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Effect of the chemical nature of fixed-bed reactor support materials on bioreactor performance and biomass accumulation

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ABSTRACT: This study investigated the effect, on reactor performance and biomass retention inside the bed, of the material used to make the supports of anaerobic fixed-bed reactors. Three inert supports of similar shape but made of three different materials polyvinyl chloride, polypropylene, high-density polyethylene were manufactured and used. All three supports had the same specific surface area but different relative densities. Three identical 10 L lab-scale up-flow anaerobic fixed-bed reactors were filled (80% of the working volume) each respectively with polyvinyl chloride, polypropylene and polyethylene support, and fed with vinasse (44 g total COD/L) for 140 days at 35 °C. The organic loading rates were increased from 0.5 g/L.d. to the maximum acceptable by each reactor. Fairly similar maximum organic loading rates were reached for each type of support, with values above 20 g of COD/L.d. and more than 80 % soluble chemical oxygen demand removal efficiency. A very large amount of biomass was entrapped and attached in all the supports and represented more than 95% of the total biomass inside the reactors. In terms of performance and biomass accumulation, this study demonstrated quite similar behavior for anaerobic fixed-bed reactors with supports made of different materials, which suggests that the nature of the material used to make the supports has no major influence. The chemical nature of the support material clearly has negligible effect and thus the size, shape, and porosity of the support must be more influential.

Keywords: *Anaerobic fixed-bed reactors (AFBRs), Organic loading rates (OLR), Polyethylene (HDPE), Polypropylene (PP), Polyvinyl chloride (PVC), Specific biomass activity, Vinasse*

INTRODUCTION

High-rate anaerobic treatment of wastewater is a commonly accepted practice in the industry due to its low energy consumption, methane production which can be used to produce energy and a lower sludge production than aerobic processes (Di Berardino *et al.*, 1997; Weiland and Wulfert, 1986). These systems are extremely efficient in the treatment of industrial and highly polluted wastewaters with short hydraulic retention times (HRTs) and high organic loading rates (OLRs) (Banu *et al.*, 2006; Rajinikanth *et al.*, 2009). High-rate anaerobic reactors may be classified into three broad groups depending on the mechanism used to achieve biomass retention, namely granulation (including UASB reactors, EGSB reactors) (Banu *et al.*, 2006; Fang *et al.*, 2011), attachment on supports (including fixed-bed reactors, fluidized-bed reactors) (Ganesh *et al.*, 2010; Nikolaeva *et al.*, 2009; Sandhya and Swaminathan, 2006) and hybrid systems (Roca *et al.*, 2007; Tawfik and El-Kamah, 2012; Tran *et al.*, 2003; Yang *et al.*, 2004).

Worldwide, there are many full-scale installations of up-flow anaerobic sludge blankets (Mirsepasi *et al.*, 2006; Parawira *et al.*, 2005), anaerobic filters,

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anaerobic attached-film expanded-bed reactors (Connaughton *et al.*, 2006), anaerobic baffled reactors (Liu *et al.*, 2010) and anaerobic sequencing batch biofilm reactors (Venkata Mohan *et al.*, 2005). The UASB and EGSB processes are the most widely applied full scale technologies for treatment of industrial wastewaters in the world (Frankin, 2001). Among all these, the up-flow anaerobic fixed-bed is a relatively simple technology with well-defined characteristics and is not as complex as fluidized-bed reactors. It can be a good option, for example, for the treatment of effluents generating granulation problems which make the application of the UASB process problematic. The anaerobic fixed-bed process is stable and resistant to stress such as organic and hydraulic shock loadings. The good performance of an anaerobic fixed-bed reactor largely depends on the design of the support medium (for e.g. media pore size and porosity) which is packed inside the working volume of the reactor (Ganesh *et al.*, 2010, Show and Tay, 1999; Tay *et al.*, 1996). The support medium serves to attach the biomass, acts as a biofiltration bed and helps avoid a washout of the biomass from the reactor (Ganesh *et al.*, 2010; Rajinikanth *et al.*, 2009).

