Start up of an anaerobic inverse turbulent bed reactor fed with wine distillery wastewater using pre-colonised bioparticles

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Abstract The long start-up period of fluidized bed biofilm reactors is a serious obstacle for their wide installation in the anaerobic treatment of industrial wastewater. This paper presents the results of an anaerobic inverse turbulent bioreactor treating distillery wastewater during 117 days of operation at a laboratory scale. The pre-colonized bioparticles for this work were obtained from a similar reactor processing the same wastewater and which had a start-up period of 3 months. The system attained carbon removal efficiency rates between 70 and 92%, at an organic loading rate of 30.6 kg m^{-3} d^{-1} (chemical oxygen demand) with a hydraulic retention time of 11.1 h. The results obtained showed that the start-up period of this kind of reactors can be reduced by 3 using pre-colonized bioparticles.

Keywords Anaerobic process; biofilm; inverse turbulent bed; phospholipid analysis; reactor start-up

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Introduction

Inverse fluidized beds present interesting features: (a) the down-flow configuration enables overcoated particles to be recovered in the bottom of the bed, (b) the liquid and the produced biogas are flowing in opposite directions, which helps for bed expansion (Garcia-Calderon *et al.*, 1998). In this kind of reactor, floating particles are fluidized by a down-flow current of liquid.

Expansion of a carrier with a specific density lower than the liquid is also possible under an up-flow current of gas, resulting in a new reactor configuration: the inverse turbulent bed. The gas bubbles generate downward liquid motions and apparent bed expansion. This phenomenon (called pseudo-fluidization) has been identified by several researchers (Legile *et al.*, 1988; Comte *et al.*, 1997). If the density of the particles remains smaller than but close to that of the liquid, the fluidization can be achieved with only an upward gas flow, counter to the liquid flow (Roustan *et al.*, 1995).

Previous studies on inverse turbulent bed reactors show several advantages compared to the inverse fluidized bed reactors. First, the bottom of the reactor can be used as a settler for recovering the sludge or the overcoated particles. Second, a gas injection is simpler than a liquid recycling, reducing clogging problems. Third, the low energy requirement, because of the low fluidization velocities required (Buffiere and Moletta, 1999; Buffiere *et al.*, 2000; Arnaiz *et al.*, 2003). In spite of these advantages, the long start-up period is a serious obstacle for their wide installation in the anaerobic treatment of industrial wastewater. This is attributed to the relatively strong hydrodynamic conditions in the reactor, which interfere with biomass adhesion during the start-up period.

This work describes the starting-up, using pre-colonized bioparticles, of an anaerobic inverse turbulent bed processing wine distillery wastewater, as a strategy for reducing the

reactor start-up period. In this reactor, a granular floating solid is expanded by an up-flow current of gas.

Methods

Laboratory-scale reactors

The experimental set-up is shown in Figure 1. The reactor consisted of a PVC tubular section of 0.08 m internal diameter and 1 m height with a conic bottom, with a total volume of 5 L. The system was equipped with a water jacket keeping the liquid temperature at $35^{\circ}C \pm 2$. The biogas production was measured by a gas flow meter (Sho-Rate^{TM}). Effluent was discharged through a port on the lower part of the column, connected to an outlet tube that kept the liquid level in the reactor. Recycling of the biogas was ensured by a peristaltic pump (Masterflex Cole Parmer).

Influent composition

The substrate used in this work was an industrial wine distillery wastewater with a mixture of trace elements and nitrogen source. The total organic carbon concentration (TOC) was 8–12 kg m^{-3}, equivalent to a chemical oxygen demand (COD) of 20–30 kg m^{-3}.

