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Biofilm technology: from support design to reactor operation

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Abstract

The aim of this work was to assess the feasibility of a Sequential Batch Biofilm Reactor (SBBR) to perform carbon and nitrogen removal: from support design to reactor operation. The experimental part was conducted in two phases. In the first phase, different supports were tested to select the most suitable one for SBBR operation. In the second phase, the most appropriate support was used in a SBBR to perform carbon and nitrogen removal. The results demonstrate that the support with the highest internal surface area presented a higher biomass accumulation. Time profiles of nitrogen ions and acetate concentration showed the typical behaviour of a SBBR performing carbon and nitrogen removal. Poly-β-hydroxybutyrate (PHB) was formed immediately after acetate depletion and was subsequently consumed for biomass growth, owing to the high oxygen concentration in the reactor.

1. Introduction

The Sequential Batch Reactor (SBR) has been used to remove organic carbon and nutrients from wastewater in one single unit under properly controlled conditions (Brito et al., 1997; Rodrigues et al., 2001). The SBR can be combined with biofilm growth on the surface of a support material, originating the Sequencing Batch Biofilm Reactor (SBBR). In SBBR systems high concentrations of biomass can be maintained independently of the sedimentation characteristics of the biological aggregates and the hydraulic retention time of the reactor. SBBRs are particularly suitable when the required microbial population grow very slowly or when the biomass yield is low (Vieira et al., 2008).

Microorganisms in SBRs are exposed to continuous periodic environmental changes, namely varying liquid volumes and substrate concentrations (donors and receivers of electrons). When confronted with such interchanging periods of high ("feast period") and low ("famine period") substrate concentrations, bacterial populations adopt specific survival strategies. In particular, they often accumulate and set aside organic carbon as internal polymers such as poly-β-hydroxybutyrate (PHB). While such storage phenomena are well studied for suspended biomass systems, as for example SBRs, they are still poorly documented in literature for systems using biofilms, such as the SBBRs (Alves et al., 2004). Nevertheless, they do play an important role in the optimization of operating strategies, which holds particularly true for multiple and interlinked degradation pathways, such as the nitrogen and carbon removal by biological nitrification and denitrification. Therefore, the aim of this work was to assess the feasibility of a SBBR to perform carbon and nitrogen removal: from support design to reactor operation.

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2. Materials and methods

2.1. Experimental set-up

In the first phase of the experimental work, biomass adhesion and biofilm formation was evaluated in a new developed thermoplastic support (*DupUM*) and in two supports currently available on the market (*Biolox10* and *Bioflow30*) (Table 1). Three SBBRs with working volumes of 2.5 L filled with different supports were operated in parallel. The bed formed by the supports occupied 47 % of the reactors volume. The reactors were operated with a constant cycle time of 6.2 h, a volume exchange ratio of 0.8 L/L and a resulting hydraulic retention time (HRT) of 7.75 h. The duration of the individual operating phases was: 102 min mixed fill, 150 min aerated, 114 min settle and 10 min draw. During the aerated phase airflow was applied through membrane diffusers, causing the reactor contents including the carrier bed to circulate. In the first operation period (days 0 – 127), a synthetic wastewater with acetate as the only carbon source and ammonium as nitrogen source was used. The carbon and nitrogen ratio (C/N) used was 6.25. At day 127, the carbon and nitrogen concentration in the system was increased from 500 mg/L and 80 mg/L to 750 mg/L and 120 mg/L, respectively. The SBBRs were inoculated with biomass from a nitrification/denitrification unit treating effluent from a brewery industry.

Table 1. Characteristics of supports.

Support	Material	Dimensions (mm)	Nº pieces/m^3	Specific surface area (m^2/m^3)
 *DupUM*	PE [a]	height: 10.0 Diameter: 17.0	324 900	407
 *Bioflow30*	PP [b] Recycled	height: 30.0 Diameter: 32.0	21 910	320
 *Biolox10*	PE Recycled	height: 10.0 diameter: 9.5	538 922	640