Several studies have been carried out on the properties of supports (Banu *et al.*, 2006; Ganesh *et al.*, 2010; Rajinikanth *et al.*, 2009; Tay *et al.*, 1996; Weiland, 1987; Young and Dahab, 1983). A number of materials have been tested as supports for biomass growth and retention in anaerobic fixed-bed reactors and the performance of these materials appear to be directly related to the ease with which bacteria can become entrapped or attached (Rajinikanth *et al.*, 2009; Show and Tay, 1999; Tay *et al.*, 1996; Weiland, 1987). The use of many natural and synthetic support materials such as coir, sand, anthracite, activated C, nylon fibres, polyurethane foam, perspex, polyvinyl chloride (PVC), sponge, carbon felt, and glass beads has been reported in literature (Hickey *et al.*, 1991; Lee *et al.*, 2007; Weiland, 1987; Yang *et al.*, 2004). It has been suggested that preferably a good support should have high porosity, a large surface area, and adequate surface properties, be lightweight, must avoid clogging due to excess biomass and should be economical (Acharya *et al.*, 2008; Weiland, 1987). Moreover, it has been reported that the most important parameter is to have supports with a high capacity for entrapping and preventing biomass washout from the reactor than a high specific surface area (Young and Dahab, 1983). Accordingly, a material with high porosity will perform better. In fact many studies have been conducted with different types of support materials for fixed-bed reactors including plastic cylinders, Raschig rings, string-shaped plastic media saddles, pall rings, and clay blocks, and each type showed different results (Choi *et al.*, 1989; Young and Dahab, 1983; Yu *et al.*, 2006).

From the above, it can be considered that several factors are responsible for biofilm growth and biomass entrapment, and the resulting good performance of fixed-bed reactors. Recent studies have shown that low-density floating supports are very good packing media in anaerobic fixed-bed reactors (AFBRs) for the treatment of effluent from the agro-food industry, distillery vinasse and winery wastewaters (Ganesh *et al.*, 2010; Rajinikanth *et al.*, 2009; Thanikal *et al.*, 2007). These studies, which have reported promising results, were conducted with loose supports of the same shape made with the same material (polyethylene). The aim of this work was to study the effect of the chemical nature of loose supports on the performance of anaerobic fixed-bed reactor for the treatment of high-strength industrial effluents. All support media were designed with the same shape (Fig. 6A) but with different materials: high density polyethylene (HDPE), polypropylene (PP) and polyvinyl chloride. The influence of the material used to make the supports on biomass retention and activity and on reactor performance was investigated.

MATERIALS AND METHODS

Design of lab-scale anaerobic fixed-bed reactors

Three up-flow anaerobic fixed-bed reactors, all of height 100 cm and diameter 12.5 cm, were used in this study. The double-walled reactors were made of plexiglass with an effective volume of 10 L and maintained at 35 °C with water recirculation from a thermostatically-regulated water bath. The feed was pumped into the bottom of each reactor by a Masterflex® peristaltic pump. The liquid inside the reactor was recirculated from top to bottom for homogenous distribution of the liquid using a Masterflex® peristaltic pump at a rate of 10 L/h. Two wire meshes, one at the bottom and one at the top of the liquid level of the reactors, were placed to maintain the supports. At the top of each reactor, an outlet port connected through a U-tube was made for separation of biogas. Temperature and pH were measured on-line using a pH probe inserted at the top of each reactor. Each reactor was filled to 80% of effective volume with randomly-distributed supports of one type. The biogas production rate was measured on-line by an Aalborg mass flow meter (0 to 50 mL/min) fitted with a 4-20 mA output. The "Modular SPC" software developed at our laboratory was used to log the data (gas output and pH).

Support media

Three support media, each made of a different plastic material (PVC, PP and HDPE), were used to fill the filter beds. Each cylindrically shaped support had a dimension of 30/35 cm x 29 cm and a specific surface area of 320 m^2/m^3, as shown in Fig. 6A. The relative densities were 1.35, 0.90, and 0.95 for PVC, PP and HDPE, respectively.

Characteristics of the wastewater and inoculum

Vinasse (residue obtained after wine distillation) was used as the substrate. Total COD of the vinasse was around 44 g/L. The feed was made alkaline with the addition of NaOH and was supplemented with nutrients, corresponding to a COD: N: P ratio of 400: 7: 1, and with 0.5 mL/L of Vitane (a commercial mixture of trace-elements). Two litres of granular sludge, with a VSS concentration of 50.8 g/L, were mixed with 8 L of effluent from a pilot-scale reactor treating distillery vinasse and then added into each reactor. The initial solid concentration in the reactors was 10 g VSS/L.

