

STAGED AND NON STAGED ANAEROBIC FILTERS: MICROBIAL SELECTION, HYDRODYNAMIC ASPECTS AND PERFORMANCE

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ABSTRACT

This work reports on the study of a staged and a non staged anaerobic filter treating a synthetic dairy waste under similar operating conditions. The effect of increasing the substrate concentration from 3 to 12 g COD/l at a constant hydraulic residence time (HRT) of 2 days was evaluated with respect to overall reactor performance, gas production and volatile fatty acids profiles along the height. The potential maximum specific methanogenic activity against acetate, H₂/CO₂, two indirect substrates (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/l). Although all microbial phases of anaerobic process were found throughout the reactors, it was possible to identify different specific sludges at different heights in both reactors. The pressure transducer technique applied was proven to be a reliable method to study methanogenic activity of different trophic groups in consortia. 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/l.

KEY WORDS

Anaerobic filter; methanogenic activity; hydrodynamic studies; dairy waste.

INTRODUCTION

The anaerobic filter, described in numerous applications after the work of Young and McCarty (1967), is a fixed bed process with biomass immobilisation by adhesion to the media surface or entrapment in its void space, as granular or flocculent aggregates. In spite of the huge increase of UASB plants world-wide in the last decade, especially for industrial waste treatment, the anaerobic filter is, in some cases, appointed as the best choice when compared with other alternatives (Hawkes *et al.*, 1995). Its relatively easy operation, high stability and performance make this system attractive for several applications. The treatment capacity of any anaerobic digester is dictated by the amount and activity of the biomass present and by the contact between substrate and biomass. One of the major drawbacks in studying anaerobic filters is the difficulty of measuring biomass quantity and quality. On the other hand, the flow through this type of digester with accumulating biomass is not usually easily determined (Young and Young, 1988).

Organic matter is anaerobically degraded to methane by a complex sequence of metabolic pathways where the microbial trophic groups are related by their substrate and product specificity. Microbial selection 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 (Morgan *et al.*, 1991). Reactor designs which may induce the accumulation of different intermediates can also be considered to affect microbial selection.

The operation of staged reactors is described in the literature from a number of viewpoints. Removal of biogas from the early stages of substrate conversion is considered to be a factor of protection against toxic gaseous products (ammonia, sulphide, and air), thereby improving conditions for methanogenesis in the later stages (Harper and Poland, 1987). These authors concluded that the removal of biogas affected the hydrogen environment, keeping it at low levels - particularly during pulse loading - and favouring acetic and propionic acid removal in the later stages. Van Lier *et al.* (1994) reported the enhancement of stability of a thermophilic treatment system by applying a staged process in a Upflow Staged Sludge Bed (USSB) reactor. In the last compartments, a very low VFA concentration and a low hydrogen partial pressure was achieved. Recently, these authors reported that the staged degradation led to a segregation of biomass in terms of its biological and physical properties (Van Lier *et al.*, 1996). The plug flow operation with consequent reduction of mixing of the staged sludges is also thought to result in a higher stability and process efficiency. Staged processes also provide an optimal environment for degradation of intermediates, such as propionate (Lettinga, 1995). According to this author, this is specially useful for thermophilic processes, but should also be beneficial for mesophilic treatment.

The anaerobic 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, their lack of reliability and reproducibility and their time consuming-nature (Morgan *et al.*, 1991; Colleran *et al.*, 1992). In this work, the biomass developed in a staged upflow anaerobic filter was studied in terms of specific methanogenic activity against direct (H_2/CO_2 , acetate) and indirect substrates (propionate and butyrate). The specific lactose activity was also measured. A control reactor, mono-staged, under the same operating conditions, was used for comparative purposes. The distribution of metabolic intermediates and microbial activity was related with the hydrodynamic behaviour of both anaerobic filters.

MATERIALS AND METHODS

Experimental set-up and operation mode

The two anaerobic filters (AFI and AFII) were constructed in Plexiglas and are schematically described in Figure 1.

