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Full Length Research Paper

Methane yield and microscopic observation as monitoring biofilm behaviour parameters, during start up phase of anaerobic inverse fluidized bed reactor

Eric Houbron[1]*, Alejandro Alvarado-Lassman[2], Alejandro Zepeda[3] and Elena Rustrian[1]

[1]Universidad Veracruzana, Facultad de Ciencias Químicas. Prol. Ote. 6, No. 1009, C.P. 94340, Orizaba, Ver., México.
[2]Instituto Tecnológico de Orizaba, División de Posgrado e Investigación. Av. Tecnológico No. 852, Col. E. Zapata. C.P. 94320. Orizaba, México.
[3]Universidad Autónoma de Yucatán. Facultad de Ingeniería Química, Campus de Ingenierías y Ciencias Exactas, periférico norte kilometro 33.5 C.P. 97203, Mérida, Yucatán, México

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Anaerobic biofilm behavior on polyethylene and Extendosphere™ supports was evaluated during start-up of an inverse fluidized bed reactor using methane yield and microscopic observation as parameter monitoring techniques. Two anaerobic inverse fluidized bed reactors were used, one filled with triturated polyethylene as solid carrier material (diameter = 380 μm, density = 926 kg/m^3) and the other with Extendosphere™ (diameter = 147 μm, density = 700 kg/m^3). Each support material was used at up to 25% of its working volume (polyethylene = 1.2 l, Extendosphere™ = 1.9 l). Both reactors were started up in sequencing batch mode, applying organic loading rates of 0.5 to 14 g COD/l.d. Both supports exhibited rapid biofilm growth during start-up. Maximum surface colonization was 46% with the polyethylene and 100% with Extendosphere™. Both supports had a methane yield of 0.298 l CH_4/g COD at 10 and 14 g COD/l.d, respectively. Digital microscopic observation results coincided with methane yield results, confirming each to be viable for parameter monitoring of biofilm growth. Data generated by these two techniques is different and complementary, and in conjunction they constitute a highly effective monitoring method of biofilm growth.

Key words: Anaerobic digestion, biofilm, inverse fluidized bed reactor, methane yield.

INTRODUCTION

Use of inverse fluidized bed reactor (IFBR) technology in anaerobic wastewater treatment has a number of advantages over traditional methods (Buffière et al., 2000; García-Encina and Hidalgo, 2005; Cresson et al., 2007). Biofilm provides interesting assets such as separation of treated water from biomass, increased solid retention time, greater surface contact between organic matter and biomass, and a compact system design (Garcia-Calderon et al., 1998; Sowmeyan and Swaminathan, 2008a). A serious disadvantage is the long required start-up period due to low biofilm growth rate (Zellner et al., 1997; Alvarado-Lassman et al., 2010). Traditional biofilm growth characterization methods involve periodic observation and biomass sampling for external analysis to determine solids, proteins, poly-saccharides, and phospholipids contents.

The methane yield (Y_{CH4}) defined as the amount of methane produced per organic matter removed, is another anaerobic biofilm behavior monitoring parameter (Michaud et al., 2005). Theoretically, maximum Y_{CH4} in a

*Corresponding author. E-mail: ehoubron@uv.mx. Tel/Fax: +52 272 7240120-7241779.

Abbreviations: IFBR, Inverse fluidized bed reactor; **OLR,** organic loading rate; **TCD,** thermal conductivity detector; **HRT,** hydraulic retention time; **ITBR,** inversed turbulent bed reactor; **VFA,** volatile fatty acid.

