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COLONISATION OF A POROUS SINTERED-GLASS SUPPORT IN ANAEROBIC THERMOPHILIC BIOREACTORS

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Abstract
Biofilm development in an open-pore sintered-glass material (SIRAN) was studied using a laboratory-scale, anaerobic fixed-film reactor under thermophilic conditions. The start-up and performance of this reactor, operating on distillery wastewater feed (vinasses), were also studied. Stepped organic loading during initial reactor start-up reduced the periods of adaptation in the colonisation process and micro-organism attachment, and biofilm formation was accelerated by the surface characteristics of the carrier. The results obtained by operating with stepped organic loading (3.81 kg COD/m^3/day) over a period of 75 days suggest that a stable operation of the process (80% COD removal) and high density of biomass immobilised on the support (89.26 g VSatt/m^3 SIRAN) was achieved. Epifluorescence microscopy demonstrated that, initially, attached growth developed in crevices where biomass was protected from shear forces and, finally, SIRAN was completely covered and biofilm developed on the entire SIRAN particles. The support, under anaerobic thermophilic conditions, due to its properties of low density, high porosity and specific area, is suitable for the immobilisation of slow-growing micro-organisms (e.g. anaerobic thermophilic organisms), and is especially adequate as a support for anaerobic fluidised beds for the treatment of high-rate organic loads. © 1997 Published by Elsevier Science Ltd.

Key words: Anaerobic digestion, biofilm, immobilisation, open-pore sintered glass, support materials.

NOMENCLATURE

COD	chemical oxygen demand
COD_r	chemical oxygen demand removal
HRT	hydraulic retention time
OLR_r	organic load rate removed
OLR_0	initial organic load rate
TSS	total suspended solids
VSatt	volatile attached solids
VSS	volatile suspended solids

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INTRODUCTION

The anaerobic treatment of industrial wastewater has a number of potential benefits, including low energy consumption, low excess-sludge production, enclosure of odours and aerosols and rapid re-start-up after prolonged shut-down (Boone, 1982). High-rate anaerobic digesters which retain biomass also have a high treatment capacity and hence low site-area requirements. The major process configurations developed are upflow anaerobic sludge blanket (UASB), both upflow and downflow stationary packed beds and fluidised- or expanded-beds (Maestrojuan, 1987; Pérez *et al.*, 1992; Breitenbucher *et al.*, 1990; Iza, 1991; Hickey *et al.*, 1991).

One drawback associated with this type of reactor is the length of time required for initial biofilm development (periods of 9 to 12 months have been reported; van den Berg & Kennedy, 1982). This problem can be minimised using a stepped organic load during initial reactor start-up (Bull *et al.*, 1983) or supplying a substrate that may be directly metabolised by micro-organisms (Balaguer *et al.*, 1992; 1993).

The nature of the media used for biofilm attachment has a significant effect on the reactor performance. A wide range of materials have been used as non-porous support media at laboratory- and pilot-scale, including glass beads (Salkinoja-Salonen *et al.*, 1983), red drain clay, sand and a number of different plastics (Nebot *et al.*, 1995) and porous materials, such as needle-punched polyester (van den Berg & Kennedy, 1982), polyurethane foam (Fynn & Whitmore, 1982) and sintered glass (Breitenbucher *et al.*, 1990).

Huysman *et al.* (1983) and Murray and van den Berg (1981) examined a variety of porous and non-porous media for the initial colonisation and

development of microbial fixed films. For non-porous media, they established that biofilm development is primarily a function of the availability of microbial-sized crevices on the surface of the material. These authors also found that the colonisation of porous material depended primarily on the size of the pores and the degree of porosity.

This study was designed to evaluate start-up and performance of an anaerobic fixed-film reactor containing a porous media (SIRAN) treating distillery wastewater (vinasses) and to follow the colonisation process on SIRAN.

