Biofilm development in down flow anaerobic fluidised bed reactors under transient conditions

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Abstract Biofilm development onto polypropylene particles (<4 mm) was studied in a laboratory-scale down flow anaerobic fluidised bed reactor. The reactor was fed with a synthetic solution containing sucrose and nutrients, and operated at 35°C during 65 days at 44% bed expansion rate and 36 h HRT. Scanning electron microscopy (SEM) monitored the biofilm development. Initial adhesion occurred within the first 6 hours and after day 44 biofilm structure was complete. The presence of attached cells morphologically similar to Methanotrichia bacilli and Methanosarcina sp. was observed by Scanning Electron Microscopy (SEM). The biofilm and the carrier surface roughness were measured by atomic force microscopy (AFM) and yielded 9.1 and 75 nm respectively. Results also showed good correlation between the SEM characterisation and the conventional anaerobic reactor parameters.

Keywords Anaerobic digestion; biofilm reactors; down flow fluidised bed; wastewater treatment

Introduction

The success of the start up of anaerobic fixed film reactors depends mainly on microbial adhesion to the support material and biofilm development (Gorris et al., 1988; Heijnen et al., 1989; Bonnet et al., 1997). The knowledge of the actual adhesion mechanisms is important for the understanding of the biofilm formation (Gorris et al., 1989). Main phenomena involved might be studied using tools from both, biochemistry and physical chemistry (Bryers, 1993). Hence, bacteria can be considered as colloidal particles covered by macromolecules (bio-polymers) with the interactions being ruled by distinct phenomena occurring at the macromolecules/support surface/aqueous media system (Rutter, 1984; Rauch et al., 1999). Microbes possess ability to interact with almost any surface by interfacial forces where the total interaction energy is the result of attraction and repulsion forces (among them, electrostatic, hydrophobic and van der Waals) (Characklis et al., 1982; Salkinoja-Salonen et al., 1983; Israelachvili, 1985).

Regarding the solid surface colonisation, the following steps may arise (Charaklis et al., 1982; Charaklis, 1984; van Loosdrecht et al., 1990; Meraz and Alvarez-Ramirez, 2000):

1. Cell transport to the surface occurring by three different pathways: Diffusive transport, convective transport and active movement.
2. Initial adhesion, a physical–chemical driven process, which can be reversible or irreversible.
3. Biofilm growth, when biopolymers bind strongly to the surface and superficial organic films are formed.
4. Colonisation, while cells become firmly attached to the support, they begin to reproduce, forming micro-colonies.
5. Actual biofilm formation.

Although the fluidised bed anaerobic reactor offers many advantages with respect to other anaerobic systems, such as high mass transfer coefficients, high biomass
concentration and stability after loading shocks, the literature reports only few examples of full scale application of these systems (Nikov and Karamanev, 1991, Marín et al., 1999). The down flow fluidisation has advantages over the conventional of less energy consumption, improved biofilm thickness control and the continuous removal of detached biomass (García-Calderón et al., 1998a; García-Calderón et al., 1998b; García-Bernet et al., 1998; Karamanev and Nikolov, 1996). The most significant bottleneck of fixed film anaerobic reactors consists of the development of a well-attached methanogenic bacteria biofilm, mainly due to their slow growth rate (Salkinoja-Salonem et al., 1983; He and Ping, 1994; Buffière et al., 1998). In this context, this paper aims to provide some contribution to the understanding of biofilm formation mechanisms and its relationship with the start up difficulties based on well-established models of adhesion.

Materials and methods

A 35 L down flow anaerobic fluidised bed reactor (Figure 1) was designed and constructed using polypropylene (\( p = 0.91, \) \( d_p = 4 \) mm) as biomass support material (Tessele et al., 2000). The reactor consisted of acrylic columns with 0.15 m diameter and 2.0 m height. Expansion was obtained by liquid phase recycling and the temperature was kept at 35°C. The hydraulic retention time was 36 h, the superficial velocity of fluid was 0.02 m.s^{-1} and the expansion rate was 44%.

The reactor started up using 10% v/v of sludge from a bench scale UASB reactor, fed with sucrose and similar nutrients. Inoculation was repeated every week to ensure the presence of methanogenic bacteria. Scanning electron microscopy (SEM) during 65 days monitored biofilm development. Samples were taken every 48 h and prepared for SEM fixing them in glutaraldehyde and then dried in a graded ethanol series, as described by Englert (1997) and Tessele (2001).

Biofilm formation was accompanied by measuring conventional anaerobic reactor parameters such as volatile organic acids production (measured by gas chromatography), alkalinity, COD removal, redox potential, pH and gas composition. The synthetic substrate was a solution of sucrose and nutrients having COD varying from 770 to 1,400 mg.L^{-1} O_2. All analytic procedures were made as described by the Standard Methods (1995).

Results and discussion

Results demonstrated, as expected, that reactor performance is related to the biofilm formation and specific organisms adhesion. Accordingly, it was shown that biofilm growth
accompanied the organic matter degradation, measured by influent and effluent COD, the early days of operation and COD removal increased as the organic loading rate (OLR) increased from 1 to 3 kg COD.m⁻³.d⁻¹ (Figure 2).

Also, alkalinity ranged between 400 and 700 mg.L⁻¹ CaCO₃; pH was maintained in neutral values, redox potential gradually decreased to –300 mV and average total organic volatile acids concentration was greater than 300 mg.L⁻¹. Figure 3 shows the average acids proportion in the reactor effluent during the start-up period.

**SEM micrographs**
The recognised adhesion mechanism model was confirmed by SEM micrographs. Initial adhesion occurred within the first 6 hours of operation (Figure 4b) and biopolymer growth
was clearly noticed after two weeks (Figure 4c). Attached bacteria reproduction was detected after day 24 (Figure 4d). Then, colonisation and biofilm formation were well defined after 30 and 44 days respectively (Figures 4e and 4f).

Conclusions

The different techniques employed in this work, permitted us to obtain results which clearly demonstrated the surface colonisation mechanisms proposed for the down flow anaerobic fluidised bed reactor start-up. Microscopic techniques followed the characteristics of a carrier surface and its interactions with microorganisms and the biopolymers, the biofilm formation steps, including suggesting the probable morphological identification of methanogenic bacteria (\textit{Methanotrix} sp. and \textit{Methanosarcina} sp.). This biofilm formation and colonisation was accompanied by changes of conventional anaerobic degradation such as redox, pH, and alkalinity, among others.

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