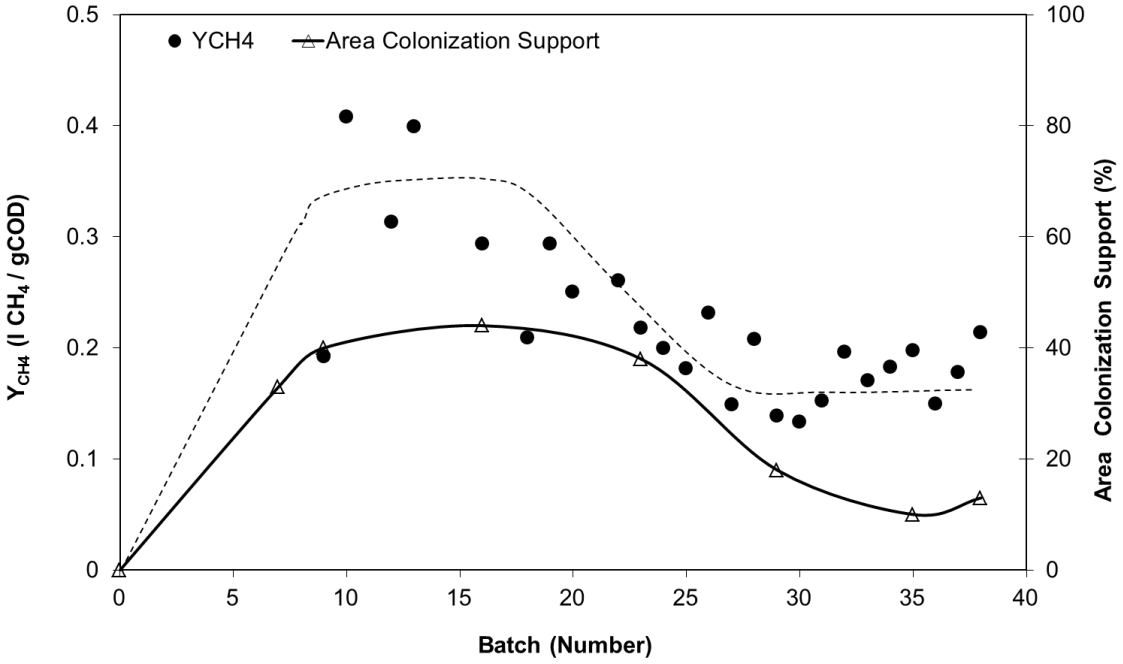

Figure 1. Batch course of methane yield (Y_{CH4}) and colonization with the polyethylene support during start-up phase.

methanogenic biomass is 0.395 l CH_4/g COD at 35°C. In the initial start-up phase, bacteria use carbon mainly to build the initial biofilm matrix, producing no methane. Y_{CH4} varies according to the biofilm formation phases (lag phase, log phase, and plateau). During the lag phase, biomass attachment for support surface colonization is reversible, and biofilm growth is made manifest as increased thickness. During the log phase, carbon is used for cell growth and maintenance, resulting in low methane production. Once steady-state conditions are reached, biofilm production may still occur to compensate for biomass losses from friction, but organic carbon is primarily converted to CH_4 near the theoretical maximum value. The present study aim is to evaluate anaerobic biofilm behavior on polyethylene and Extendosphere™ supports during start-up of an inverse fluidized bed reactor using methane yield and microscopic observation as monitoring parameters.

MATERIALS AND METHODS

Two anaerobic IFBRs were used, each reactor consisting of acrylic tubular columns (internal diameter = 4.48 cm; height = 112 to 137 cm) with a settling zone at the bottom. These were connected to a Marriott flask system to measure volumetric methane production. Liquid in the reactors was recycled at a constant flow velocity (6.8 m/h), and both were maintained at 35°C and pH 7.0. One reactor was filled with triturated polyethylene as a solid carrier material (particle diameter = 380 μm; density = 926 kg/m^3) (Figure 2A) and the other with Extendosphere™ (Figure 6A) (glass granules including an air bubble, supplied by PQ Hollowsphere Limited, England) (particle diameter=147 μm; density=700 kg/m^3). The carrier (support) materials were loaded at 25% of their working volume (polyethylene=1.2 l; Extendosphere™=1.9 l) as described in previous study (Alvarado-Lassman et al., 2008, 2010). Both reactors were inoculated with anaerobic granules from a UASB reactor treating paper wastewater, and fed with brewery wastewater. Initially, the reactors were started up in sequencing batch mode, applying an organic loading rate (OLR) of 0.5 g COD/l.d and then operated in continuous mode, with OLR increasing to 10 g COD/l.d. A batch was considered finished at 80% chemical oxygen COD removal. Routinely assayed system performance parameters included COD, and solids analysis in influent and effluent samples (Michaud et al., 2003). Biogas composition was analysed using gas chromatography (BUCK 310), with a thermal conductivity detector (TCD) equipped with a Packed Buck CTR-1 column, with the Peaksimple software for data acquisition and control (APHA, 1995). A digital microscope (Intel® Play™ QX3™ Computer Microscope) was used to view the support materials. Gram coloration was applied to the colonized polyethylene support, and surface colonization biomass was estimated by direct observation of digital images.