METHODS

Reactor

The colonisation process of support media was carried out in an anaerobic digester treating vinasses of wine and using SIRAN as fixed-film support. The schematic diagram of the anaerobic fixed-film system used in the laboratory study is shown in Fig. 1. The anaerobic filter reactor consisted of a vertical cylindrical tank (length 25 cm, internal diameter, 10 cm). The active liquid volume was 2 l, and the empty volume was 2.4 l. The temperature was maintained at 55°C (precision of ± 1°C) and the biogas generated was collected in a gas meter. The operation was semicontinuous and feed was supplied by a peristaltic pump connected to a programmable timer. Effluent recirculation was used to mix and homogenise the liquid in the system.

Feed composition

The feed utilised was wine-distillery water coming from an ethanol producing wine-distillery plant situated in Tomelloso (Ciudad Real, Spain). The vinasses were transported and maintained at 4°C before their utilisation. This feed was diluted with tap water to attain the required feed chemical oxygen demand (COD) concentration to be used in this experiment (around 18 g COD/l) and was supplemented with sodium hydroxide to maintain a neutral pH.

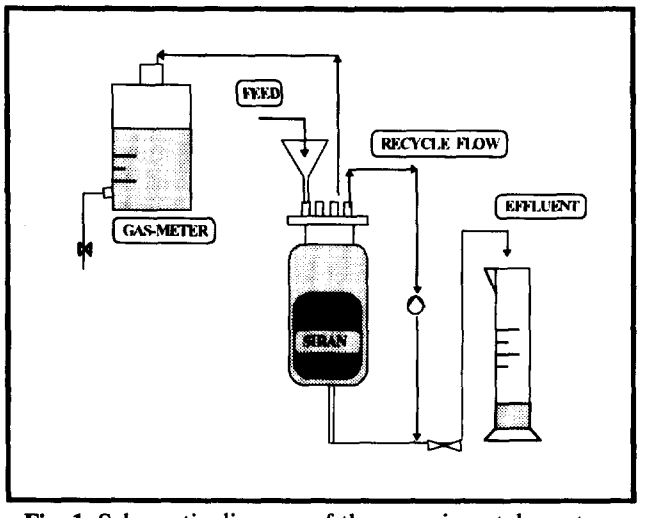

Fig. 1. Schematic diagram of the experimental reactor.

Vinasse biodegradation batch experiments (Pérez *et al.*, 1996) indicated that this was a complex medium formed by two substrates of different nature and biodegradability. Firstly, S1, the easily biodegradable substrate fraction (80% of the total), and secondly, S2, the non-easily biodegradable substrate fraction (recalcitrant substrate), in the conditions under which the experiments were carried out.

Characteristics of the support media

The media support used consisted of open-pore sintered-glass beads, 'SIRAN'. This carrier has been developed and marketed by Schott Glaswerke and it is produced by sintering of a mixture of glass and salt powder followed by a washing process which elutes the non-sinterable salt. The resulting glass sponge has well-defined pore-size distribution (double-pore structure).

The particles were sieved for uniformity and the resulting particles had an apparent diameter of approximately 1.5–2 mm. This material was chosen because of its uniformity and also because it could be calcined to measure dry organic matter concentrations. Although at the moment it does not represent a practical material for full-scale application because of its currently high cost, demonstration of its high performances as a biofilm support could contribute to a widespread use of this kind of media. The main characteristics of SIRAN carrier are shown in Table 1.

Experimental procedure

The reactor was seeded with active biomass from a conventionally operational steady-state digester. Every day, 500 ml of medium were replaced with 500 ml of new fresh vinasses (diluted and neutralised vinasses with 18 g COD/l, HRT: 4 days). When the reactor operated at stationary-state conditions, approximately 275 ml of support medium were added to the reactor and occupied a total volume of 110 ml in an unexpanded mode. Subsequently, and due to the evolution of the process, the organic load was reduced to 3.81 g COD/l/day (HRT: 4.7 days).

Analytical methods

All analytical determinations were performed according to *Standard Methods* (APHA, 1989). For liquid samples, the parameters analysed in both the

Table 1. Main characteristics of SIRAN carriers

SIRAN characteristics	
Sphere diameter (mm)	1.5
Bulk density (g/l)	570.0
Pore diameter (μm)	60–300
Pore volume (%)	55–60
Surface area (m^2/l)	87

effluent and the influent were pH, chemical oxygen demand (COD), both total and volatile suspended solids (TSS, VSS) and attached microbial mass (VSatt). For gaseous samples, the parameters analysed were the volume of biogas produced at STP and its composition.