The initial liquid volume of each reactor was 14.2 and 17.7 L for AFI and AFII, respectively. Both configurations had an equal volume of support medium which consisted of PVC raschig rings of 21 mm in size, with a specific surface area of 230 m²/m³ 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 sludge digester. Both reactors were inoculated with equal 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 achievement of pseudo- steady state conditions, profiles of COD and volatile fatty acids along the height of each reactor were assessed in duplicate. For influent concentration of 3 and 9 gCOD/l, biomass was withdrawn from three points in each reactor (first

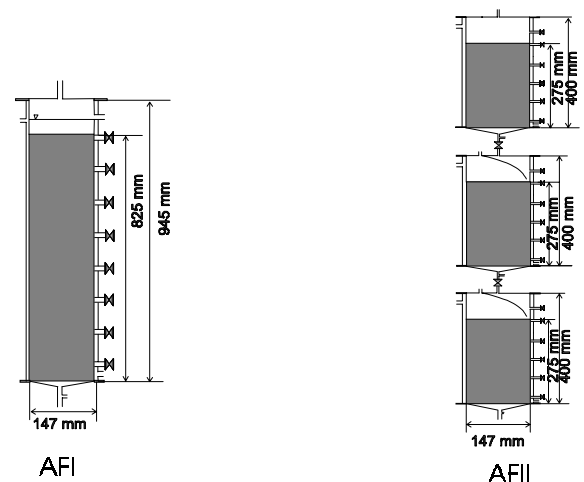


Figure 1. Schematic configuration of AFI and AFII.

sampling port of each stage of AFII and equivalent points in AFI - located at 5, 32 and 65 cm from the bottom). These sludges were analysed for specific methanogenic activity against acetate, H₂/CO₂, propionate and butyrate. The specific rate of lactose consumption was also determined. For these two operating conditions tracer experiments were conducted and the flow pattern was determined for both configurations.

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 - MgSO₄.7H₂O:30.2 g/l; KH₂PO₄: 28.3 g/l KCl: 45 g/l. 0.6 ml of this solution was added per gram of COD fed. Micronutrients - FeCl₂.6H₂O: 2 g/l; H₃BO₃: 0.05 g/l; ZnCl₂: 0.05 g/l; CuCl₂.2H₂O: 0.038 g/l; MnCl₂.4H₂O: 0.5 g/l; (NH₄)₆Mo₇O₂₄.4H₂O: 0.05 g/l; AlCl₃.6H₂O: 0.09 g/l; CoCl₂.6H₂O: 2 g/l; NiCl₂.6H₂O:0.092 g/l; Na₂SeO₃.5H₂O: 0.164 g/l; EDTA: 1g/l, Resazurin: 0.2 g/l; HCl 37%: 1 ml/l. The composition of this solution was based on the work of Zehnder *et al.* (1980). Micronutrients were supplemented to the influent feed by addition of 1 ml per litre of feed.

Analytical methods

Routine analysis. COD, volatile and total solids (VS and TS) were determined by Standard Methods (APHA, AWWA, WPCF, 1989). VFA were determined by HPLC (Jasco, Japan) using a column Chrompack (cat n°28350); the mobile phase was sulphuric acid (0.01N) at a flow rate of 0.7 ml/min. The column temperature was set at 40°C and the detection was made spectrophotometrically at a wave length of 210 nm. The methane content of the biogas was measured by a Pye Unicam GCD gas chromatograph (Cambridge, England) using a column Chrompack Haysep Q (80-100 mesh). N₂ was used as carrier gas (30 ml/min) and the temperatures of injection port, column and flame ionisation detector were 120, 40 and 130°C, respectively.