RESULTS AND DISCUSSION

Polyethylene support

During IFBR sequencing batch start-up, using the polyethylene carrier, a total of 38 batches were applied during a period of 225 days. During the first 26 batches, Y_{CH4} decreased from 0.3 to 0.15 l CH_4/g COD, then reached a constant value < 0.2 l CH_4/g COD (Figure 1). This indicated rapid support colonization during the initial

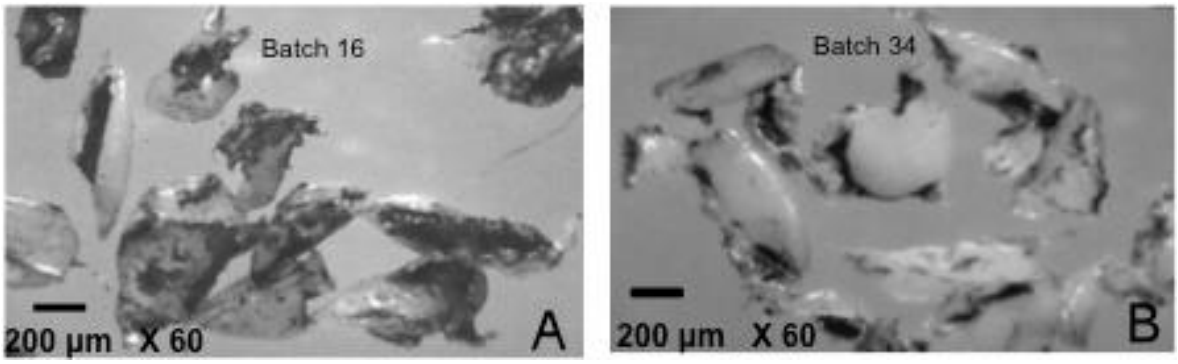

Figure 2. Microscopic image of polyethylene support during start-up phase: (A) Batch 16; (B) batch 34.

batch, and predominant CH_4 production, followed by a progressive decrease in CH_4 production and bacterial growth. Similar results have been reported previously in a study using synthetic wastewater (Hidalgo and García-Encina, 2002). The virgin polyethylene support was rapidly colonized, reaching 40% of the area during batch 16 (Figure 2A), followed by a regular decrease in surface colonization in batch 34 (Figure 2B). This behaviour coincided with methane production, confirming rapid initial biofilm growth during the first period (before batch 16) using the batch mode (Figure 1). Pressure generated by permanent change in the So/Xo ratio during the batch cycle may have caused this response. During a second period, high hydraulic retention time (HRT) favoured bacterial growth in the liquid phase associated with reversible biofilm detachment.

During the steady-state phase on the polyethylene carrier, OLR increased to 10 g COD/l.d, HRT decreased to 6 h, and consequently Y_{CH4} increased gradually to the theoretical maximum value (0.395 l CH_4/g COD) (Figure 3A and B). Until the 30th day, the majority carbon was used for biofilm formation, and then methane produced. Support surface colonization and Y_{CH4} profiles correlated well; for example, surface colonization stopped (maximum value=46 %) when Y_{CH4} reached the theoretical maximum value at 10 g COD/l.d (Figure 3B). However, when this change was made, the Y_{CH4} decreased to 0.298 l, and the surface colonization remained constant. This could indicate an increased thickness of the biofilm due to increasing Y_{CH4} and not increase of solids in the effluent. (Figure 3B). Moreover, COD and pH values were maintained in 180 ± 5 mg/l and 7.1 ± 0.1, respectively, in all the assays (Figure 3A and B).

As shown in Figure 4, polyethylene supported colonization efficiency differed according to support bed depth, with the higher portions exhibiting low colonization efficiency and the lower portion a maximum value of 46%. This gradient was observed throughout the experiment, suggesting that biofilm loss on the bed's upper portion may have resulted from the shear stress generated by water flowing down the support. The use of the two monitoring parameters provided different and complementary data. Direct microscopic observation produced data on support material colonization efficiency and biofilm growth, but no biofilm metabolism data. In contrast, Y_{CH4} values produced biomass growth and metabolism data but no colonization quality information. The combination of these two monitoring techniques provided excellent parameter data when using the polyethylene carrier material.