COD was determined by the dichromate reflux methods. For soluble CODs, the sample was first filtered as in the TSS analysis and the filtrate was used for the COD analysis. TSS and VSS were determined by the glass fibre filter method as described in *Standard Methods* (APHA, 1989). Gas production was measured continuously by water displacement. Measurements of methane and carbon dioxide were obtained using gas chromatography separation accomplished using a stainless-steel column packed with Carbosieve SII (diameter 1/8 in., length 2 m) and a thermal conductivity detector (TCD). The injected sample volume was 1 cm^3 and the operational conditions were as follows: 7 min at 55°C; rammed at 27°C/min until it reached 150°C; detector temperature: 255°C; injector temperature: 100°C. The carrier was helium and the flow rate used was 30 ml/min. A standard gas (by Carburos Metalicos, S.A.) of 4.65% H_2; 5.33% N_2; 69.92% CH_4 and 20.10% CO_2 was used for the calibration of the system.

Attached biomass concentrations were determined by removing a representative sample from the reactor and then ashing the dried sample to measure the total volatile solids both attached to the particles and entrapped among them (Shieh *et al.*, 1981).

In several stages of the colonisation process, a small fraction of colonised particles was utilised for their morphologic characterisation using optic microscopy and epifluorescence microscopy (wavelength excitation at 400–440 nm with barrier filter at 460 nm).

RESULTS AND DISCUSSION

The experimental protocol was designed to examine the effect of organic loading rate on the efficiency of the anaerobic thermophilic fixed-film reactor (with SIRAN media) process and evaluate the evolution of the attached biomass concentration in the reactor.

The organic volumetric loading and removal rates, OLR_0 and OLR_r respectively, the organic removal efficiency (as percentage of initial COD), volumetric gas and methane production (l/l digester/day) compared to the hydraulic retention time are shown in Fig. 2.

During the early start-up period of the reactor (period T1), the organic removal efficiency (OLR: 4.4 g COD/l/day) was 80% soluble COD removal, corresponding to the maximal biodegradation efficiency of the substrate S1 content in the feed. This COD removal included two processes: organic matter conversion to methane and synthesis of new microbial biomass that increased suspended biomass. The support added to the reactor (period T2), at 15 days from inoculation, brought about a decrease in the useful reactor volume (1.89 l) and, in consequence, an organic overload of 4.7 g COD/l/day. This caused the flora to adapt to the new conditions of operation, an abrupt decrease in the pH of the medium and a decrease of organic removal efficiency to 70% (percent of initial COD).

The direct neutralisation, as well as the decrease of the organic load applied (3.81 g COD/l/day) in the T3 period, permitted the stabilisation of the process, reaching a level of efficiency of 81% and a pH value of 8. At this point, the effluent COD was stabilised at residual constant values slightly greater than 3 g COD/l.

The volumetric rate of methane generation during the process showed a tendency to stabilise in the range approaching 0.9 l/l digester/day. Methane yield, as litres of methane produced per gram of COD removal, diminished from the period T1 to T2, improving in T3 until it reached 0.29 l/g COD_r. Nevertheless, this value was significantly lower than the stochiometric theoretical value of 0.35 l CH_4/g COD_r (1 g of COD is equivalent to 0.35 l of methane under STP conditions).

In this sense, the synthesis of new micro-organisms and the initial biomass attachment processes on the support surface involved the initial production of polysaccharide to bind the material. This phase involves a high consumption of organic material through the synthesis route (anabolism), thereby diminishing the quantity of substrate that it transforms into methane. For this reason, the theoretical value of 0.35 l CH_4/g COD is higher than the calculated experimental value.

The monitoring of the colonisation and attachment biomass processes on SIRAN was studied by evaluating the modifications that were produced in the total content of volatile suspended solids in the liquid medium and attached to the support throughout the temporal extension of the experiment, according to the protocol described by Shieh *et al.* (1981).