Activity measurements. Methanogenic activity tests were performed using a pressure transducer technique (Colleran *et al.*, 1992). The test involves the monitoring of the pressure increase developed in sealed vials fed with non-gaseous substrates or pressure decrease in vials previously pressurised with gaseous substrates (H₂/CO₂). Strict anaerobic conditions were maintained. The hand held pressure transducer used was developed at University College Galway, Ireland and was capable of measuring a pressure increase or decrease of two atmosphere (0 to ± 202.6 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, catalogue number 986119.

Tracer experiments. The residence time distribution (RTD) studies were performed by the stimulus-response technique (Levenspiel, 1972) using lithium chloride as tracer. 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 "Optimization 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.

RESULTS AND DISCUSSION

Reactor Performance

Table 1 summarises the operating conditions and performance data of AFI and AFII. As is evident, the overall performance of the two reactors was very similar over the trial period. The slightly higher biogas production from AFI is evidenced by a marginally higher methane yield from AFI (average value over the trial period 0.340±0.023 m³CH₄/KgCOD_{removed}) than from AFII (average value over the trial period 0.312±0.057 m³CH₄/KgCOD_{removed}). Figure 2 represents the evolution of COD and volatile fatty acids (VFA) along the reactor height for the two configurations and for influent concentrations of 3 and 9 g COD/l. The

soluble COD and the COD of volatile fatty acids decreased sharply at the inlet of each reactor and their evolution through the reactors were identical for the two operating conditions. However, as can be seen, increasing the influent COD concentration altered the aspect of VFA evolution. At an influent COD of 3 g/l, a net decrease of acetic and propionic acids was observed at the bottom of AFI and in the 1st stage of AFII. In the upper sections, practically no VFA were detected for both reactors. This may be explained by the relatively low load applied and by the hydrodynamic behaviour of each configuration.

Table 1. Operating conditions and performance data of AFI and AFII

	Time (days)	HRT (±0.01days)	Influent COD (mg/l)	Effluent COD (mg/l)	Organic loading rate (KgCOD/m ³ .day)	Efficiency (%)	CH ₄ (%)	biogas (m ³ /m ³ .day)
AFI	[343-430]	1.93	2948±61	62±7	1.55±0.07	97.9±0.3	65.3±0.5	0.86±0.04
	[430-495]	1.93	5911±293	130±32	3.02±0.15	97.7±0.6	64.8±0.6	1.77±0.12
	[495-596]	1.93	8837±411	157±33	4.52±0.22	98.2±0.4	62.3±1.3	2.65±0.09
	[596-628]	1.93	12143±402	215±26	6.25±0.24	98.2±0.2	61.1±0.5	3.51±0.13
AFII	[343-430]	2.05	2952±60	59±8	1.44±0.40	98.0±0.3	67.0±6.9	0.72±0.05
	[430-495]	2.05	5911±293	68±16	2.81±0.13	98.8±0.3	64.4±5.0	1.50±0.08
	[495-596]	2.05	8837±411	175±56	4.34±0.21	97.9±0.7	61.0±3.2	2.29±0.08
	[596-628]	2.05	12143±402	200±55	6.09±0.24	98.3±0.5	58.3±5.1	3.30±0.19

As discussed later, AFI was found to be well mixed in 75% of its volume, but in the same conditions AFII showed a more plug flow behaviour. At an influent COD of 9 g/l, the bottom of AFI and the first stage of AFII became more acidogenic with a visible production of acetate and propionate. Lactate was degraded very quickly in all situations and formate, which was considered to be an important intermediate formed in early compartments of the anaerobic baffled reactor (Gorbicki and Stuckey, 1991), disappeared just after the inlet of the reactors. This fact may be attributed to the relatively low loading rates applied and to the lower number of stages in AFII, compared to the baffled reactor studied by these authors.