Extendosphere™ support

The reactor with extendspohere support was operated in mode batch during 10 batches, with a duration of 10 days for each one. Y_{CH4} was of 0.25 l CH_4 /g COD at the first batch, suggesting the immediate support colonization (Figure 5). This was confirmed by observation of initial biofilm growth at the end of batch one (Figure 6B). Michaud et al. (2005) observed similar biofilm growth only after 40 days, using a continuous mode during the start-up phase. Introduction of fresh support material during batch two provoked an immediate reduction in Y_{CH4}, a characteristic of new biomass production (Figure 5). After batch seven, a thin biofilm had covered the entire surface of the Extendosphere™, and by batch eight, OLR had increased to 1 g COD/l.d (Figure 6C). This renewed biomass production was manifested in decreased Y_{CH4}. Biomass production in the liquid phase did not occur until batch ten. As was observed with the polyethylene support material, initial biofilm growth on the Extendosphere™ was rapid during the first batch cycle. The operating conditions increased biofilm development during all assays.

Increases in OLR were correlated with Y_{CH4}, which reached a maximum of 0.35 l CH_4/g COD (Figure 7A and B). Similar result was obtained by Michaud et al. (2002) with Extendosphere™ support in an inverse turbulent bed reactor. With an OLR of 14 g COD/l·d and HRT of 6 h, free cells were completely washed out and biofilm

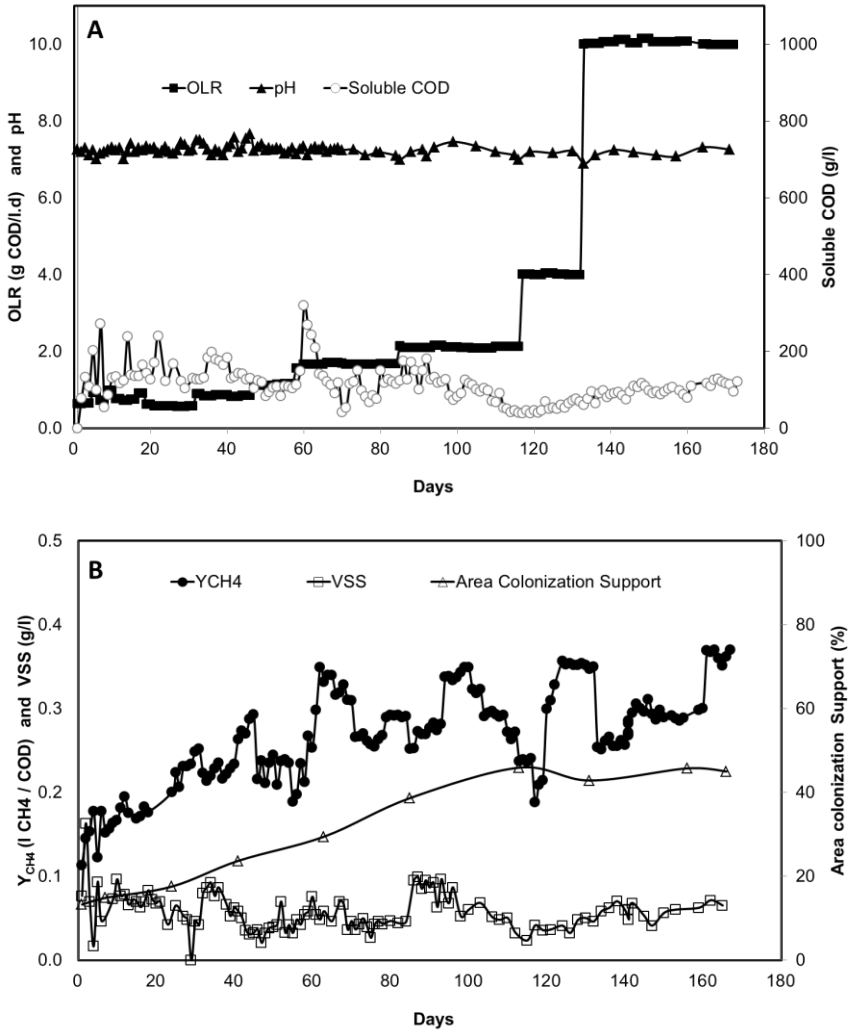

Figure 3. (A) Time course of OLR, pH, and COD; (B) time course of methane yield (Y_{CH4}). Volatile suspended solids (VSS) and percentage of area colonization support with the polyethylene support during steady-state phase.