a) Polyethylene; b) Polypropylene

In the second phase, a SBBR with a working volume of 28 L was operated with a constant cycle time of 5 h, a volume exchange ratio of 0.36 $L \cdot L^{-1}$ and a HRT of 14 h. The duration of the individual operating phases was: 115 min mixed fill, 165 min aerated and 20 min draw. The biofilm was formed on the support selected in the previous phase of the experimental work. The SBBR was operated with synthetic water. The composition of the synthetic substrate solution was: 643 $mg \cdot L^{-1}$ $NaCH_3COO \cdot 3H_2O$, 130 $mg \cdot L^{-1}$ NH_4Cl, 210 $mg \cdot L^{-1}$ $NaHCO_3$, 44 $mg \cdot L^{-1}$ KH_2PO_4, and 1 $mL \cdot L^{-1}$ of a trace element solution in accordance with Vishniac and Santer (1957). The reactor was inoculated with the selected supports coming from the previous phase.

2.2. Analytical methods

The thermodynamic characterization of the interaction support material-biomass was evaluated using contact angle measurements. Contact angles were measured using sessile drop method at 20ºC with distilled water, formamide and 1-bromonaphthalene.The measurements were carried out in a standard contact angle apparatus (Kruss-GmH, Hamburg).

Grab samples were taken and analyzed for ammonium, nitrite, nitrate and chemical oxygen demand (COD) according to *Standard Methods*. The biofilm accumulation on supports was

estimated as dry weight measurements. PHB content of suspended biomass and biofilm (external and internal) was measured by gas chromatography (GC) using the method developed by Smolders et al. (*1994*).

2.3. Calculations

The Extended Derjaguin-Landau-Verwey-Overbeek theory (XDLVO) was used to assess the affinity of the individual support materials to biomass adhesion (van Oss, 1989).

Mass balance calculations were based on an assumed elemental composition of $CH_{1.8}O_{0.5}N_{0.2}$ for 1 Cmol biomass (Beun et al., 2002). The carbon consumption of hereotrophic growth was calculated based on the observed ammonium consumption and a biomass formation rate per mole of acetic acid of 0.40 Cmol·Cmol^{-1} (Beun et al., 2002). Stoichiometric considerations for energy production lead to the following rates of acetate consumption with respect to nitrate, nitrite, and oxygen: 1.25 Cmol acetate·mol^{-1} NO_3^-, 0.5 Cmol acetate· mol^{-1} NO_2^- and 1 Cmol acetate·mol^{-1} O_2, respectively. It was assumed that nitrification is performed by ammonium and nitrite oxidizing bacteria with a biomass formation yield of 0.057 Cmol mol^{-1} NH_4^+ and 0.034 Cmol mol^{-1} NO_2^-, respectively (Henze et al., 1995).

3. Results and Discussion

3.1. Supports surface properties

The free energy of adhesion between the biomass and the support surface immersed in water was calculated to foresee the biofilm adhesion on the supports. According to the results obtained, the surface properties of *Biolox10* and *DupUM* (with values of ΔG of -34.60 mJ.m^{-2} and -30.16 mJ.m^{-2}, respectively) are more favourable to biomass adhesion than the one of *Bioflow30* (-4.04 mJ.m^{-2}) due to their lower free energy of adhesion (Salerno et al., 2004).

3.2. Biofilm formation in supports

The biofilm accumulation profile expressed as dry weight per specific surface area is presented in figure 1.

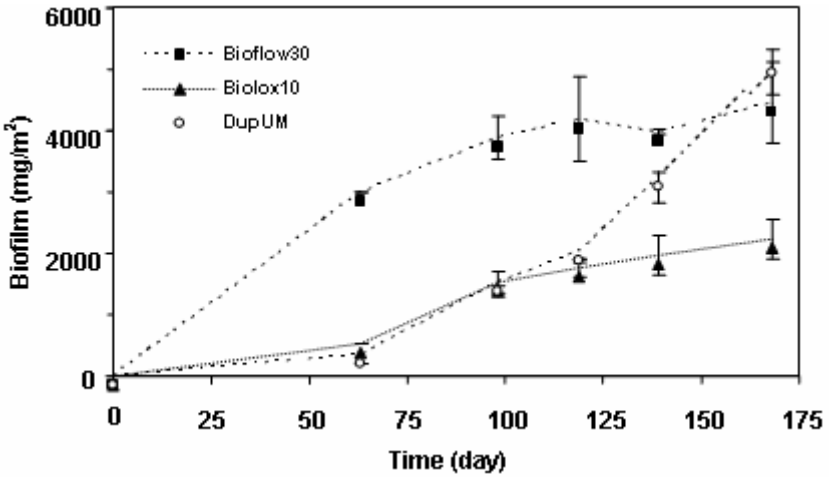

Figure 1. Biofilm accumulation on supports surfaces expressed as dry weight per specific surface area.