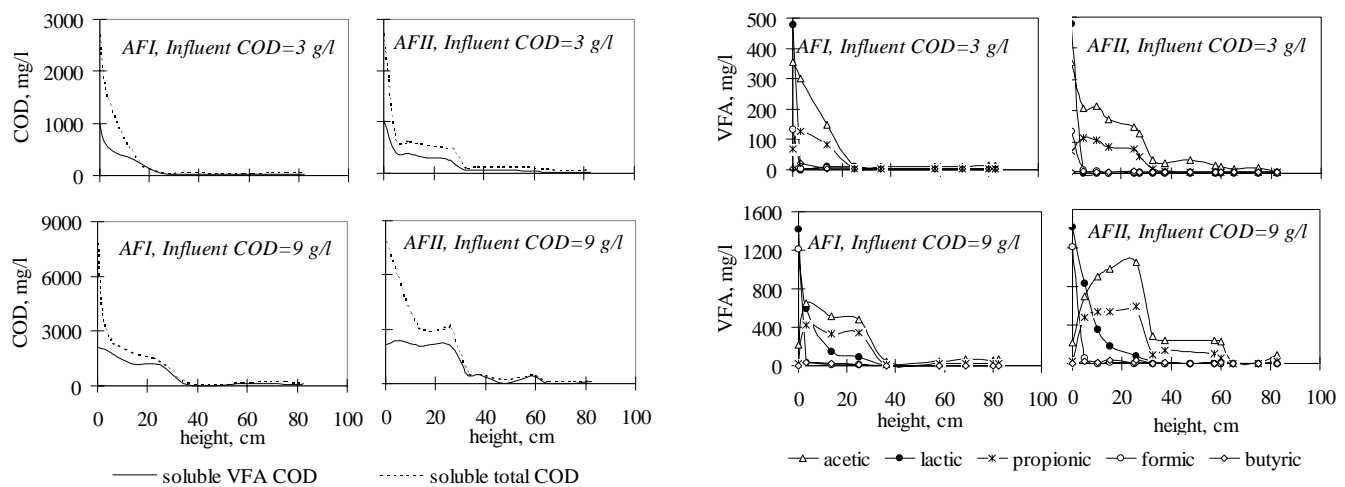


Figure 2. Profiles of COD and Volatile Fatty Acids along the height of reactors AFI and AFII at two different influent concentrations.

Biomass analysis

At influent concentrations of 3 and 9 g COD/l, samples of biomass were removed at three points of each reactor and analysed for VS concentration and microbial activity. Table 2 represents the average VS determined after collection of a representative volume of entrapped solids showing the effect of influent concentration on the entrapped VS accumulated in the reactors. For influent concentration of 3 g COD/l, the ash content was slightly lower in the bottom section, but differences from the later sections were not very significant. At an influent concentration of 9 g COD/l, a net increase of ash content in the middle and top sections was observed. The increase in pH towards the top (6.6 to 7.4, in average) can partially justify the accumulation of inorganic precipitates in the same direction. Higher values of VS in the top of AFI were

observed when compared with AFII but growth of biomass was, in average, slightly lower in AFI than in AFII. This could be explained by a shift of COD removal towards methane production in this reactor, justifying the marginally higher methane yield obtained. However, as the values are very close it is impossible to make definitive conclusions. It should be emphasised that these values represent only entrapped biomass. Other experiments using the same support media and substrate, but under different operating conditions, have demonstrated that the amount of adhered biomass was not more than 15% of the total biomass retained in reactors of the configuration under study.

Table 2. VS distribution along the height for AFI and AFII

		entrapped VS, g/l		%VS/TS	
		Influent COD=3 g/l	Influent COD=9 g/l	Influent COD=3 g/l	Influent COD=9 g/l
AFI	Bottom	5.02±0.60	17.10±1.04	76.9	82.7
	Middle	2.95±0.09	9.71±0.19	73.7	53.6
	Top	0.66±0.01	5.22±0.56	73.5	59.5
	average	2.88±0.61	10.7±1.20	-	-
AFII	Bottom	5.92±0.45	16.10±1.48	77.9	73.0
	Middle	2.38±0.26	17.5±0.04	70.2	48.9
	Top	0.21±0.11	3.81±0.04	74.4	58.7
	average	2.84±.53	12.5±1.48	-	-

Figure 3 represents the specific methanogenic activity against direct substrates (acetate and H₂/CO₂) for the two operating conditions tested. At an influent COD of 3 gCOD/l, both 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. As expected, higher specific activity values against both substrates were obtained in the upper than in the lower section of the two reactors.