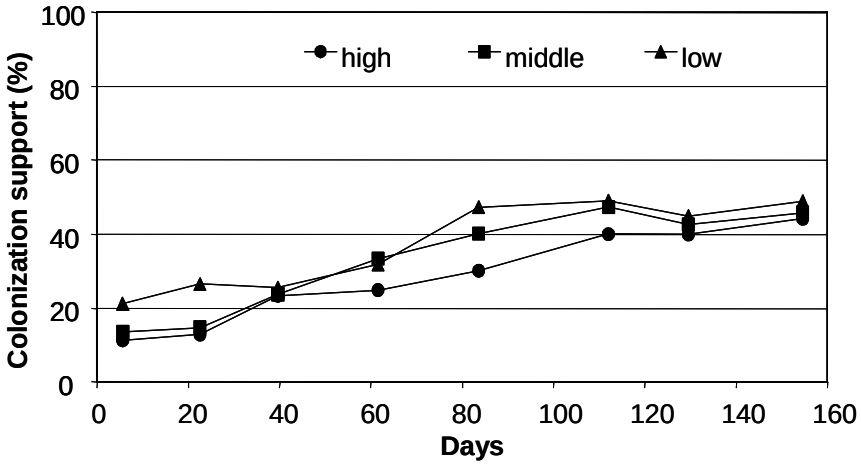

Figure 4. Time course of colonization with polyethylene support during continuous phase at different bed depth.

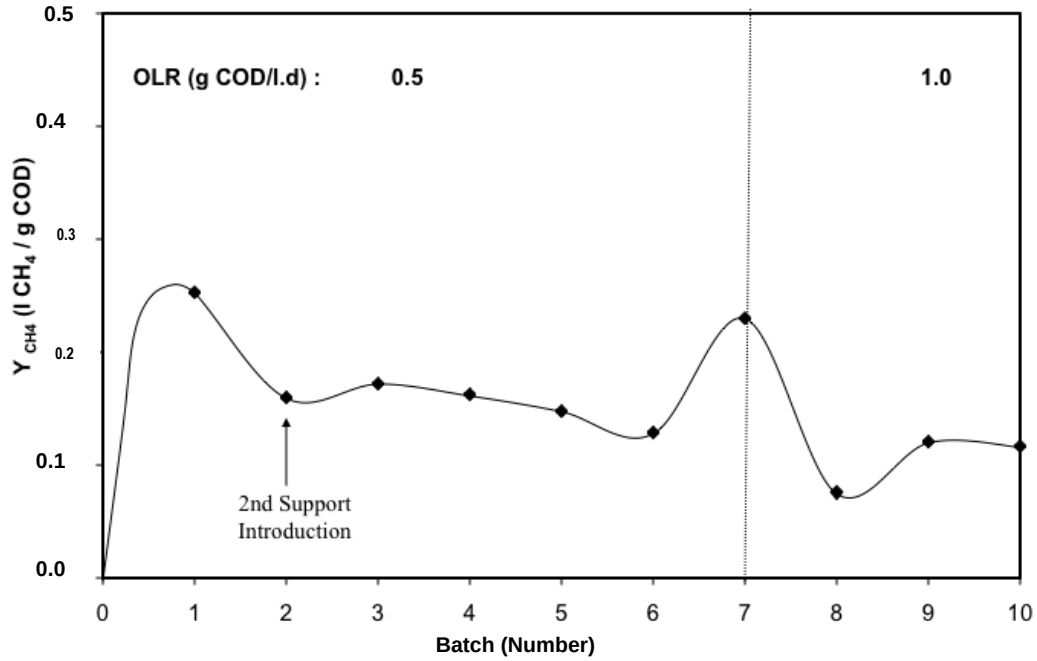

Figure 5. Time course of methane yield (Y_{CH4}) with the Extendosphere™ support during start-up phase.

