}, the comparison between AFI and AFII flow pattern (Figs 6(b) and 7(b)) revealed that differences were only marginal. This agrees with the slight differences found for the COD and VFA distributions, microbial segregation and for the performance of the two configurations.

4 CONCLUSIONS

For the range of operating conditions tested, there was no clear advantage on the use of staged systems. The stratification of microbial activity increased with the increase in the influent concentration and was more

TABLE 7
Results of Tracer Experiments. Application of the Tanks in Series Model and Young and Young's Model[21]

Digester Configuration		Influent COD 3 g dm^{-3}	Influent COD 9 g dm^{-3}
Non-staged (AFI)	$\tau a1$ (h)	3·2	2·6
	τp (h)	6·7	7·7
	β	0·032	0·032
	$\tau a2$ (h)	36·5	36·3
	Correlation coefficient, R^2	0·983	0·992
Staged (AFII)	$\tau a1$ (h)	—	7·6
	τp (h)	—	9·2
	β	—	0·138
	$\tau a2$ (h)	—	31·2
	Number of tanks, J	3·0	—
	Correlation coefficient, R^2	0·923	0·962

pronounced in the staged digester. However, for both configurations, the maximum specific acetoclastic and hydrogenophilic activities were observed at the top of the reactors. The specific methanogenic activity against propionate, corresponding to the activity of syntrophic population, was maximal in the middle sections for both configurations and the lactose specific activity was maximal at the bottom. The microbial aggregates present in the middle section were the most dense, which was related to the higher specific methanogenic activity against propionate. Tracer experiments revealed that for the non-staged reactor the flow pattern was unchanged with the increase of influent concentration, whereas for the staged reactor the amount of dispersion detected increased significantly.

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