Figure 3 plots the temporal evolution of the biomass colonisation process on SIRAN support. The graphic shows, in logarithmic scale, the volatile suspended solids in the medium (kg VSS/m^3) and the volatile attached solids on the support (kg VSatt/m^3 SIRAN) over a period of 75 days.

As is shown in Fig. 3, the effluent volatile suspended solids increased only slightly from 0.59 g VSS/l to a maximum of 1.9 g VSS/l after 36 days of operation. Subsequently, VSS decreased smoothly until it stabilised at 1.0 g VSS/l. Therefore, the whole reactor contained approximately 2 g VSS.

In contrast, the physical characteristics of the SIRAN support (double-pore structure and high surface area) favoured the adequate conditions for biomass attachment from the initial stage of the pro-

cess. So, in just 1 day of contact between micro-organism and support, values of 1.09 g VSatt/m^3 SIRAN were obtained. This quantity evolved until stabilisation was reached in the range from 20 to 25 kg VSatt/m^3 SIRAN, increasing to 89.26 kg VSatt/m^3 SIRAN in 75 days. Therefore, considering a volume of support of 275 ml, this supposed a quantity of 24.55 g VSatt in the total active reactor.

The results published by Gorris *et al.* (1988) indicate that the colonisation processes proceeded in three consecutive phases: viz. lag phase, biofilm production phase and steady-state phase, irrespective of the type of inoculum applied. This pattern reflected the overall rate of colonisation and biofilm production for beads present in the reactor. These stages were observed in the present study, although the stepped COD load reduced the duration of the initial period of induction to a few hours.

The final values of biomass density on the SIRAN support were comparable with values published by Fox *et al.* (1990) in colonisation processes on GAC (granular activated carbon) in fluidised-bed systems, with similar profiles of evolution, although the GAC support required a longer period of time to reach the same density of attached biomass. Thus, at the end of the experiment (12 weeks), SIRAN retained over seven times the biomass retained on 0.7 mm sand, and over three times the attached biomass of anthracite (Fox *et al.*, 1990) during a 43 week period treating a synthetic feed containing acetate as the only carbon source under mesophilic conditions.

Finally, Bull *et al.* (1983) indicated that stepped organic loading with co-substrate addition improved performance during the initial start-up time and appeared to aid bacterial development. The feed utilised in this study was a complex medium that provided all the macro- and micro-nutrients necessary for an adequate colonisation process of the carrier. In this sense, the vinasses showed an adequate relationship between the different macro- and