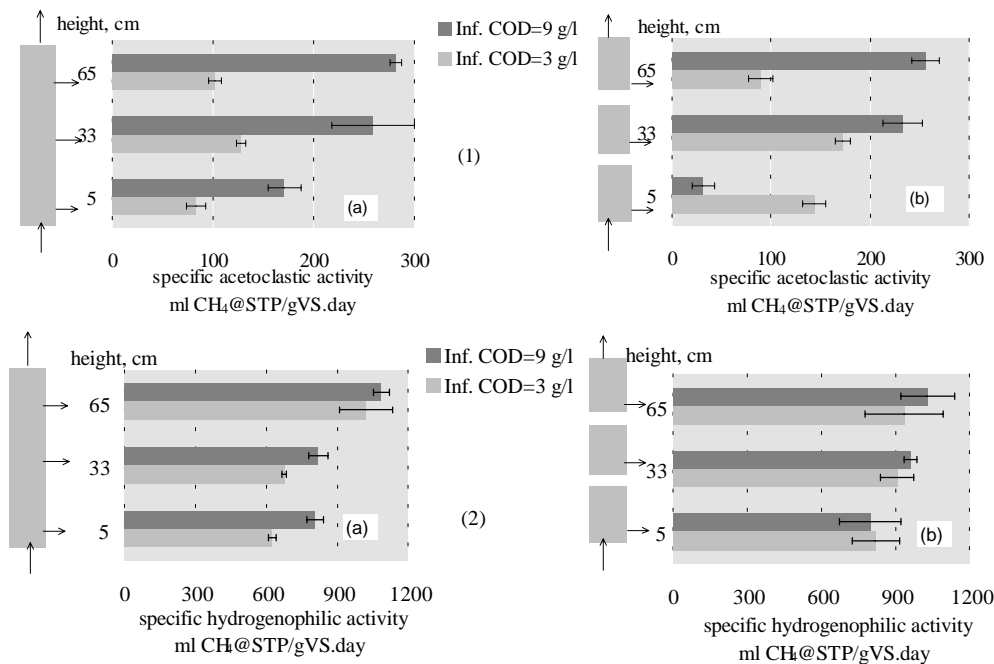
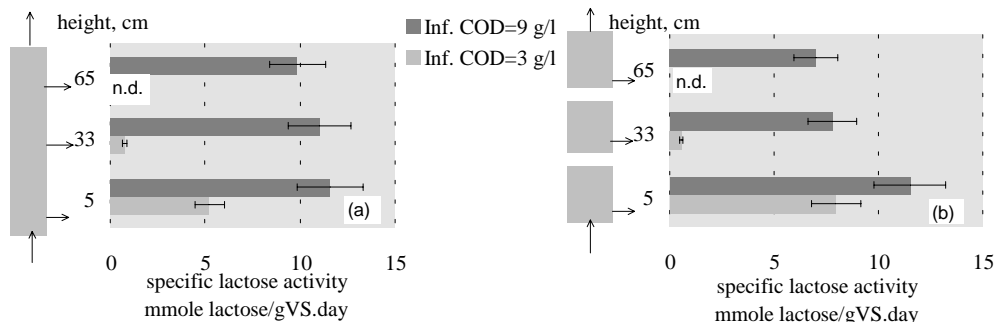


