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A new method to study interactions between biomass and packing material in anaerobic filters

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A new anaerobic, random-packed, fixed-bed reactor, where the fixed bed matrix is distributed between up to 36 independent mini-bioreactors, has been developed to investigate biomass-support interactions in anaerobic filters. Glass, Plexiglas and polyvinyl chloride (PVC) of three sizes all gave similar results though entrapped biomass was maximal at 3 g/L of matrix void volume for the smallest size and a maximum of 1 g adhered biomass per m^2 was obtained for the largest size. In a second run, by periodically removing 3 mini-bioreactors, potential specific methanogenic activities against individual substrates were determined along the time and a continuous growth of the adhered biomass was observed, achieving a maximum of 40% of the total biomass.

Introduction

In anaerobic filters the biomass immobilization is achieved by entrapment in the void space and by adhesion to the surface of the packing material. In the upflow mode, the adhered biomass is usually considered to play a minor role due to its low proportion as compared with the total accumulated biomass (Young and Dahab, 1983). However, depending on support properties such as bed and carrier porosity, surface area, and type of material, a thick biofilm can be formed increasing its importance on the overall performance (Anderson *et al*., 1994). If hydraulic or toxic shocks are applied the adhesion of biomass provides a more stable immobilization than the entrapment.

So far the comparative studies of different supports for biomass colonization have been made either in continuous mode, operating one reactor with each support (Anderson *et al*., 1994) or in batch tests run simultaneously (Bonastre and Paris, 1988). Although the former methodology provides the most directly applicable data, it became a rather hard task to operate several anaerobic filters in parallel, especially if long term operation is to be evaluated. On the other hand, the comparison of colonization properties in batch assays does not account for all the environmental conditions prevailing in a real reactor.

On the other hand, the study of anaerobic filters is limited by the difficulty of determining biomass quantity and quality as well as its evolution with time and operating conditions. It is believed that a general behaviour can not be predicted and it is proposed that biomass-support interactions should be characterized for each particular application.

In the present work a methodology based on the placement of several mini-bioreactors in parallel in the same bioreactor was tested with two different applications: (I) Comparison of three materials (glass, Plexiglas and polyvinyl chloride (PVC)) and three carrier sizes in terms of biomass distribution between the adhered and entrapped fractions. (II) Evolution of biomass characteristics (distribution in the support and consortium composition) by regular withdrawal of some accumulated biomass with non-detectable operation disturbances.

Materials and methods

Experimental set-up

The bioreactor was a cylindrical tank constructed of PVC with a working volume of 86 liters. In the central section a large number of mini-bioreactors were arranged in parallel. The feeding was continuously introduced at the bottom and a stirrer allowed the homogenization of the camera below the support section. The mini-bioreactors were placed over a perforated plate located at a distance of 10 cm from the bottom. In both studies Raschig rings were used as support. The temperature was kept at 35 °C by water circulation in an internal jacket (Figure 1).

Supports and packing arrangement in experiment I

The supports were Raschig rings of glass, Plexiglas and PVC. The small, medium and large sizes of the supports were 0.82, 1.2 and 2.02 cm for the glass and the Plexiglas,

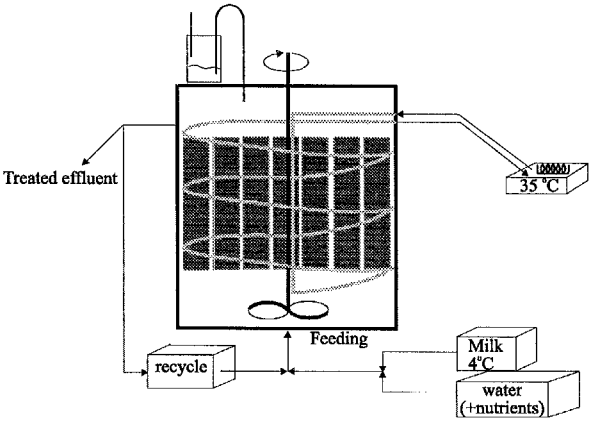

Figure 1 Schematic representation of the fixed bed reactor.

Table 1 Average porosity (of four replica) of each combination material/size.

	porosity of the mini-bioreactors, (cm^3 void/cm^3)		
	small size	medium size	large size
glass	0.77	0.83	0.89
Plexiglas	0.69	0.83	0.80
PVC	0.87	0.86	0.85

but for the PVC, due to commercial availability, the sizes were 1.2, 1.6 and 2.1 cm. The diameters of the mini-bioreactors were 2.5, 4.4 and 6.2 cm for the small, medium and larger sizes respectively. The combination size/kind of material resulted in nine case studies and four replica were performed. The porosity was individually calculated for each mini-bioreactor and Table 1 represents the average values of the four replica.