Reactor performance

The reactor was filled with pre-colonized bioparticles up to 40% of its active volume (working volume) and at a gas velocity for being expanded of 1.5 m h^{-1}. Influent was kept in a refrigerator to avoid fermentation and it was constantly agitated by a magnetic stirrer to ensure homogenization. The reactor was monitored for temperature, flow rate, pH and biogas production. Biogas composition, total suspended solids (TSS), volatile suspended solids (VSS), suspended lipid phosphate (SLP), volatile attached solids to the carrier (VAS), attached lipid phosphate to the carrier (ALP), volatile fatty acids (VFA) and TOC were

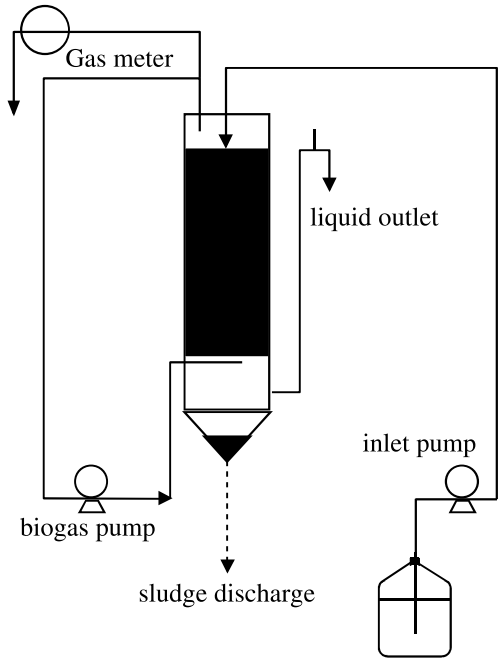

Figure 1 Experimental anaerobic inverse turbulent bioreactor

routinely analyzed. Organic loading rate (OLR) was increased by reducing hydraulic retention time (HRT) while maintaining constant the COD concentration in the influent. HRT, based on expanded bed volume, was fixed at 22.8 h and it was reduced stepwise to 11.1 h at the end of the study.

Pre-colonized bioparticles

The pre-colonized bioparticles (biocovered Extendosphere^{TM}) for this work were obtained from a similar reactor processing the same wastewater and for which the start-up period was 3 months. At the end of the start-up period, the OLR reached by that previous reactor was 23.1 $kg_{COD}\ m^3\ d^{-1}$ with a HRT of 15.4 h and a COD removal of 83%. The amount of attached biomass within that previous reactor was 48.3 $mg_{VAS}\ mL^{-1}_{CARRIER}$ and 165.3 $nmol_{ALP}\ mL^{-1}_{CARRIER}$.

Measurements and analysis

VFA and TOC of the discharged effluent and biogas composition were determined daily through off-line analysis. Liquid samples were centrifuged at 10,000 rpm for 10 min before analysis to remove suspended solids. VFA analysis was done using a gas chromatograph with a flame ionization detector (Chrompack CP 9000), nitrogen being the carrier gas (335 kPa). TOC was titrated by UV oxidation with a Dohrman DC 80 apparatus. Gas was analyzed by gas chromatography with a Shimadzu GC-8A apparatus with argon carrier (3 bar) using a catharometer detector (90 mA). pH was measured with a Mettler Toledo 1100 Calimatic pH meter.

Biomass determination

TSS and VSS in the effluent were measured according to *Standard Methods* (APHA-AWA-WPCF, 1992). Biofilm development within the reactor was measured by determining VAS on washed samples of bioparticles. The procedure used in this study in order to determine SLP and ALP was a modification of that by Findlay *et al.* (1989). Bioparticles were observed with an optical microscope Olympus BX 60 and a 2 mm Leitz-Wetzlar graduated slide with 0.01 mm intervals.

Results and discussion

Reactor performance

The reactor was initially operated at an OLR of 15.8 $kg_{COD}\ m^3\ d^{-1}$ and a HRT of 22.8 h. After 31 days of operation, OLR achieved by the reactor was 23,1 $kg_{COD}\ m^3\ d^{-1}$ with a HRT of 15.6 h. COD removal ranged during the start-up from 78 to 87% (Figure 2a). These operational conditions were reached by the reactor started-up with virgin support after 88 days of operation (Figure 2b; quoted in Buffiere and Moletta, 1999).

After the start-up period, the input OLR was fixed at 11.0 $kg_{COD}\ m^3\ d^{-1}$ (HRT of 29.2 h) and it was increased stepwise to 28.2 $kg_{COD}\ m^3\ d^{-1}$ (HRT of 11.8 h) in 28 days (from day 37 to day 65). It can be seen from Figure 2a that the system was not affected and COD removal was always between 83 and 92%.

