COMPARISON OF TWO CONFIGURATIONS OF UPFLOW ANAEROBIC FILTERS: SPECIFIC METHANOGENIC ACTIVITY PROFILES

Alves, M.M.[*], Pereira, M.A.[*], Mota, M.[*], Novais, J.M.[⁜], Colleran, E.[✦]
[*]University of Minho, Department of Biological Engineering, Largo do Paço, 4709 Braga codex-Portugal
[⁜]Technical University of Lisbon, Laboratory of Biochemical Engineering, Av Rovisco Pais, 1096 Lisboa codex-Portugal
[✦]Department of Microbiology, University College, Galway-Ireland

ABSTRACT

The aim of this work was to determine the distribution of microbial activity along the height of two configurations of anaerobic filters treating a synthetic dairy waste. A traditional configuration was compared with a staged system which had biogas removal from each of the three stages. The effect of increasing the substrate concentration from 3 to 12 g COD/l at constant hydraulic residence time (HRT) of 2 days was evaluated with respect to overall reactor performance, gas production and effluent volatile fatty acids profiles. The potential maximum specific methanogenic activity against acetate, H_2/CO_2 and an indirect substrate (propionate) was determined for sludge sampled from three different points in each reactor, under two operating conditions (influent COD of 3 and 9 g COD/l). The increase in influent concentration was shown to promote a stratification of the specific acetoclastic activity more pronounced in the staged reactor. Both the hydrogenophilic and acetoclastic activities were highest at the top of the filters, whereas the methanogenic activity against propionate was maximum in the middle section and was very similar for both reactor configurations. The results confirmed the reliability of the pressure transducer technique to study methanogenic activity of different trophic groups in consortia.

KEY WORDS: Anaerobic Filter; Methanogenic Activity; Dairy waste.

1-INTRODUCTION

The anaerobic degradation of organic matter to methane involves 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 design 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 regarded from different points of view. The 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 removal in the later stages. However, enrichment of the latter stages in acetic and propionic acid degraders was not proven.

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 this process, the relatively low superficial biogas load in the final compartment improved the settling conditions of the sludge. 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 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 they 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 an indirect (propionate) substrate. A control reactor, mono-staged, under the same operating conditions, was used for comparative purposes. Methanogenic activity tests were performed using a pressure transducer technique (Colleran *et al.*, 1992).

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 schematically described in Fig. 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 with a porosity of 92.5%. In the staged reactor (AFII) a gas-solid separator was fitted to allow biogas release from each compartment.

The released biogas was individually measured for volume produced and methane content. The substrate was stored at 4 °C in order to minimise acidification. Several sampling ports were fitted to allow 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.

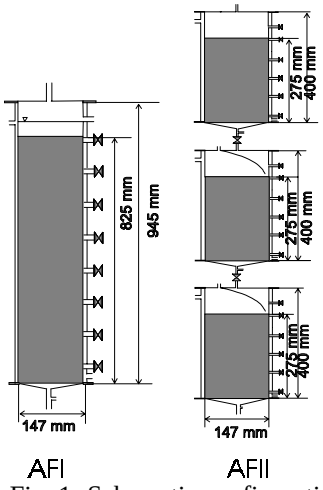

Fig. 1 -Schematic configuration of AFI and AFII.

Routine reactor performance was monitored by determining influent and effluent Chemical Oxygen Demand (COD), influent flow rate, effluent volatile fatty acids (VFA) from each reactor (including each stage of AFII), the rate of gas production and its methane content. All the routine analyses were determined three or four times a week, except the gas flow rate that was measured on a daily basis. 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 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, H_2/CO_2 and propionate.

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 - $MgSO_4.7H_2O$:30.2 g/l; KH_2PO_4: 28.3 g/l KCl: 45 g/l. 0.6 ml of this solution was added per gram of COD fed. Micronutrients - $FeCl_2.6H_2O$: 2 g/l; H_3BO_3: 0.05 g/l; $ZnCl_2$: 0.05 g/l; $CuCl_2.2H_2O$: 0.038 g/l; $MnCl_2.4H_2O$: 0.5 g/l; $(NH_4)_6Mo_7O_{24}.4H_2O$: 0.05 g/l; $AlCl_3.6H_2O$: 0.09 g/l; $CoCl_2.6H_2O$: 2 g/l; $NiCl_2.6H_2O$:0.092 g/l; $Na_2SeO_3.5H_2O$: 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.

2.3- Analytical methods

2.3.1- Routine analysis: COD and volatile suspended solids (VSS) were determined by Standard Methods (APHA; AWWA; WPCF, 1989). VFA were determined by HPLC (Jasco, Japan) using a column Chrompack (cat nº28350); 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. Methane content of biogas was measured by a Pye Unicam GCD gas chromatograph (Cambridge, England) using a column Chrompack Haysep Q (80-100 mesh). N_2 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.

2.3.2 - 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 (H_2/CO_2). 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.

3 -RESULTS AND DISCUSSION

3.1 - Reactor Performance**:** Tables I and II summarise the operating conditions and performance data of AFI and AFII, respectively.