Each replica was separated in a basket made with a plastic grid (aperture 1 cm) and the space limitations led to the layout presented in Figure 2 (a). The height of the support section in the bioreactor was 15 cm, and the total volume was 27.1 liters. In this experiment the bioreactor was opened at the end of the operation, the mini-bioreactors were removed and the fractions of biomass determined.

Supports and packing arrangement in experiment II

In the experiment designed to follow the biomass development, the fixed bed section was composed of 27 similar mini-bioreactors, which were numbered, as presented in Figure 2 (b). The support consisted of PVC Raschig rings of 21 mm diameter, with a specific surface area of 230 m^2/m^3 and a porosity of 92.5%. Each mini-bioreactor had 7.1cm internal diameter, a total volume of 989 cm^3 and

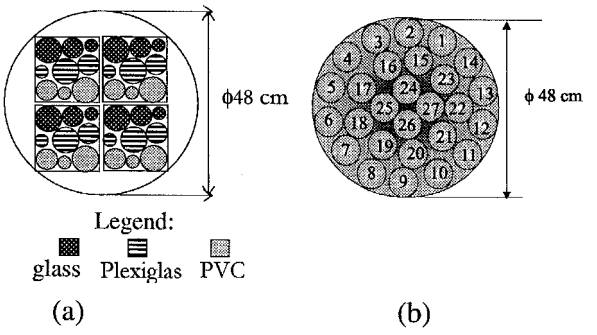

Figure 2 Layout of the fixed bed matrix distribution. (a) experiment I, (b) experiment II.

accommodated 89 pieces of support material. The bioreactor was opened at four different operation times and 3 of the 27 mini-bioreactors were randomly selected and replaced by new similar mini-bioreactors, which were not accounted for, in the next selection.

Substrate and inoculum

In both experiments, skim milk diluted with tap water was used as substrate and 5 g $NaHCO_3$/l were added to give suitable buffer capacity. In experiment I, no nutrients or trace minerals were added, but in experiment II, 0.6 ml of the following solution were added (per g of COD fed): 30.2 g $MgSO_4.7H_2O$/l, 28.3 g KH_2PO_4/l, 45 g KCl/l. A trace mineral solution based on the work of Zehnder *et al.* (1980) was also supplemented to the influent feed by addition of 1 ml per litre. In both experiments the seed sludge was obtained from a local municipal sludge digester.

Analytical methods

Routine analysis

COD, volatile and total solids (VS and TS) were determined by Standard Methods (APHA, AWWA, WPCF, 1989). Volatile fatty acids (VFA) were determined by HPLC using a Chrompack column (300 × 6.5 mm) and a mobile phase of 5mM H_2SO_4 at 0.7 ml/min. The column was set at 40°C with detection at 210 nm. Methane content of biogas was measured by GC using a Chrompack Haysep Q (80–100 mesh) column, with N_2 as carrier gas at 30 ml/min and a flame ionisation detector.

Biomass characterization

(i) Biomass separation and quantification In both experiments, the entrapped biomass was considered to be the fraction which was unattached to the support after being freely dispersed in a distilled water bath and subjected to circular manual movements with alternated senses for 1 minute. The total volume of liquid and its volatile solids

content were determined. In experiment I this procedure was repeated individually for each of the 36 mini-bioreactors. In experiment II, after centrifugation at 6000 rpm for 10 min, this fraction was ressuspended in an anaerobic buffer and total volume and volatile solids (VS) content were determined. Activity tests were performed with this fraction of biomass. The adhered biomass was removed from the support by sonication for 10 min.

(ii) Activity measurements Methanogenic activity tests were performed using the pressure transducer technique proposed by 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 (H_2/CO_2). The non gaseous substrates were acetate, propionate, butyrate and ethanol. Strict anaerobic conditions were maintained. The hand held pressure transducer was capable of measuring a pressure increase or decrease of two bar (0 to $\pm$202.6 kPa) over a range of $-$200 to $+$200 mv, with a minimum detectable variation of 0.005 bar. A sensing element consisting of a 2.5 mm square silicon chip with integral sensing diaphragm is connected to a digital panel meter module and the device is powered by a 7.5 V DC transformer. The same technique was used to characterize the methanogenic activity of the inoculum. All tests were performed in triplicate.