From day 75 up to the end of this study, the mode of operation was changed in order to verify the stability of the system facing disturbances. The input OLR was dropped down from 27.2 $kg_{COD}\ m^3\ d^{-1}$ (HRT of 11.7 h) to an average of 16.4 $kg_{COD}\ m^3\ d^{-1}$ (HRT of 19.3 h) since day 75 to day 90. After this period, the input OLR was roughly increased to an average of 28.1 $kg_{COD}\ m^3\ d^{-1}$ (HRT of 11.8 h) since day 91 to day 117. It can be seen from Figure 2a that the system was partly affected. Nevertheless, COD removal was always between 70 and 84%.

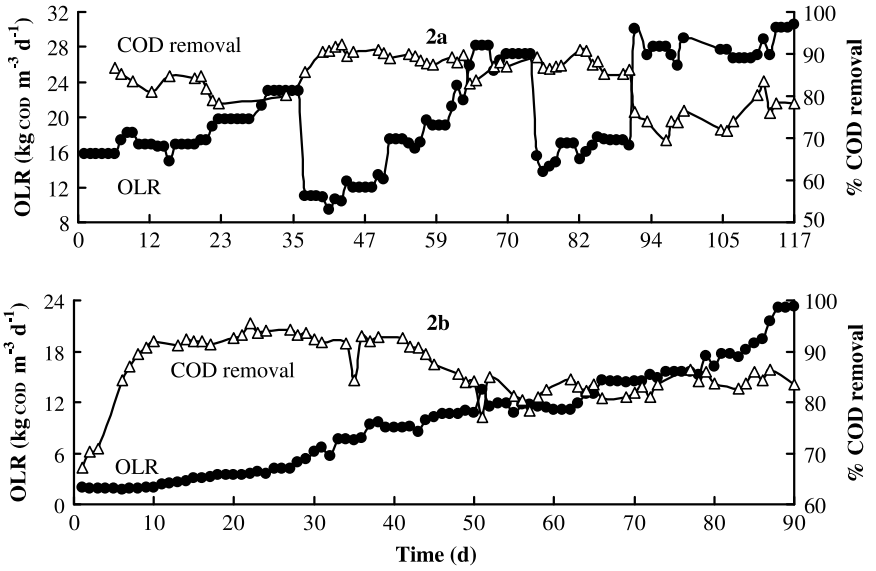

Figure 2 Performance of two anaerobic fluidised bed reactors treating wine distillery wastewater. (a) Start-up with pre-colonized bioparticles. (b) Start-up with virgin support

A comparison with other reactors can be done. The initial start-up period obtained in this study working with an inverse turbulent bed bioreactor was better than those obtained in some fluidized bed treating the same wastewater. The initial start-up is lower than the observed with a classical up-flow fluidized bed working with 385 μm pozzolana particles (Buffiere *et al.*, 1995). In that work, the OLR was increased from 2 to 18 $kg_{COD}\ m^3\ d^{-1}$ in 70 days and the carbon removal ranged between 75 and 92%. In an inverse fluidized bed with a down-flow liquid fluidization using perlite as biomass carrier, the OLR was increased from 3 to 15 $kg_{COD}\ m^3\ d^{-1}$ in 60 days, but the reactor was destabilized and the carbon removal was only 55% at the end of the experiment and the input load had to be decreased (Garcia-Calderon *et al.*, 1998). In an inverse turbulent bed with an up-flow current of gas using Extendosphere™ as biomass carrier, the OLR was increased from 2 to 23.1 $kg_{COD}\ m^3\ d^{-1}$ over the three first months of operation (Buffiere and Moletta, 1999).

The results obtained in this work showed that, using pre-colonized Extendosphere™, the start-up period of an anaerobic inverse turbulent bioreactor could be reduced by a third.

Biomass analysis

In this study, total biomass amount is measured in terms of volatile solids concentration and lipid-phosphate concentration. The main disadvantage of the first method is that it estimation includes not only active microorganisms, but also inert mass, exopolymers and absorbed organic matter on flocs and biofilms. Phospholipids, present on bacterial membrane up to 90–98%, do not form part of cell reserves and are easily degraded during bacteria lysis (White *et al.*, 1979). Therefore, their estimation only includes living biomass.