Figure 3. Acetoclastic(1) and hydrogenophilic (2) activity. (a) AFI; (b)AFII

The specific acetoclastic activity was more stratified than the hydrogenophilic activity and this effect was more pronounced in the staged reactor (Figure 3). As suggested for staged reactors by other authors, the dilution of methanogenic bacteria with acidifying biomass can explain the low value of acetoclastic activity in the first stage of AFII (Guiot *et al.*, 1995; Van Lier *et al.*, 1996). In fact, from Figure 4, the acidogenic activity measured by the activity against lactose, was predominantly located at the bottom of each reactor, for an influent COD of 3 g/l, but for 9 g/l it was extended to all sections of both configurations with a slightly higher stratification in AFII than in AFI. The hypothesis of methanogenic activity dilution with acidifying biomass was not observed in the latter sections and a similar effect was not observed for the specific

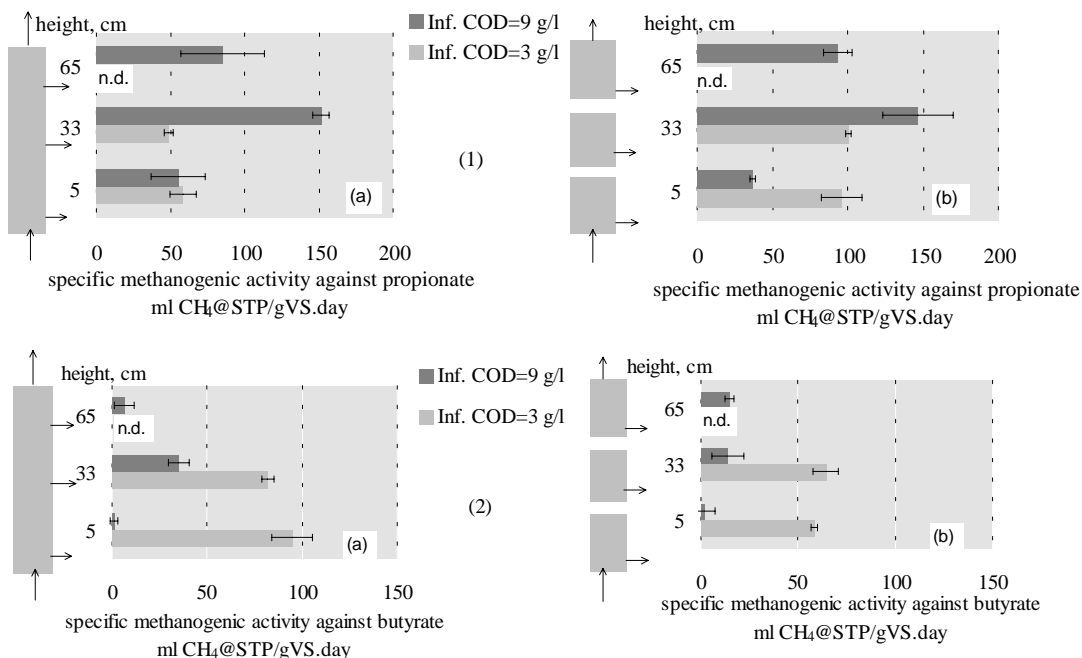
hydrogenophilic activity in the bottom section of AFII which remained at approximately the same value. In AFI, the specific hydrogenophilic activity at the base of the reactor increased with increasing substrate concentration. The two genera of acetate utilising bacteria, *Methanosaeta* (formerly *Methanothrix*) and *Methanosarcina* differ with respect to substrate affinity, maximum growth rate and substrate specificity. While *Methanosarcina* is able to utilise several substrates, including H_2/CO_2 and acetate, *Methanosaeta* can only degrade acetate. Harper and Pohland (1986) suggested that the multiple substrate utilisation capability of *Methanosarcina* genera (Hydrogen Oxidising Acetotroph or HOA) confers a higher survivability on these species.



n.d. - not determined

Figure 4. Specific lactose activity. (a) AFI; (b) AFII

These authors also commented on the inhibitory effect of hydrogen on acetate degradation by HOA species and on the preference of HOA species for H_2/CO_2 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/l, *Methanosarcina* should be the predominant acetate degrader exhibiting, however, a low acetate activity. The pH susceptibility of *Methanosaeta* may be another factor justifying the possible washout of these bacteria from the first stage of AFII. pH values of 6.5-6.6 were recorded in the AFII bottom section. Figure 5 represents the specific activity values obtained against propionate and butyrate.