(iii) Electron microscopy A scanning electron microscope (Leica Cambridge S360) was used to observe the adhered biomass in experiment I. Samples were fixed with a 3% (W/V) glutaraldehyde solution in cacodylate buffer, pH 7.2 and washed with increasing concentration ethanol solutions.

Operating conditions

In the experiment I, the influent COD was increased from 3000 to 6000 mg/l and the hydraulic retention time (HRT), based on the active volume was set at 1.3 days. In the experiment II, the influent COD was increased from 3000 to 12000 mg/l and, after the start-up the HRT varied between 0.92 and 1.4 days. In both experiments a recycle was applied to increase the upflow velocity through the bed. Upflow superficial velocity was 0.27 m/day in experiment I and 0.43 m/day in experiment II.

Results and discussion

Performance

Tables 2 and 3 present the average operating conditions and performance of the reactor during the experiment I and II respectively. The higher overall performance in experiment II was expected due to the presence of macro and trace nutrients. A decreasing trend in the removal efficiency was observed between the 90th and 114th days reaching an average pseudo-steady state value as low as 77%. To increase and stabilize the overall performance, the hydraulic retention time was increased from 0.9 to 1.4 days after this period. After biomass removal no disturbances were observed in the operation.

Biomass characterization in experiment I

In experiment I, after 115 days of operation, all mini-bioreactors were removed and the retained biomass was characterized. Results are presented in terms of total biomass (gVS/l), entrapped biomass (gVS/l of matrix void volume) adhered biomass (gVS/m^2 of support) and fraction of the adhered biomass. The average values of the different fractions of biomass obtained for the four replica were considered and the associated 95% confidence intervals

Table 2 Operating conditions and reactor performance (experiment I).

Time (days)	*Hydraulic retention time (HRT) (days)*	*Influent COD (mg/l)*	*Organic loading rate, (kg COD/m^3.day)*	*Removal efficiency, (%)*
0–48	1.4 ± 0.2	2723 ± 331	2.4 ± 0.8	61 ± 9
49–115	1.3 ± 0.1	5619 ± 274	4.3 ± 0.5	73 ± 4

Table 3 Operating conditions and reactor performance (experiment II).

Time (days)	*Hydraulic retention time (HRT) (days)*	*Influent COD (mg/l)*	*Organic loading rate, (kg COD/m^3.day)*	*Removal efficiency, (%)*
0–29	1.4 ± 0.1	3043 ± 38	2.2 ± 0.1	92 ± 1
29–90	0.9 ± 0.1	3043 ± 38	3.3 ± 0.1	94 ± 1
90–114	0.9 ± 0.1	5899 ± 56	6.5 ± 0.1	77 ± 3
114–132	1.4 ± 0.1	5899 ± 56	4.3 ± 0.1	93 ± 2
132–162	1.4 ± 0.1	8685 ± 82	6.3 ± 0.1	90 ± 1
162–246	1.4 ± 0.1	12099 ± 72	8.7 ± 0.2	86 ± 1