Measurements and analysis

pH and biogas were measured on-line. VFA concentrations, soluble COD, the alkalinity of the discharged effluent were determined through off-line analysis after centrifugation at 15,000 rpm for 15 min (APHA, 2005). VFAs were measured using a flame ionization detector gas chromatograph Varion 3900 attached with an automatic sampler. A semi-capillary Econocap FFAP column of 15 m length, 0.53 cm diameter, and Phase EC^{TM} 1000 film 1.2 micro m was used. The temperatures of the injector and detector were, respectively, 250 °C and 275 °C. The carrier gas was nitrogen (25 kPa). The margin for error for this measurement was between 2% and 5%, with a quantification threshold of 0.1 g/L. Biogas composition (CO_2, O_2, N_2 and CH_4) was determined using a gas chromatograph (Shimadzu GC-8A) connected to a C-R8A integrator. The carrier gas was argon at 2.8 bars. The temperatures were 30 °C for the oven and 100 °C for the injector and the detector. The margin for error for this measurement was 5%. Soluble COD was measured by a colorimetric method using Hach 0-1,500 mg/L vials. Other parameters (SS and VSS) were measured following (APHA, 2005). Biomass development within the reactors at the end of the experiment was determined by measuring the amount of solids attached and entrapped by the supports after 24 h at 105 °C.

Experimental design

A "classical" start-up strategy was followed and the OLR was increased from a low value of 0.5 g/L.d. (for avoiding reactor overloading during the first day of start-up) to the maximum OLR acceptable by each reactor, that is to say the maximum OLR with a minimum COD removal efficiency of 80 %. The OLR was increased 15-20% each week on condition that COD removal efficiency was 80% or more. To increase the OLR, the HRT was decreased by increasing the feed flow-rate while maintaining the feed concentration constant throughout the experiment.

RESULTS AND DISCUSSION

Performance of the reactors

Evolution of OLR, soluble COD and COD removal efficiency

The three reactors, each with a different support material (PVC, PP or HDPE), were started initially with a low OLR of about 0.5 g COD/L.d. with a corresponding HRT of more than 60 days. The reactor operation with a low OLR of 0.5 g COD/L.d. was useful in the biomass growth and retention, resulting in a successful start-up. The OLR was then raised by increasing the feed pump flow rate, once or twice a week at the rate of 15-20% at each step, on condition that the COD removal rate was at least 80%. A COD removal of above 80% was kept as the cut-off value as suggested elsewhere (Thanikal *et al.,* 2007). Figs. 1, 2 and 3 present for each reactor the evolution over time of its OLR applied, soluble COD and COD removal efficiency. During the first 60 days, the soluble COD at the outlet of all the reactors was very low (0.67 g/L on average) and quite constant. Average COD removal

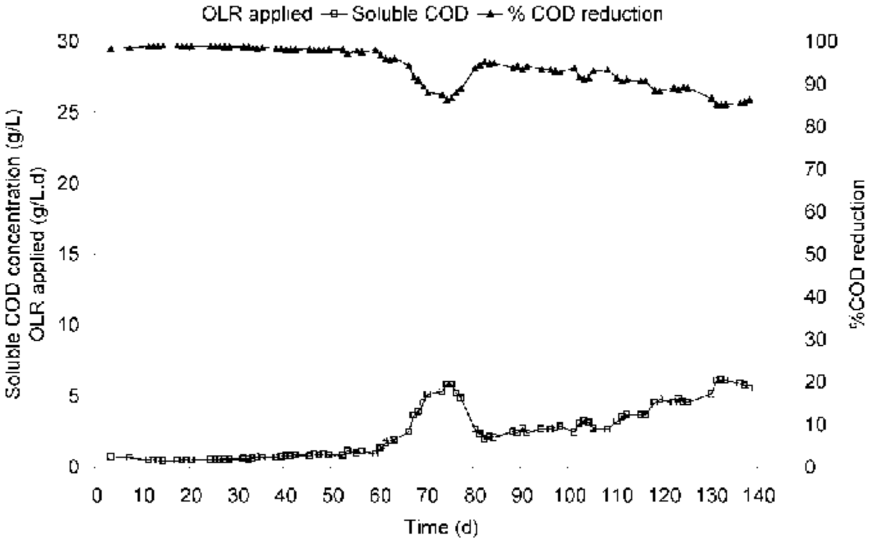

Fig. 1: Evolution over time of OLR, soluble COD and purification efficiency of the anaerobic fixed-bed reactor filled with supports made of polyvinyl chloride (PVC)