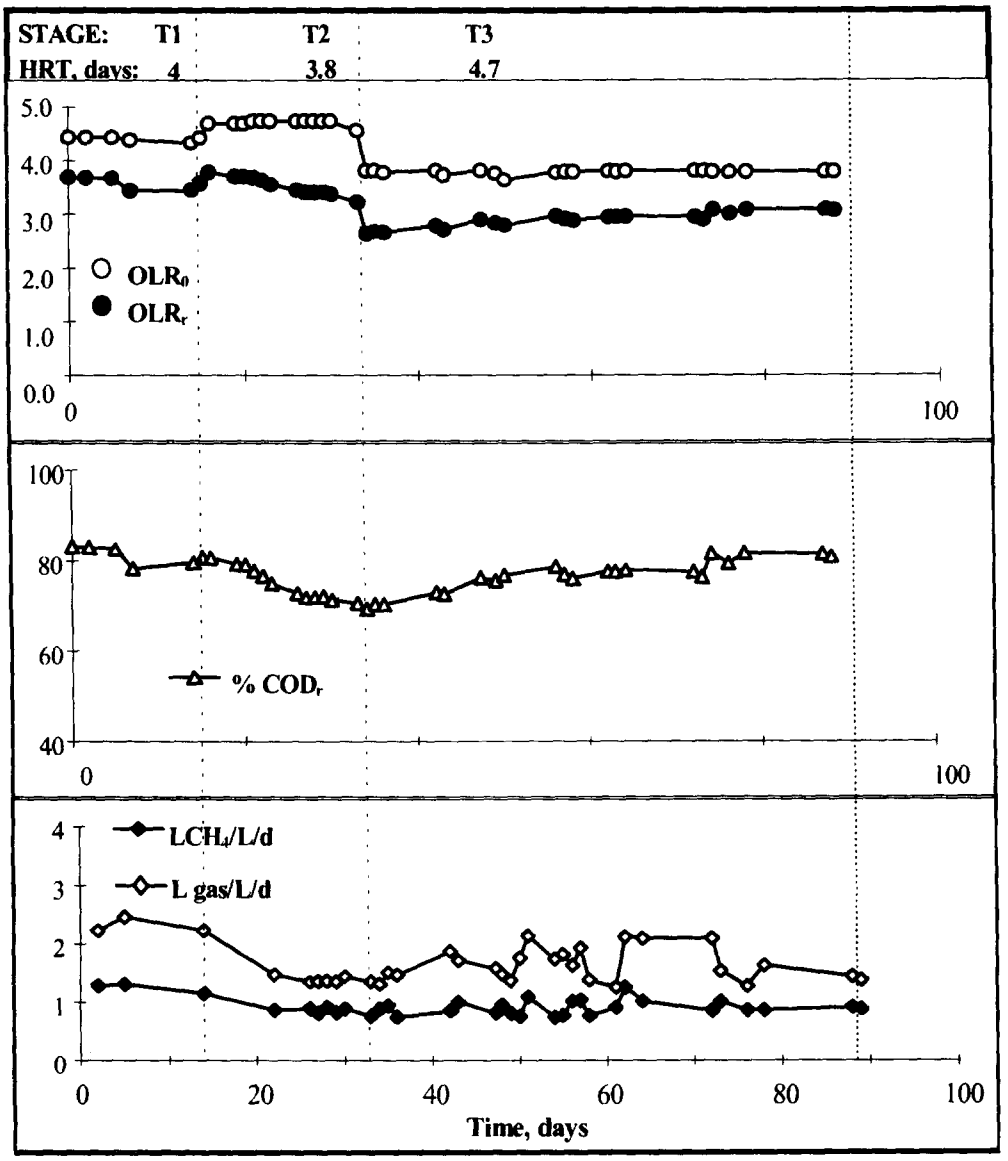

Fig. 2. Variations of the characteristic parameters of the anaerobic process: (a) organic loading and removal rate, OLR_0 and OLR_r, as kg COD/m^3/day; (b) COD_r, organic removal efficiency (as a percentage of initial COD); (c) volumetric CH_4 and biogas production rate, l/l digester/day. Feed: diluted vinasses 18 g COD/l.

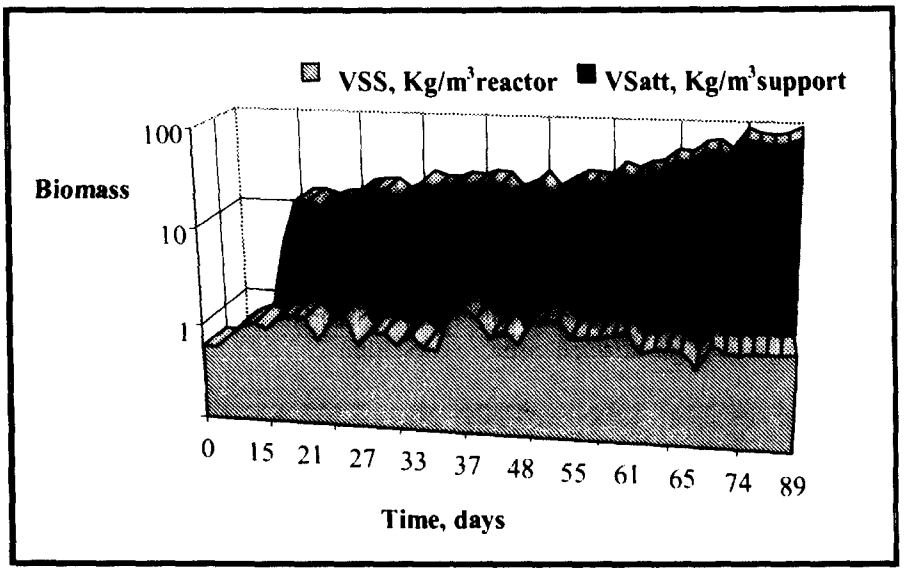

Fig. 3. Profiles of effluent volatile suspended solids (kg VSS/m^3 digester) and volatile attached solids (kg VSatt/m^3 SIRAN) during the colonisation process.