Initial biomass concentration of pre-colonized bioparticles used in this study were 48.3 $mg_{VAS}\ mL^{-1}_{CARRIER}$ and 165.3 $nmol_{ALP}\ mL^{-1}_{CARRIER}$. At the end of the start-up period, final biomass concentration was 23.4 $mg_{VAS}\ mL^{-1}_{CARRIER}$ and 30.7 $nmol_{ALP}\ mL^{-1}_{CARRIER}$. Microscopic observations confirmed that strong loss of biomass (Figure 3).

It means, comparing with the reactor started-up with virgin support, that a biomass concentration reduced by half in terms of VAS treated almost the same organic matter. That

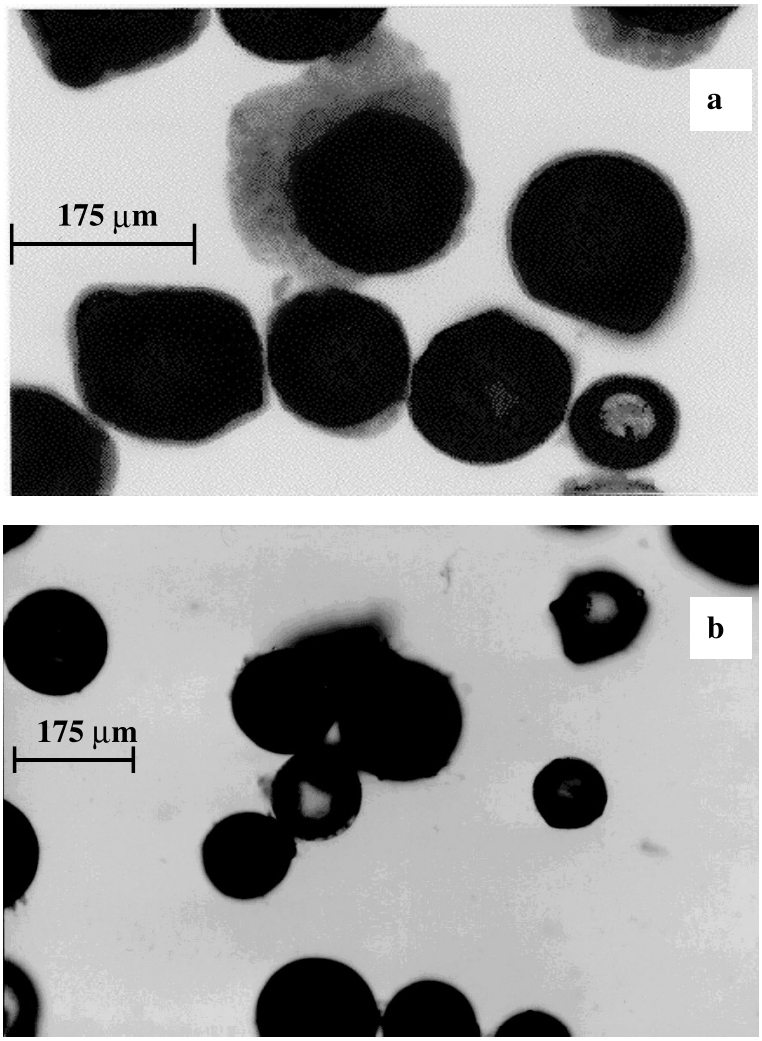

Figure 3 Biofilm coverage of the carrier material. (a) Start-up period. (b) End of the study

increase in the biomass activity cannot be explained by a greater ratio of cells in the biofilm matrix, since ALP also decreased significantly, but by a change in biofilm composition. This strong selection is not uncommon in reactors fed with industrial wastewater, in which very specific substrata could select very specific groups of bacteria, progressively more active (Arnaiz *et al.*, 2003).

Conclusions

The results obtained in this study showed that the start-up period of an inverse turbulent bed reactor can be reduced by a third using pre-colonized bioparticles. The biofilm at the end of the study was very active and specialized, which can explain the stability of the reactor facing disturbances.

The inverse turbulent bed reactor appeared to be a good option for anaerobic treatment of wine distillery wastewater. The systems attained high OLR with good COD removal rates and it exhibited a good stability to the variations in OLR and HRT.

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