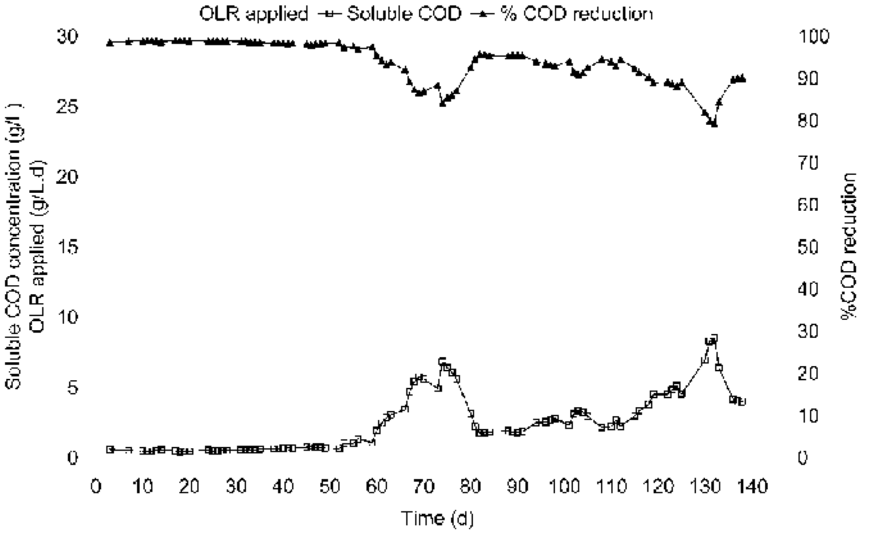

Fig. 2: Evolution over time of OLR, soluble COD and purification efficiency of the anaerobic fixed-bed reactor filled with supports made of polypropylene (PP)

efficiency was very high (98.5%) as the COD of the feed was more than 40 g/L. After day 60, a rapid increase of the COD at the outlet of the reactors was observed for all three reactors simultaneously. All the reactors showed very similar behaviour. The use of a new batch of vinasse was the reason for this sudden overloading. The OLR was first maintained constant for 15 days but as the COD at outlet continued to increase during that period, the OLR was then decreased from day 75 to day 80. After day 75, a rapid decrease in the COD at outlet was observed, indicating that the reactors were recovering from the

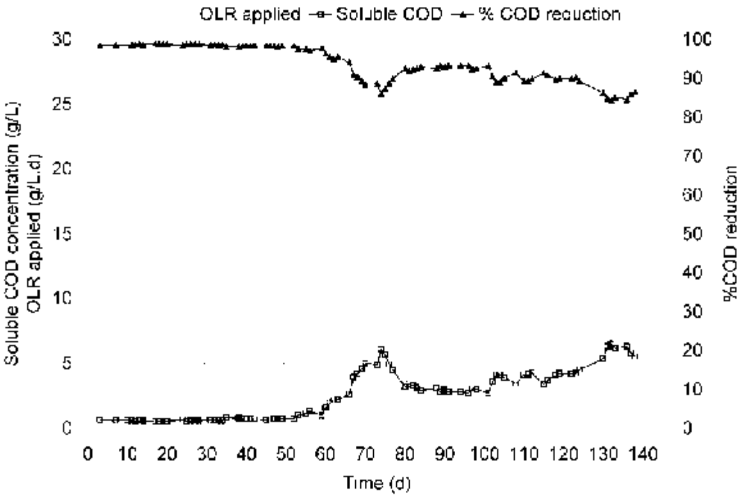

Fig. 3: Evolution over time of OLR, soluble COD and purification efficiency of the anaerobic fixed-bed reactor filled with supports made of high-density polyethylene (HDPE)

change of feed and, thus, from day 80 the OLR was increased regularly for the next 60 days, from 3.3 g/L.d on day 80 to around 20 g/L.d at the end of the experiment. During this period, the soluble COD at outlet increased very slowly (from 3.2 g/L on day 80 to around 6.5 g/L on day 130) and the COD removal efficiency always remained above 80%.