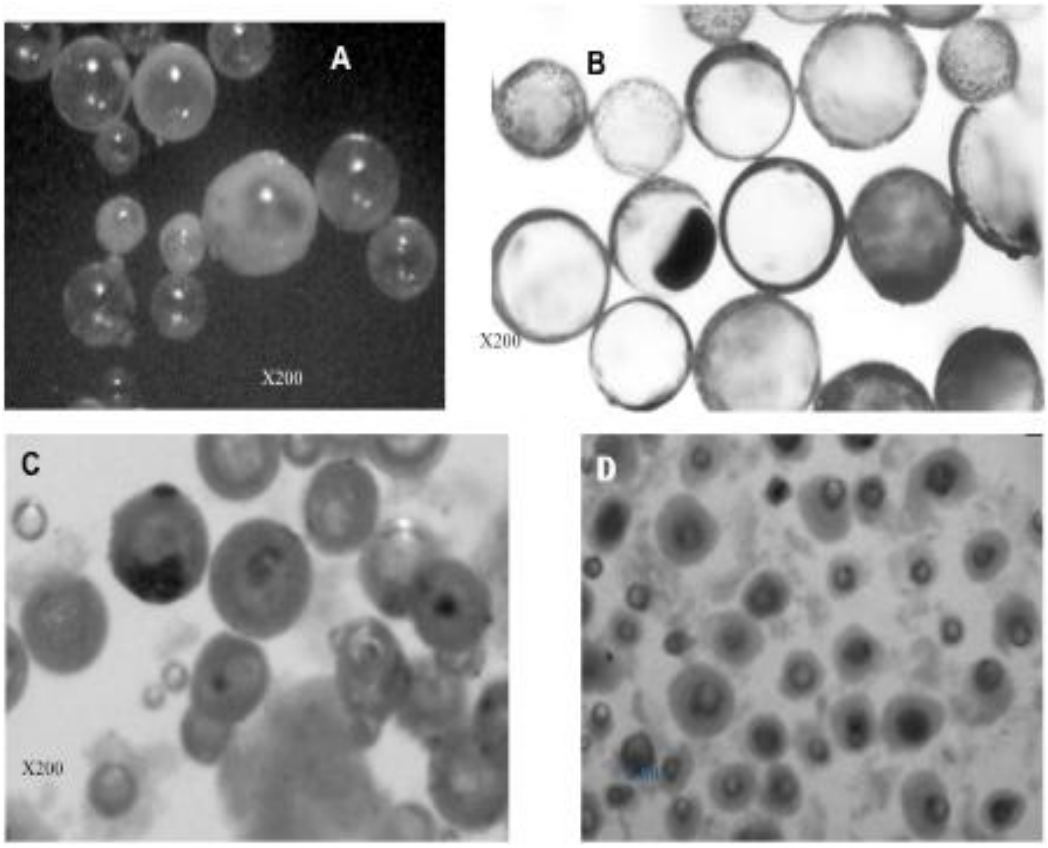

Figure 6. Microscopic observation of extendosphere. A: Fresh support, B: batch number. 1, C: batch number. 7, D: continuous O.L.R. 10 g COD/l.d.