n.d. - not determined

Figure 5. Specific methanogenic activity against propionate (1) and butyrate(2). (a) AFI; (b) AFII

Since these substrates 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 (Dolfing and Bloemen, 1985). A comparison of Figures 3 & 5 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/l where the acetoclastic activity (Figure 3) was lower than the measured propionate utilising activity. The distribution of propionate-utilising activity was similar for both configurations along the height of the reactors, with a definite maximum in the middle section at an influent COD of 9 g/l. No effect of removing the biogas was observed in the distribution of the specific methanogenic activity against acetate and propionate (Figures 3 & 5). 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/l, a net maximum in the middle section was observed.

Tracer experiments

Tracer experiments were performed for influent concentrations of 3 and 9 g COD/l, in order to evaluate the effect of increasing gas production (Table 1) and biomass (Table 2) in the flow pattern for both reactors. Table 3 represents the results obtained for the application of the “dispersion model” (Levenspiel, 1972), a simple model with a unique parameter (Dispersion Number= D/uL). This model predicts flow patterns from ideal plug flow (dispersion number=0) until ideal completely mixed flow (dispersion number =infinite). 0.2 is defined as a large degree of dispersion and 0.02 as an intermediate degree of dispersion.

Table 3. Results of tracer experiments - application of the dispersion model

	Influent COD=3 g/l			Influent COD=9 g/l		
	D/uL (residual sum of squares)	recovered tracer (%)	MRT (hours)	D/uL (residual sum of squares)	recovered tracer (%)	MRT (hours)
AFI	0.85 (3.94)	71.2	38.3	0.96 (2.09)	97.6	38.5
AFII	0.16 (1.17)	91.5	55.0	0.61 (0.17)	106.6	42.4

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

Although the parameter obtained represents an optimum for each experiment, it can be observed from Table 3 that, in some situations, the residual sum of squares is high, indicating that the model is not suitable to predict flow in such cases. Attempts were made to find a more complex, multiparameter model. Figure 6 represents the application of the compartmental model proposed by Young and Young (1988) to the experimental data obtained for AFI, for influent concentration of 3 g COD/l (a) and 9 g COD/l (b).

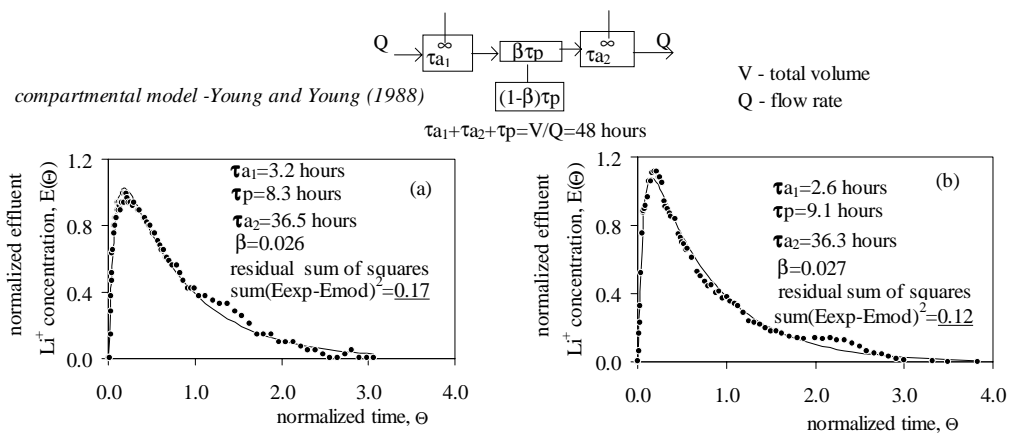


Figure 6. Application of the model proposed by Young and Young (1988) to the residence time distribution experiment for AFI. (a) Influent COD=3 g/l. (b) Influent COD=9 g/l