High OLR could be applied after 135 days of operation for the three reactors with an OLR of more than 20 g COD/l.d. The minimum HRT was 1.7 days for PVC, 2.2 days for PP and 1.8 days for HDPE. These results indicate that the behaviour of the three reactors was quite similar and they could treat quite a high OLR without any sign of overloading at an OLR of around 20 g/L.d. The experimental evidence indicates that the nature of the support material has negligible effect on reactor performance. Therefore the shape and size of supports are more crucial than the material which determines the differential behaviour of reactors in wastewater treatment (Ganesh *et al.*, 2010; Weiland, 1987). Besides, it has been also shown that pore size and porosity also plays a critical role than media surface area in the performance of anaerobic packed-bed reactors (Tay *et al.*, 1996).

Evolution of volatile fatty acids concentration

The concentration of VFAs inside the reactor determines the pH of the process. Any accumulation of VFAs may lead to a drop in pH resulting in the drop of methane yield and reactor performance (Chen *et al.*, 2008). The VFA profiles of the three reactors are shown in Fig. 4 and can be divided into three phases. In the first phase (days 0-60), no VFAs were measured in the liquid phase as the reactors were operated at quite a low OLR. During the second phase (days 60-80), an increase in soluble COD was observed after feeding of the reactors with a new vinasse (Figs. 1, 2 and 3). This was linked to an increase in the propionic acid concentration (Fig. 4), a phenomenon which is often observed during an overloading of a reactor fed with distillery vinasse. A previous study on winery effluent also showed accumulation of propionic acid with increased OLR (Ganesh *et al.*, 2010). However, the decrease in the OLR helped to rapidly eliminate the propionic acid accumulated in the reactor. In the third phase (days 80-135), acetic acid and propionic acid concentrations had a tendency to increase slightly with the increase in the OLR, but their concentrations always remained lower than 1.8 g/L and 3.2 g/L, respectively. Similar values of VFAs concentrations below 3.2 g/L are also reported in a previous study on anaerobic fixed bed reactors (Ganesh *et al.*, 2010).

Evolution of total suspended solids at outlet

The measurement of TSS at outlet is an important parameter because it reflects the filtration capacity of

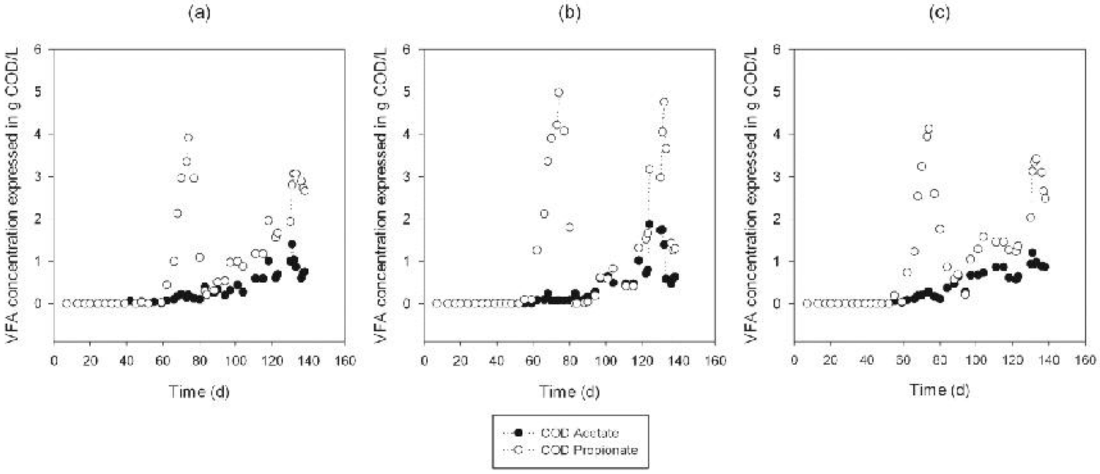

Fig. 4: Acetate and propionate accumulation during anaerobic digestion of vinasse in fixed-bed reactors packed with (A) PVC (B) PP and (C) HDPE.