micro-nutrients with a favourable COD/N/P ratio suitable for microbiological treatment. An exhaustive study of the characteristics and properties of vinasses can be found in a previous paper by the authors (Sales *et al.*, 1982).

At the end of the process, the attached biomass was more than 93% of the total volatile solids contained in the reactor. Surface roughness increased both the rate of attachment and the rate of accumulation of biomass on the packing media. This was most probably due to the bacteria initially colonised in the pores being protected from shear forces and changes in environmental conditions. Under these conditions, no COD removal was caused by suspended growth.

These findings indicate that SIRAN is a suitable support medium for fluidised-bed, anaerobic reactors treating high-rate organic loadings.

Epfluorescence microscopic observations (λ_{exc}: 400–440 nm, $\lambda_{barr.}$: 460 nm) were made on both virgin media and on media samples obtained from each stage of the process. This visual observation corroborated the results obtained: the SIRAN carrier showed a high colonisation density at a faster rate during start-up. The surface roughness was critical to initial micro-organism attachment and biofilm formation (Breitenbucher *et al.*, 1990; Fox *et al.*, 1990; Yee *et al.*, 1992).

The morphologic biofilm characterisation (40 × magnification) in different colonisation stages is shown in Fig. 4. Biofilm growth on SIRAN media was located primarily in areas with irregular shapes and large crevices, where protection from shear forces existed. A mature biofilm completely covered the rough surface. Figure 4 shows the original, non-populated particle of SIRAN, showing the double-pore structure.

The micropores in the range of 1–10 μm provided the initially submerged micro-organisms with a population area from which the entire carrier could be populated. So, 24 h after the beginning of the seeding [Fig. 4(b)], the initial attachment by anaerobic micro-organisms was observed on large crevices. After 12 days, the colonisation had increased [Fig. 4(c)]; the biofilm began to grow out of the crevices, and after 21 days [Fig. 4(d)] the biofilm filled in the crevices and biofilm growth from neighbouring crevices joined together. A mature biofilm completely covered the rough surface [Fig. 4(e)]. In this situation, when the biomass occupied the whole extension of the material, the surface had a smoother aspect than that of the non-colonised particles.

Fox *et al.* (1990) presented a schematic hypothesis explaining how biofilm accumulation occurs on a rough surface such as GAC, which has similar physical characteristics to SIRAN. The scheme proposed by this author could correspond, therefore, to a visualisation of the superficial colonisation process on SIRAN. Indeed, the visual observation of the media by optical microscopy confirmed the results presented.

CONCLUSIONS

Anaerobic fixed-film digesters can achieve 80% COD reduction at a COD loading of 3.81 kg COD/m^3/day, within 75 days. Therefore, very short start-up periods are followed by a stable operation: the high biomass concentration is protected against wash-out and remains on the large inside surface area of the SIRAN carriers.

The sponge-like characteristics of the sintered-glass carrier enable very fast attachment of the micro-organisms, so the start-up time was very short, with high COD removal right from the beginning of the feed. Stepped organic loading during initial reactor start-up gave rapid biofilm development and

reduced the lag periods: in just 24 h of incubation quantities of 1.09 g VSatt/l SIRAN were obtained.

The open-pore structure of the carrier offered high surface areas to be colonised by active biomass and the entire carrier could be populated. This characteristic favours a high biomass colonisation capacity in short periods of time: 89.26 kg VSS/ m^3 SIRAN in a period of 75 days.

The previously colonised supports in anaerobic thermophilic conditions, due to their properties of drop density, high porosity and high surface area, are adequate for utilisation as a support for fluidised-bed anaerobic reactors treating high-rate organic loads.