Table I - Operating conditions and performance data of AFI

Time (days)	HRT (days)	Influent COD (mg/l)	Effluent COD (mg/l)	Organic loading rate (KgCOD/m3.day)	Efficiency (%)	CH_4 (%)	biogas (m^3/m^3.day)
[343-430]	1,93±0,01	2948,1±60,6	62,2±7,3	1,55±0,07	97,9±0,3	65,3±0,5	0,86±0,04
[430-495]	1,93±0,01	5911,3±293,3	130,0±32,4	3,02±0,15	97,7±0,6	64,8±0,6	1,77±0,12
[495-596]	1,93±0,01	8836,6±410,9	157,0±33,6	4,52±0,22	98,2±0,4	62,3±1,3	2,65±0,09
[596-628]	1,93±0,01	12143,1±402,5	215,0±26,4	6,25±0,24	98,2±0,2	61,1±0,5	3,51±0,13

Table II - Operating conditions and performance data of AFII

Time (days)	HRT (days)	Influent COD (mg/l)	Effluent COD (mg/l)	Organic loading rate (KgCOD/m3.day)	Efficiency (%)	CH4 (%)	biogas (m^3/m^3.day)
[343-430]	2,05±0,01	2952,5±59,6	59,4±7,8	1,44±0,40	98,0±0,3	67,0±6,9	0,72±0,05
[430-495]	2,05±0,01	5911,3±293,3	67,6±16,4	2,81±0,13	98,8±0,3	64,4±5,0	1,50±0,08
[495-596]	2,05±0,01	8836,6±410,9	175,1±55,8	4,34±0,21	97,9±0,7	61,0±3.2	2,29±0,08
[596-628]	2,05±0,01	12143,1±402,55	200.4±55.2	6,09±0,24	98,3±0,5	58,3±5,1	3,30±0,19

As is evident from Tables I and II, the overall performance of the two reactors was very similar over the trial period. The slightly higher biogas production from AFI is evidenced in Figure 2, where a marginally higher methane yield from AFI than from AFII is observed.

Fig. 3 represents the evolution of volatile fatty acids (VFA) along the reactor height for the two configurations and for influent concentrations of 3 and 9 g COD/l. 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, a fact that can be partially explained by the flow pattern determined for the two reactors (under the operating conditions, AFI was found to be well mixed in 75% of its volume whereas AFII showed a more plug flow behaviour - data not shown).

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

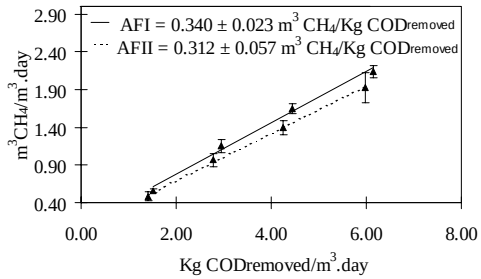

Fig. 2 - Methane yield for AFI and AFII

loading rates applied and to the lower number of stages in AFII, compared to the baffled reactor studied by these authors

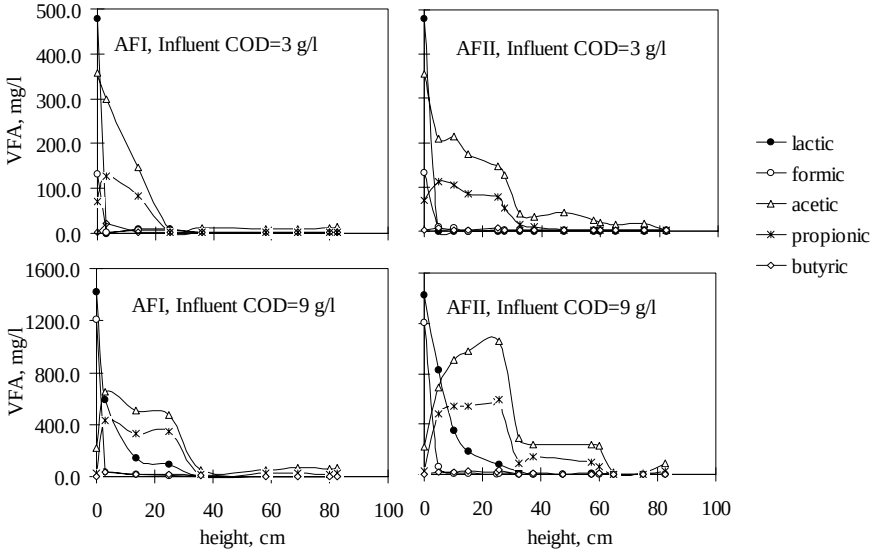

Fig. 3 - Profiles of Volatile Fatty Acids along the height of reactors AFI and AFII at two different influent concentrations.