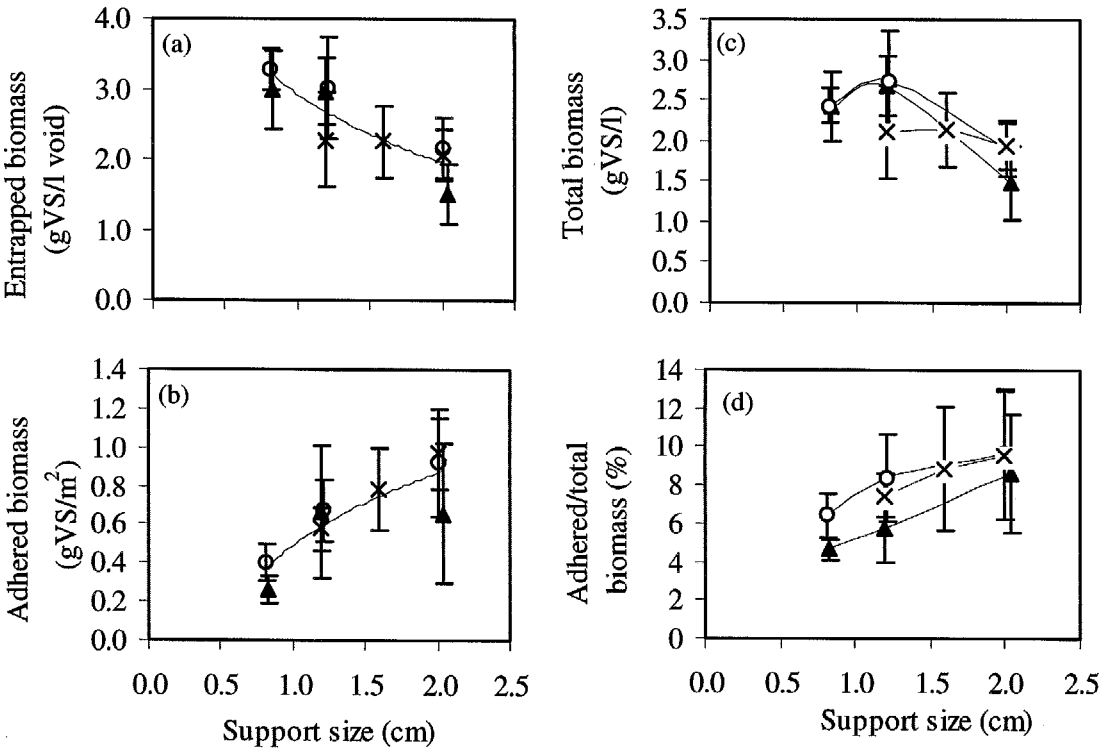

Figure 3 Effect of size and material on: (a) entrapped biomass per volume of matrix void space, (b) adhered biomass per m^2 of support surface, (c) total accumulated biomass per liter of active volume and (d) fraction adhered/total biomass.(▲ – glass, ○ – Plexiglas, × – PVC).

were determined. Figure 3 represents the average values for the biomass distribution for all the materials and sizes. It was observed that entrapped biomass was more concentrated in the smaller supports and that thicker biofilms were formed in the larger supports.

A lower retention capacity of the larger pores was observed. This trend suggests that smaller pore sizes lead to a faster bed clogging. A maximum in the total accumulated biomass for the medium sizes of each material was observed (Figure 3 c). Despite the wide range of the associated 95% confidence interval, the ratio adhered/total biomass was lower for the glass than for the other two support materials and for all the materials it increased with the size of the support. Furthermore, in average, 92% of total biomass was entrapped and only 8% grown as a biofilm. This result highlights the major importance of entrapped biomass in upflow anaerobic filters, as reported in earlier literature (Young and Dahab, 1983).

The effect of support size can not be interpreted independently of the bed porosity effect. In fact different bed porosities (Table 1) induced different upflow interstitial velocities which were calculated considering that all mini-bioreactors were under similar superficial upflow velocity. The possibility of different superficial velocities induced by different head losses resulting from differences in bed porosities or biomass concentration was not considered. Figure 4 presents the effect of the interstitial velocity on the entrapped and adhered biomass. A clear trend between the entrapped biomass and the interstitial velocity was observed. Higher interstitial velocities favoured the biomass entrapment and, by comparison with Figure 3 (a) this happened for the smaller support sizes, but there is no clear evidence about which was the dominant effect. Two hypothesis can be put forward to explain this apparently unexpected result: (i) the different head losses could not be neglected and for the smaller supports, lower superficial velocities were, in fact, applied or (ii) the effect of higher interstitial velocity was, in fact, to favour the biomass compactness and density as observed for the effect of bulk velocity in the biofilm formation (Vieira *et al.*, 1993). However, this last hypothesis was not observed for the adhered biomass which did not follow any clear trend with the interstitial velocity (Figure 4 (b)).

Microphotographs obtained for the cleaned surfaces revealed that glass is the smoothest surface and PVC is the roughest one (not shown). The microbial population pres-