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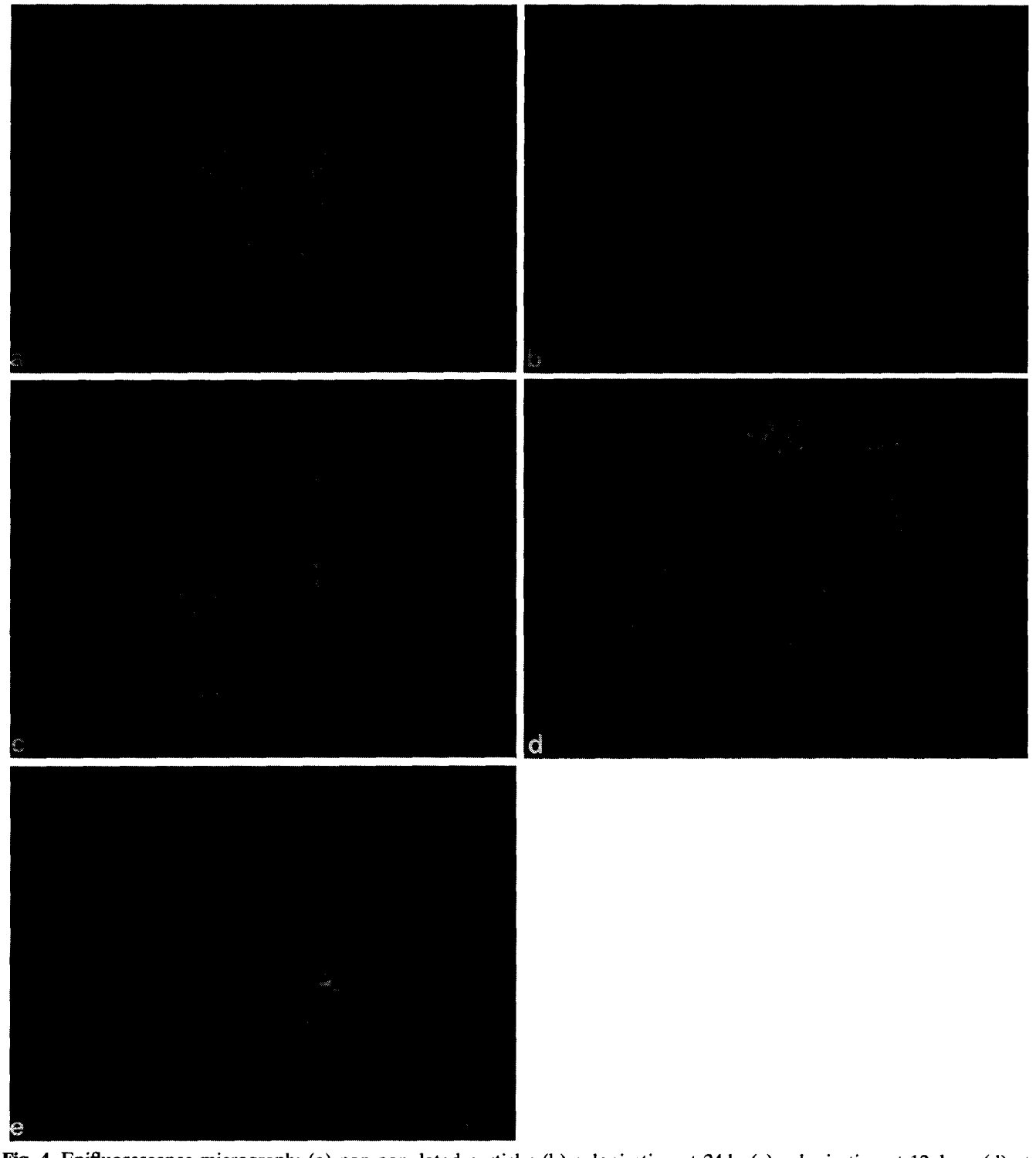

Fig. 4. Epifluorescence micrograph: (a) non-populated particle; (b) colonisation at 24 h; (c) colonisation at 12 days; (d) at 21 days from incubation; (e) at the end of the colonisation process (75 days).

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