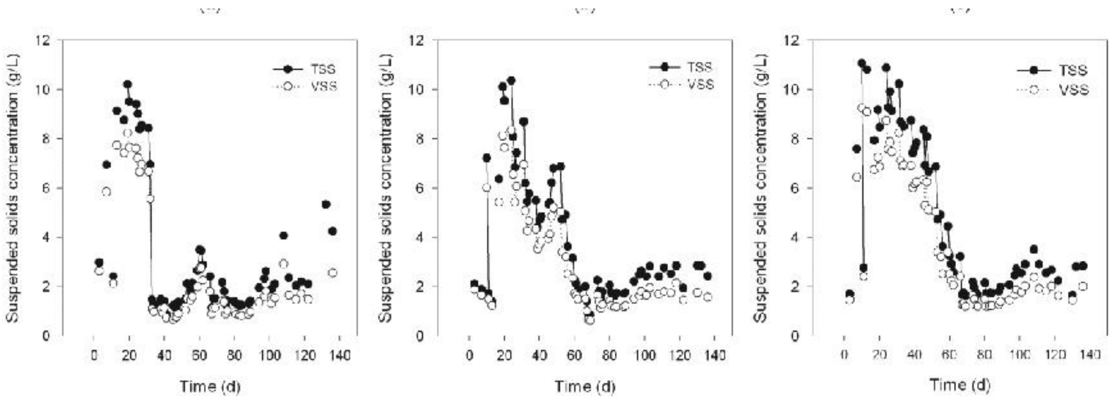

Fig. 5: Changes in the volatile suspended solids concentration at the outlet of three reactors packed with (A) PVC (B) PP and (C) HDPE.

the supports inside the reactor. The evolution of suspended solids concentrations for the three reactors is shown in Fig. 5. During the first 30 days, the three reactors behaved identically with very high TSS concentrations at outlet (up to 11 g/L), showing loss of biomass and low filtration of the suspended solids by the supports. After day 30, two kinds of support (HDPE and PP) showed quite similar results. For both these reactors, TSS slowly decreased until day 60-70 and then remained low (2 g/L on average) until the end of the experiment. For PVC supports, the filtration effect started much earlier and from day 33 until day 103 the solid concentrations at the outlet were low (1.2 g/L on average), except for an increase (3 g/L on average) on days 55-65. This increase could be attributed to low capacity of PVC to retain the increase in organic matter due to organic overload from feeding new vinasse. It can therefore be assumed that PP and HDPE are good materials for organic load shocks during reactor operation.

From day 108, solid concentrations at the outlet of all the three reactors started to increase, indicating a loss of solids from the reactor, consequently, the reactor needed to be unclogged as high concentrations of biomass started to be discharged from the reactor. The concentrations of TSS in the effluent was found to be independent of OLR shifts or flow rates as also reported in a previous study (Rajinikanth *et al.*, 2009). Although the performance of the three reactors was satisfactory, the concentration of solids in the effluent remained above the discharge limits. This necessitates the post-treatment of the effluent from the AFBR for safe discharge into the environment.

Evaluation of the quantity of biomass inside the reactors

After 140 days of operation, the three reactors were stopped and opened to quantify: (i) the biomass attached to the supports or entrapped inside the supports and; (ii) the biomass in the liquid phase and the biomass trapped between the supports. The results are presented in Table 1. The average weight of the modules was 5 g for HDPE, 4.83 g for PP and 8.58 g for PVC. The supports were removed in batches from the top of the reactor. Six batches of supports were removed, first 5 batches with 40 supports each and the last batch with 19 supports for HDPE, 13 supports for PP and 29 supports for PVC. Photographs of the three supports taken from the reactors are presented in Fig. 6 (B-D). Table 1 shows that the quantity of total solids attached/entrapped was 406 g for PVC supports, 387 g for PP supports and 368 g for HDPE supports. The difference between the supports is not significant, with only a 10% difference between the reactor with the lowest quantity of attached/entrapped biomass (HDPE supports) and that with the highest (PVC supports). The volatile fraction of the attached/entrapped biomass was around 50% of the total solids for all the three supports.

It was found that most of the biomass adhering to the three kinds of supports was easily washed away under normal tap water flow. This clearly suggested that the adhered biomass was not a real biofilm but, rather, was entrapped in the support media, an observation also recorded in the case of the commercial Bioflow® 30 (Thanikal *et al., 2007)*. The fractions of total biomass in the liquid phase were very low in all three reactors compared to the attached/entrapped biomass, representing only 3.6% of the total biomass for HDPE and PVC and 4.2% for PP. This indicates that the major fraction of reactor biomass was inside the support media (>95%). Specific biomass activities were

Table 1: Overview of the biomass distribution inside the reactors and specific activity