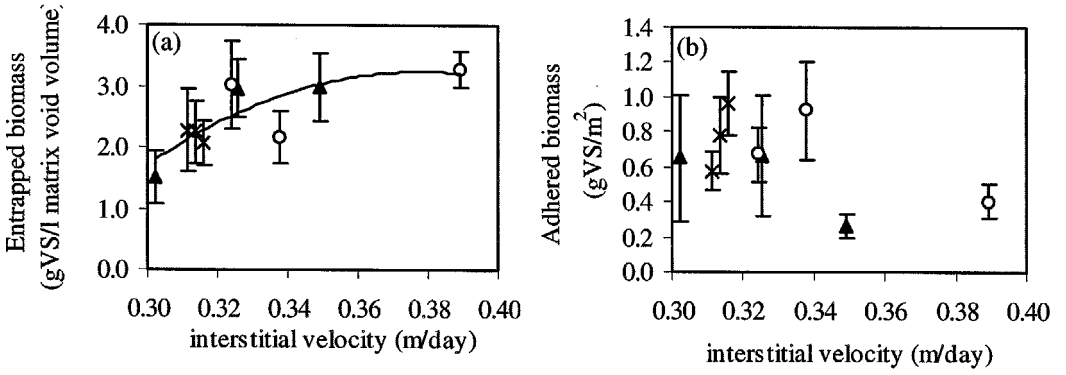

Figure 4 Effect of interstitial velocity on the (a) entrapped and (b) adhered biomass. (▲ – glass, ○ – Plexiglas, × – PVC).

ent on glass, the most hydrophilic surface seemed to be predominantly similar to *Methanospirillum* genera, but in Plexiglas and PVC, long rods of *Methanosaeta* (formerly *Methanothrix*) like bacteria were observed. This agrees with the observed by Verrier *et al.* (1988) who studied the adhesion of pure cultures of these bacteria.

Biomass characterization in experiment II

During this experiment the biomass was analyzed at four different operating times, on the 90th, 132nd, 162nd and 212th days. It can be observed that the biofilm was continuously built on the support and the entrapped biomass concentration achieved a maximum of 2.2 g VS/l void and no further increase was observed with the increase in the organic loading rate (Table 4). Curiously, and in spite of different operating conditions, namely different upflow velocities, in the experiment I values of 2.2 and 2.1 g VS/l void were obtained for the entrapped biomass concentration in the larger supports of Plexiglas and PVC, respectively, which had sizes similar to those used in experiment II. This suggests that size effect was probably more dominant than interstitial velocity effect on the entrapped biomass concentration in the experiment I. The increasing importance of the adhered biomass is evidenced in Figure 5 where the time course of the ratio adhered/total biomass is presented.

Values ranging from 19.8 ± 3.9 to 40.3 ± 2.7% where obtained, which are rather higher than those found in experiment I (average value of 9.6 ± 3.3% for the largest PVC support). Two reasons can be pointed out to explain these differences: (i) the difference in substrate composition (nutrients were added in experiment II, but not in experiment I), and (ii) the difference in operating conditions, namely upflow velocity and operating time.

Table 4 Distribution between the adhered and the entrapped biomass (expressed as volatile solids-VS) in the experiment II (±95% confidence interval).

day	*adhered biomass (gVS/m2)*	entrapped biomass (gVS/l matrix bed volume)	*total supported biomass (gVS/l reactor)*
90	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
132	1.4 ± 0.2	1.0 ± 0.1	0.8 ± 0.1
162	2.8 ± 0.3	2.2 ± 0.1	1.8 ± 0.1
212	5.5 ± 0.3	2.1 ± 0.1	2.2 ± 0.1

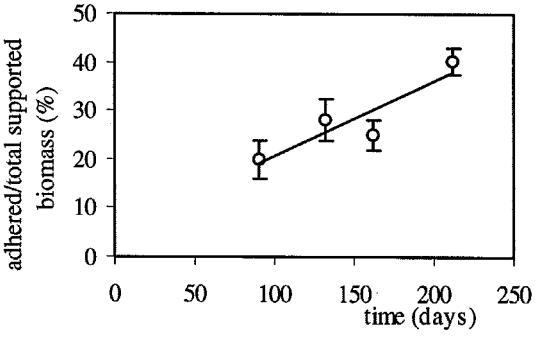

Figure 5 Time course of the ratio adhered/total supported biomass.

The observed growth yields ranged from 0.11 to 0.15 ±0.01 gVS/g removed COD which are in good agreement with those reported in the literature for non acidified substrates (Pavlostathis and Giraldo-Gomez, 1991, Hanaki *et al.*, 1994).