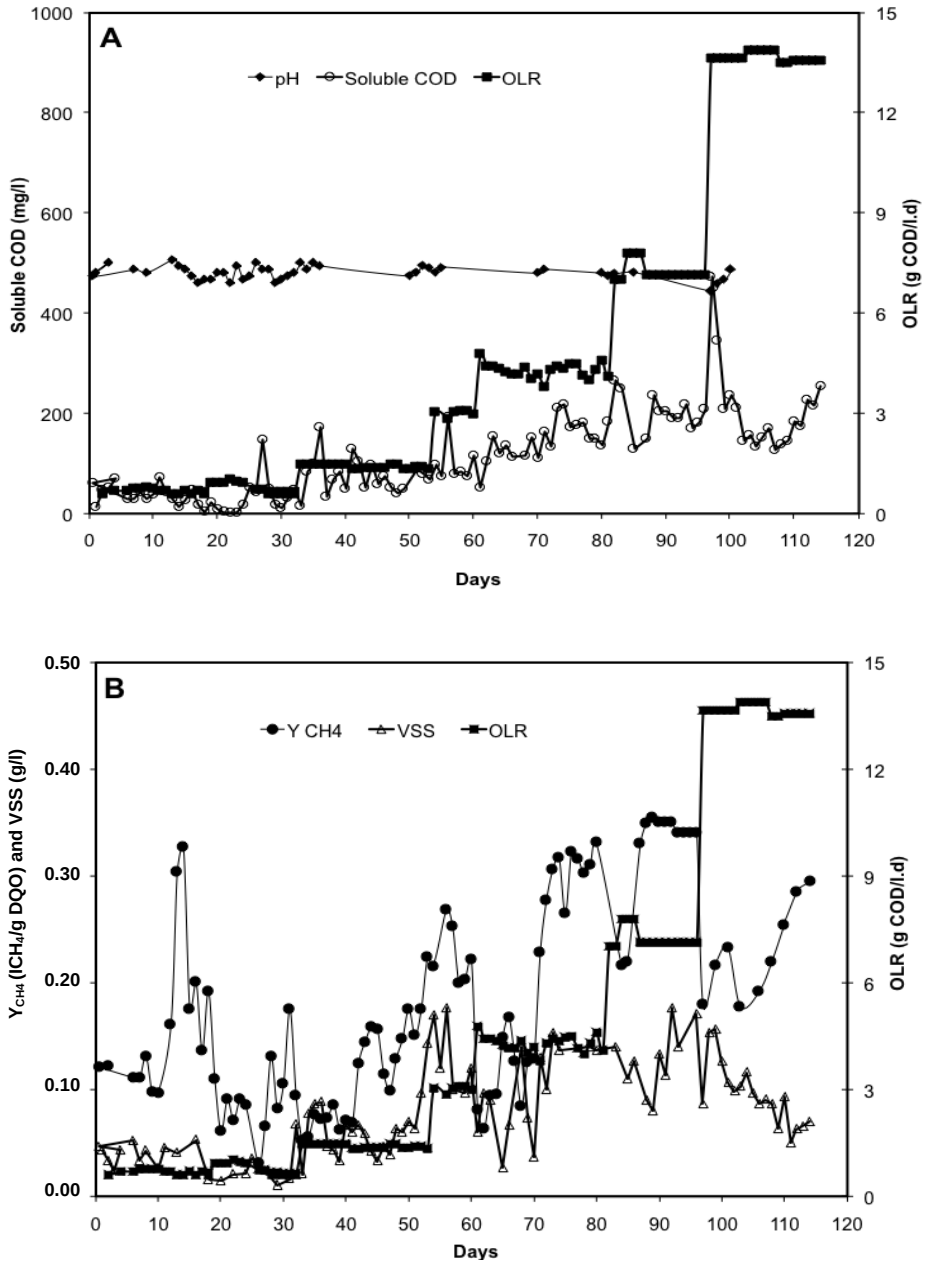

Figure 7. (A) Time course of pH, COD and OLR; (B) time course of methane yield (Y_{CH4}), VSS and OLR, with the Extendosphere™ support during steady-state phase.

metabolism was dedicated completely to CH_4 production. This occurrence was associated with a decrease in the VSS concentration to a value of 0.053 g/l (Figure 7B). However, support surface colonization (100%) and excellent biofilm maturation were observed using the Extendosphere™ support material (Figure 6D). Indeed, after 115 days, attached biomass reached was of 0.21 g VS/g support. Similar results were reported by Garcia-Calderon et al. (1998) in IFBR and by Buffière et al. (2000) in an inversed turbulent bed reactor (ITBR). Each time OLR was raised, especially between days 60 and 70, as well as days 95 to 110, Y_{CH4} and COD removal rate exhibited the same behaviour profile; an initial reduction corresponding to an adaptation period, followed by an increase to a constant value when the balance was reached between biomass growth and methane production. This pattern was observed until reaching the maximum Y_{CH4} value (0.35 l CH_4/g COD), when the carbon flux was used for anaerobic respiration rather than biopolymer synthesis. During this period, pH and soluble COD do not present significant variations (Figure 7A), an outcome of the non-accumulation of volatile fatty

acid (VFA) and COD removal efficiency of over 90%. This behaviour was similar in the polyethylene support assay. Considering that observed specific activity reached maximum levels, biofilm growth was apparently the best treatment option for organic overload in the studied wastewater.

With Extendosphere™ support, Y_{CH4} reached was of 0.298 l CH_4/g COD at 35°C when OLR was raised to 14 g COD/l.d. Similar result was obtained when using the polyethylene support to 10 g COD/l.d, since steady state for a colonized support corresponds to equilibrium between biofilm growth and detachment by hydrodynamic forces such as shear (Michaud et al., 2003, 2005). Optimum methane yield can be used as a parameter to compare the capacity of different supports to fix biomass and generate biofilm thickness at similar OLRs. Furthermore, constant Y_{CH4} values measured under steady state conditions could also function as an indicator of biofilm detachment rate. Overall, Extendosphere™ support promoted more desirable biofilm behaviour than the polyethylene support. Of the two carrier materials, surface colonization and biofilm growth reached complete levels only on the Extendosphere™, allowing for greater OLR removal. Variation in surface colonization versus support depth was observed, but the generally excellent biofilm adhesion levels with Extendosphere™ resulted only in differences in biofilm thickness; thinner at the top and thicker at the bottom (Sowmeyan and Swaminathan, 2008b).