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 completely mixed volume. The application of this model to AFI revealed that, on average for the two operating conditions tested, the mixed inlet represents 6% of the total volume, the plug flow was 18% of the total volume (where there is visible substrate depletion - Figure 3) and 76% of the total volume was completely mixed. The dead volume was 17.5% of the total volume or 97.3% of the plug flow volume. As observed with the dispersion model (Table 3), no effect of increasing biogas

(Table 1) and biomass (Table 2) was detected on the model parameters. Although unexpected, this can be explained by the impossibility of distinction between hydraulic and biological dead space with this technique stimulus-response. It is possible that a shift from hydraulic to biological dead volume had occurred with the increase in influent concentration. Figure 7 represents application of the “tanks in series” model and the model proposed by Young and Young (1988) to the tracer experiment for AFII. While for influent COD of 3 g/l, a series of three completely mixed volumes described well the flow pattern in this reactor, for influent COD of 9 g/l, the model of Young and Young (1988) was applied with good agreement to the experimental data. As can be seen in Table 3, and in Figure 7 the amount of dispersion was clearly increased between the two situations and the comparison between AFI and AFII flow pattern for influent COD of 9 g/l (Figures 6 (b) and 7 (b)) revealed that differences were only marginal. This is in accordance with the slight differences encountered for microbial COD and VFA distribution as well as for the performance between the two configurations.

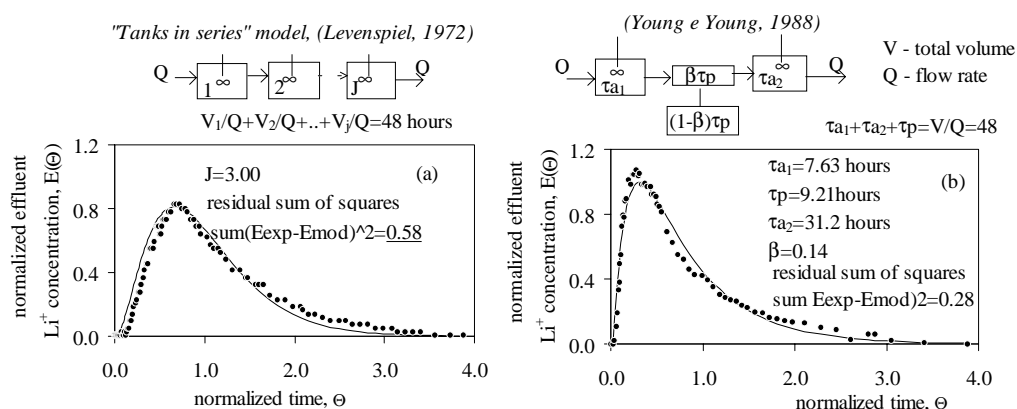


Figure 7. Application of the “tanks in series” model and the model proposed by Young and Young (1988) to the residence time distribution experiment for AFII. (a) Influent COD=3 g/l. (b) Influent COD=9 g/l

CONCLUSIONS

The application of a pressure transducer technique to study the behaviour of anaerobic consortia has proven to be very useful due to its relatively easy utilisation and good reproducibility compared with other techniques reported in the literature. For the range of operating conditions tested, no differences were observed in overall performance between the staged and non-staged anaerobic filters, except that a slightly higher methane yield was observed for the non-staged configuration. Maximum specific acetoclastic and hydrogenophilic activities were observed at the top of the reactors. The specific methanogenic activity against propionate was higher in the middle sections for both configurations and the lactose specific activity was higher at the bottom section and was slightly more stratified in the staged reactor. Tracer experiments revealed that for the non staged reactor the flow pattern was unchanged with the increase of influent concentration and the consequent increase in biomass and gas production, while, for the staged reactor the amount of dispersion detected increased significantly.

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