Biolow10 and *DupUM* displayed a very slow biofilm growth, whereas *Bioflow30* presented an initial biofilm growth about six times higher. This may be related to the different detachment forces in the reactors as a result of the support geometry. For the same fraction of support

(47 %) and as a result of the different dimension of the supports, the number of support pieces was considerably different in each reactor. This difference is higher for *Biolox30*, where there were only 26 support pieces in the reactor, which is much less than the number of pieces in the reactors with *Biolox10* and *DupUM* (around 633 pieces and 381 pieces, respectively). Hence, the occurrence of collisions and their intensity are expected to be different in the reactors. Probably, the abrasion forces which result from the collisions caused by the high number of pieces of *Biolox10* and *DupUM* in the respective reactors have conditioned initially the biofilm formation on the respective supports.

From day 127, the concentration of carbon and nitrogen in the reactors was increased. Biofilm accumulation on *Bioflow30* and *Biolox10* did not change significantly compared to the increase of biofilm mass observed on *DupUM*. These results have shown that biofilm accumulation on *DupUM* was limited by the amount of available substrate. After 168 days, *DupUM* presented a higher biomass accumulation per unit of surface area (5096 $mg.m^{-2}$) than *Biolox10* and *Bioflow30* (2231 $mg.m^{-2}$ and 4454 $mg.m^{-2}$, respectively). Based on these experimental results *DupUM* was selected for the next phase of the experimental work.

The thermodynamic approach did not allow us to foresee the biofilm formation on the supports. The results obtained through the thermodynamic characterization indicated that *Bioflox10* and *DupUM* had more favourable surface properties to the initial adhesion of biomass than *Bioflow30.* However, after 120 days of reactors operation, *Bioflow30* showed higher biofilm accumulation than the other supports. According to Gjaltema et al. (1997), in airlift reactors the biofilm adhesion and formation is dominated by the reactor hydrodynamic conditions and by collisions among particles. The results from biomass adhesion and biofilm formation studies obtained in the present work suggest that the hydrodynamic conditions established in the reactors and the geometry of the supports played a crucial role in biofilm formation. Biofilm growth was favoured in the supports that presented a higher internal surface area that protected biofilms from erosion and abrasion detachment mechanisms.

3.3. Reactor performance

Figure 2 depicts profiles of nitrogen ions, acetate as COD and dissolved oxygen (DO), during a typical SBBR cycle.

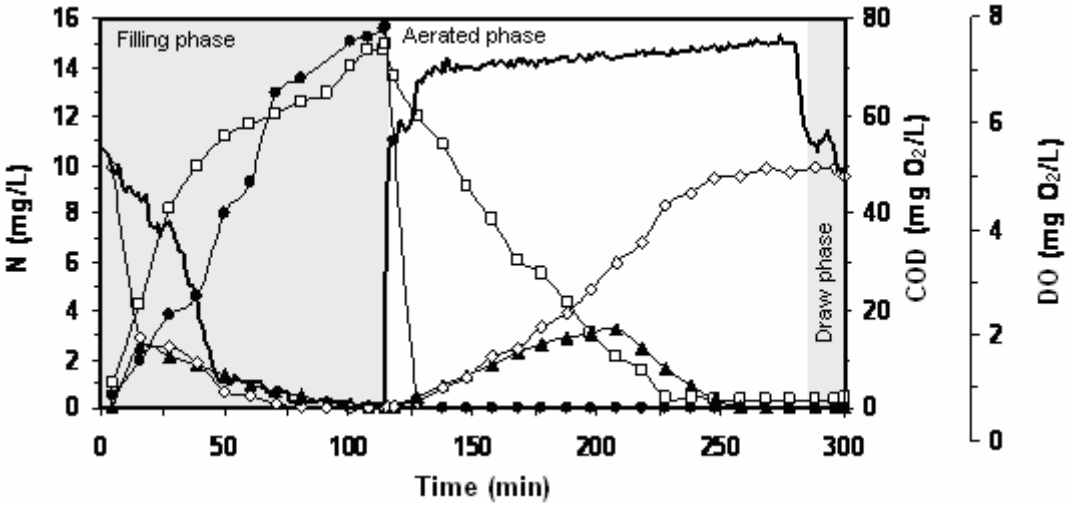

Figure 2. COD (●), DO (-), ammonium (□), nitrite (▲) and nitrate (◊) profiles during a SBBR cycle.