	Reactor with PVC modules	Reactor with PP modules	Reactor with HDPE modules
Number of modules	229	213	219
Weight of 1 module (g)	8.58	4.83	5.0
Attached/entrapped biomass:			
Quantity of attached/entrapped total solids (g)	406	387	368
Quantity of attached/entrapped volatile solids (g)	198	192	184
Organic fraction of the total solids: Volatile solids/Total solids (%)	48.7	49.7	50
Average quantity of total solids/module (g/module)	1.81	1.92	1.75
Biomass in the liquid phase:			
Volume of liquid in the reactors at the end of the experiment (L)	7.1	7.3	6.9
Quantity of total solids in the liquid phase (g)	12	12.4	10.4
Quantity of volatile solids in the liquid phase (g)	7.5	8.4	6.9
Maximum OLR (g COD/L.d.)	24	23	22
Soluble COD removal (%)	86	80	86
Specific activity (g COD/g VSS.d.)	1.17	0.92	1.15

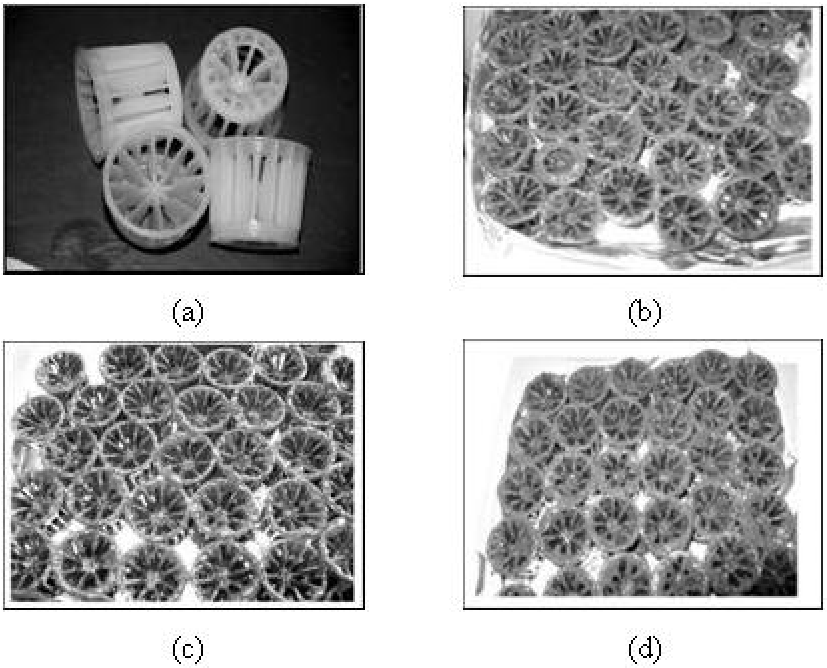

Fig. 6: Photograph of support module (A) before biomass entrapment (B) PVC module after biomass entrapment (C) PP module after biomass entrapment and (D) HDPE module after biomass entrapment.

around 1.0 g COD/g VSS.d. which is much higher than those earlier reported in anaerobic SBR reactors with suspended biomass (Ruiz *et al.,* 2002) and anaerobic filters with floating supports (Rajinikanth *et al.,* 2009). The results show that the supports made it possible to increase dramatically the quantity of biomass inside the reactors while maintaining quite a high biomass specific activity.

CONCLUSION

Three anaerobic fixed-bed reactors of 10 L, each one filled with loose 3-cm supports, were followed for 140 days to study the influence of the chemical nature of the support on reactor performance and biomass accumulation. The supports had the same shape but each one was made with a different material, PVC, PP or HDPE. The results did not show significantly different behavior and after four months of operation all three reactors were able to reach an OLR above 20 g COD/L.d. with more than 80 % removal efficiency. Quite a high quantity of biomass was entrapped inside the reactors (between 370 and 410 g solids/reactor) and there was no major difference according to the chemical nature of the material used to make the supports. These results show that the behavior of the reactors in terms of OLR and quantity of accumulated biomass was not different enough to decide that any one material was really better than another. Indeed, at industrial scale, anaerobic fixed-bed reactors will be designed with an OLR of around 10 to 15 g COD/L.d. and, for the three reactors, this OLR was reached after 100 days of operation and no significant difference was observed between the three materials. The results also suggest that the supports' main mechanism of biomass retention is entrapment inside the supports, with only very limited biofilm formation on the supports. It was shown in a previous study that the size of the support (0.9, 3 and 4 cm) had a clear influence on reactor performance and biomass retention (Ganesh *et al.*, 2010) but the present study shows that for a given size and shape, the material used is not an important parameter. Thus, the main criteria for the choice of the material used for the supports should be their ease of use and their production cost.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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