3.2 - Biomass analysis: At influent concentrations of 3 and 9 g COD/l, biomass was removed at three points of each reactor and analysed for VSS concentration and potential methanogenic activity. Fig. 4 represents the effect of influent concentration on the entrapped VSS accumulated in the reactors. Other experiments using the same support media and substrate, had demonstrated that the amount of adhered biomass was not more than 15% of the total biomass retained in reactors of the configuration under study. Consequently the entrapped floculant biomass was considered to be representative in quality and quantity of the population present in each point Figs. 5 and 6 represent the specific methanogenic activity against direct substrates (acetate and H_2/CO_2) 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 activities values against both substrates were obtained in the upper than in the lower section of the two reactors. The specific acetoclastic activity was more stratified than the hydrogenophilic activity and this effect was more pronounced in the staged reactor (Fig.5). 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). However, a similar effect was not observed for the specific hydrogenophilic activity in the bottom stage of AFII which remained at approximately the same value despite an increase in influent COD concentration to 9 g/l. 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*

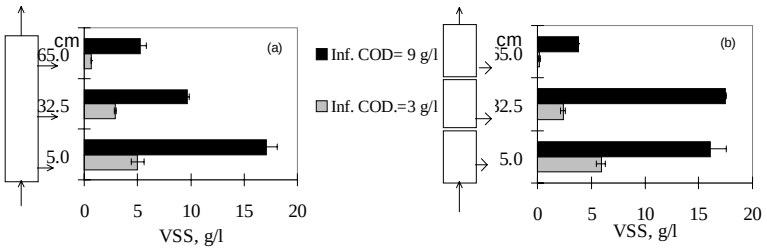

Fig. 4 - VSS distribution along the height .(a)AFI;(b)AFII

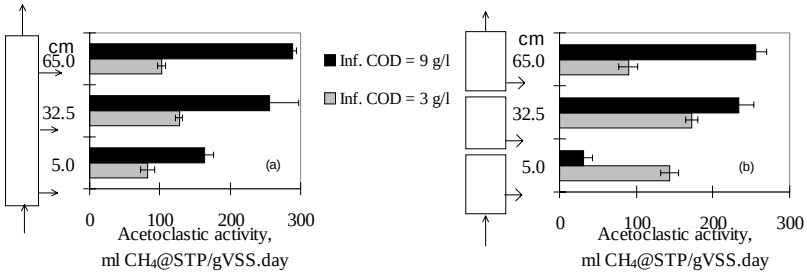

Fig. 5 - Acetoclastic activity. (a) AFI; (b)AFII

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. These authors also commented 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.

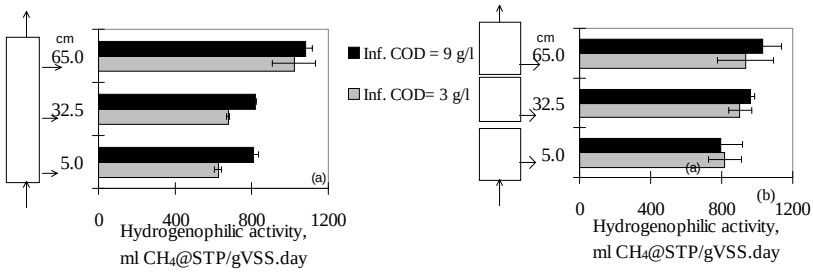

Fig. 6 - Hydrogenophilic activity.(a)AFI;(b)AFII

Fig. 7 represents the specific activity values obtained against propionate for sludge sampled from both reactors. Since propionate is an indirect methanogenic substrate, a valid measurement of the maximum specific methanogenic activity against propionate can only be obtained when the acetoclastic and hydrogenophilic activities are not rate-limiting (Dolfing and Bloemen, 1985). A comparison of Figs 5, 6 & 7 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. In the latter case, the acetoclastic activity (Fig.5) was substantially lower than the measured propionate utilising activity in the biomass sampled studied. 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. At influent COD concentration of 3 g/l, the specific methanogenic activity against propionate was slightly higher for AFII than for AFI in both the bottom and middle sections and in the upper section it was not determined. No effect of removing the biogas was observed in the specific methanogenic activity against acetate and propionate (Fig. 5 & 7) in opposite to the postulated by Harper and Pohland, (1987).

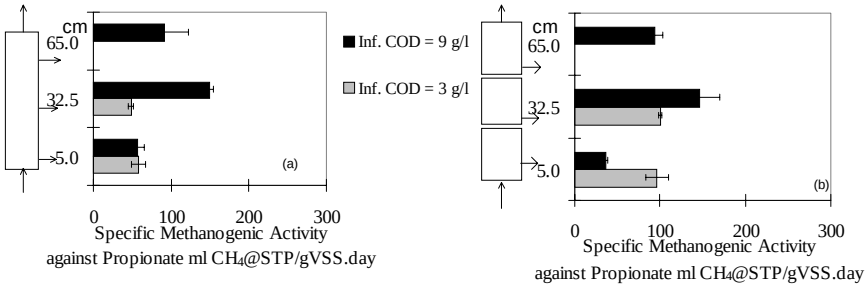

Fig. 7 - Specific methanogenic activity against propionate.
(a) AFI; (b) AFII

4 - 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 literature. In the range of operating conditions tested, no differences were observed in overall performance between the staged and non-staged anaerobic filter, 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 and the specific methanogenic activity against propionate was higher in the middle sections for both configurations.

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