Figure 6 presents the evolution of methanogenic activities against direct (Fig. 6 a and b) and indirect substrates (Fig.6

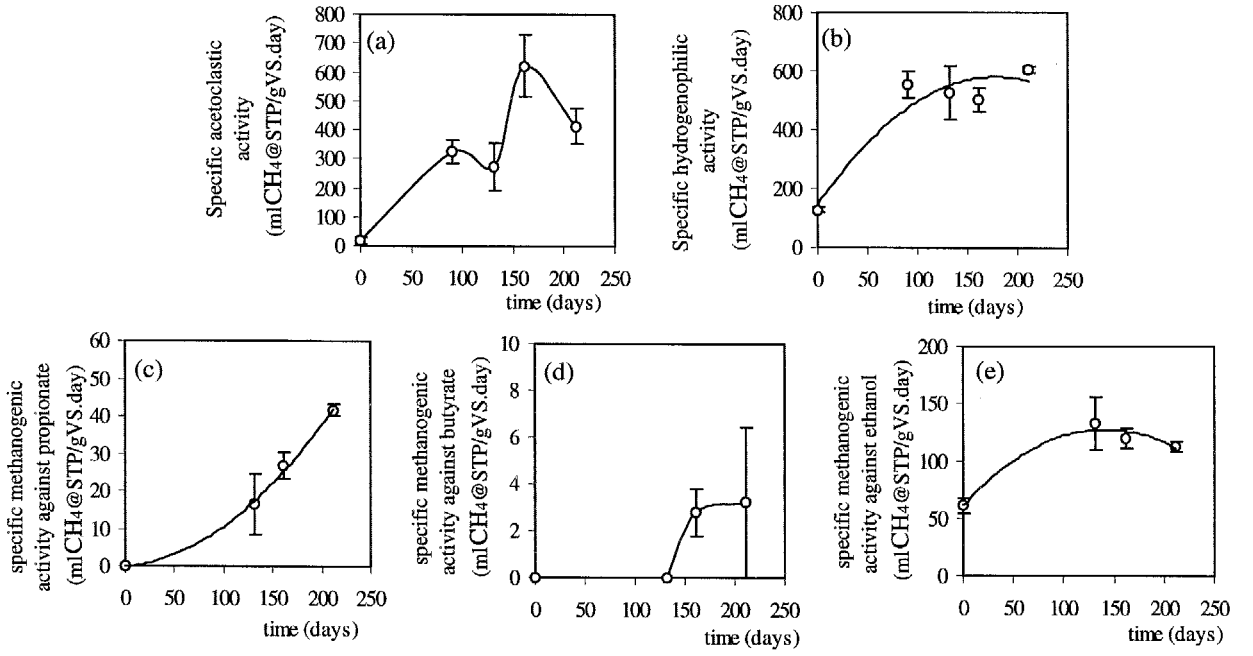

Figure 6 Potential specific methanogenic activity against direct ((a) acetate and (b) H_2/CO_2) and indirect ((c) propionate, (d) butyrate and (e) ethanol) substrates.

c, d and e). The acetoclastic activity reached a maximum of 621.4±106.6 ml of methane (measured at standard temperature and pressure conditions – STP) per gram of volatile solids (VS) and per day on the day 162. After this maximum a clear decrease was observed. It is suggested that the increase in the influent COD from 3 to 12 g/l induced a propagation of acidogenic bacteria from the bottom to the support section. Acetoclastic activity suffered an effective dilution process whereas hydrogenophilic activity, probably balanced by the relatively high growth rates of these bacteria remained stable.

Concerning the methanogenic activity against propionate, butyrate and ethanol, it should be said that, 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). In the present work this condition prevailed for all samples. Due to the little amount of sludge available, these activities were not measured for the first point (day 90). It was observed that the inoculum had no detectable specific methanogenic activities against propionate and butyrate (Figure 6 c and d), but a recovery of these activities was observed during the trial period, specially for the case of propionate. The specific methanogenic activity against butyrate was always very low (<5 ml CH_4@STP/gVS.day) and the specific methanogenic activity against ethanol remained aproximately constant after the start-up (Figure 6 e).

Conclusions

A new methodology to study interactions between biomass and packing material in anaerobic filters is presented. In a first experiment where three materials with three different sizes were compared under equal operating conditions, it was possible to conclude that the entrapped biomass (expressed as g VS/l void) was more concentrated in the smaller carriers and the adhered biomass showed an inverse behavior and a thicker biofilm was observed in the larger supports. In a second experiment, where the evolution of biomass distribution and consortium composition was evaluated during a trial period of 246 days, it was possible to conclude that the biofilm increased in thickness over all the trial period, achieving a maximum of 40% of the total supported biomass. After a maximum of 621.4 ml CH_4@STP/gVS.day, the acetoclastic activity decreased, whereas the specific hydrogenophilic activity increased during the start up and remained nearly constant over the trial period.The specific methanogenic activity against propionate, butyrate and ethanol, which accounts for the syntrophic activity was practically absent in the seed sludge, but showed a significant development troughout the trial period.

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