As described above, the complementary analyses of Y_{CH4} (biofilm growth and detachment rate, and metabolism data) and microscopic observation (support colonization and biofilm thickness data) proved an effective biofilm monitoring method in IFBR. For example, support biomass adherence generates data on mass loading rate, but no other parameter is measured, whereas the surface or volumetric loading data collected here are more informative for IFBR. In fact, the basic principles of this technology are based on a particle density < 1, which allows support flotation and liquid down flow without particle loss. Support density in the present study (polyethylene = 926 kg/m^3; Extendosphere™ = 700 kg/m^3) changed as biofilm thickness increased. This change in density was calculated using support diameter and apparent density, and biofilm width. The polyethylene (d = 380 μm) reached a 1 kg/m^3 density when biofilm thickness was 80 μm, while the Extendosphere™ (d = 147 μm) reached this density when the biofilm was 260 μm thick. Loss of support material due to sedimentation can occur under these conditions. When polyethylene supports of different densities are used (380 and 4000 μm), particle diameter becomes the principal variable affecting changes in density, since larger particle diameter allows for greater biofilm thickness. Therefore, measuring biofilm thickness is more informative than biomass quantity when sampling particles. Using microscopic observation, horizontal and vertical diameter can be easily measured, particle diameter subtracted, and biofilm width and apparent density calculated. For instance, Extendosphere™ particle diameter was 147 μm, meaning average biofilm thickness could be calculated as 95 μm with no anticipated particle sedimentation (Arnaiz et al., 2003).

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REFERENCES

Alvarado-Lassman A, Rustrian E, Garcia-Alvarado MA, Rodriguez-Jimenez GC, Houbron E (2008). Brewery wastewater treatment using anaerobic inverse fluidized bed reactors. Bioresource Technol. 99:3009–3015

Alvarado-Lassman A, Sandoval-Ramos A, Flores-Altamirano MG, Vallejo-Cantu NA, Mendez-Contreras J (2010). Strategies for the startup of methanogenic inverse fluidized-bed reactors using colonized particles. Water Environ. Res. 82:387-391.

APHA (American Public Health Association, American Water Works Association, Water Pollution Control Federation) (1995). Standard Methods for the Examination of Water and Wastewater. 19th ed. Washington, DC.

Arnaiz C, Buffiere P, Elmaleh S, Lebrato J, Moleta R (2003). Anaerobic digestion of diary wastewater by inverse fluidization: the inverse fluidized bed and the inverse turbulent bed reactors. Environ. Technol. 24:1431-1443.

Buffière P, Bergeon JP, Moletta R (2000). The inverse turbulent bed: a novel bioreactor for anaerobic treatment. Water Res. 34:673-677.

Cresson R, Escudié R, Carrère H, Delgenès JP (2007). Influence of hydrodynamic conditions on the start-up of methanogenic inverse turbulent bed reactors. Water Res. 41:603-612.

Garcia-Calderon D, Buffièrre P, Moletta R, Elmaleh S (1998). Anaerobic digestion of wine distillery wastewater in down-flow fluidized bed. Water Res. 32:3593-3600.

García-Encina PA, Hidalgo MD (2005). Influence of substrate feed patterns on biofilm development in anaerobic fluidized bed reactors (AFBR). Process Biochem. 40:2509-2516.

Hidalgo MD, García-Encina PA (2002). Biofilm development and bed segregation in a methanogenic fluidized bed reactor. Water Res. 36:3083-3091.

Michaud S, Bernet N, Buffière P, Delgenès JP (2005). Use of the methane yield to indicate the metabolic behaviour of methanogenic biofilms. Process Biochem. 40:2751-2755.

Michaud S, Bernet N, Buffière P, Moletta R (2003). Influence of Hydrodynamic Conditions on Biofilm Behavior in a Methanogenic Inverse Turbulent Bed Reactor. Biotechnol. Prog. 19:858-863.

Michaud S, Bernet N, Buffière P, Roustan M, Moletta R (2002). Methane yield as a monitoring parameter for the start-up of anaerobic fixed film reactor. Water Res. 36:1385-1391.

Sowmeyan R, Swaminathan G (2008a). Evaluation of inverse anaerobic fluidized bed reactor for treating high strength organic wastewater. Bioresour. Technol. 99:3377-3380.

Sowmeyan R, Swaminathan G (2008b). Performance of inverse anaerobic fluidized bed reactor for treating high strength organic wastewater during start-up phase. Bioresour. Technol. 99:6280-6284.

Zellner G, Macario AJL, de Macario EC (1997). A study of three anaerobic methanogenic bioreactors reveals that syntrophs are diverse and different from reference organisms. FEMS Microbiol. Ecol. 22:295-301.