During the mix fill phase, the nitrate left over from the previous cycle was completely denitrified with acetate, so that no nitrite accumulation was observed. Time profiles of ammonium, nitrite and nitrate concentration in the aerated phase showed the typical behaviour of nitrification reactions, via nitrite formation and subsequent oxidation to nitrate. A

nitrogen balance of this phase showed that 66 % of ammonium supplied was oxidized to nitrate with the remainder being used for biomass growth.

According to these results, 46 % of carbon supplied was consumed during the mixed fill phase with the remainder being consumed in the aerated phase. Based on mass balances in the fill phase, it was possible to estimate that 15 % of carbon was used to the growth of biomass, 25 % was used as a source of energy and the remaining 60 % were removed by unknown mechanisms. The quantification of PHB in the biomass has allowed complementing the balance of carbon during the operation cycle illustrated in figure 2. Figure 3 presents schematically the main carbon fluxes in the SBBR.

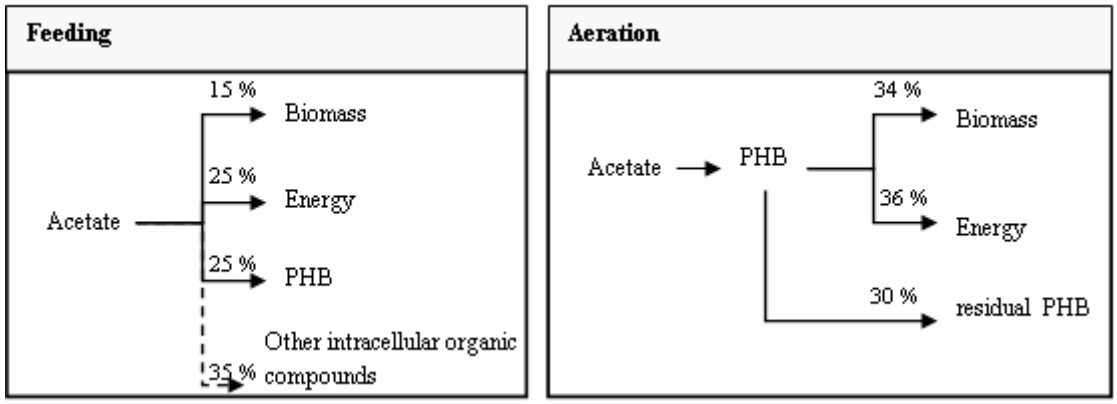

Figure 3. Fate of carbon added as acetate in the feeding and the aeration phase of the SBBR.

As illustrated, the carbon portion missing at the end of the feeding phase was stored as PHB (25 %), while the remaining 35 % were removed by another mechanism such as conversion to intracellular intermediary composts of a low molecular weight or storage as another PHA, besides PHB (Dionisi et al., 2001).

The acetate present at the beginning of the aeration phase is quickly converted and stored in the biomass as PHB. After acetate depletion, a part of the PHB (70 %) is consumed as a source of carbon and energy, while the rest is stored in the biomass (30 %). According to the reactions' stoichiometry, the organic carbon transformed into PHB during the aeration phase would be sufficient to denitrify the entire nitrate previously produced. This did not occur, however, probably due to the high concentration of oxygen dissolved in the liquid. Instead the PHB was consumed in the production of biomass and in energy needs.

Suspended biomass was revealed to play a significant role in the carbon removal mechanism: about 79 % of the total PHB formed was stored in the suspended biomass, although this biomass represented only 20 % of the total biomass present in the reactor. This important result might be attributed to the higher accessibility of suspend biomass to acetate, due to existence of lower mass transfer limitation.

4. Conclusions

Biomass adhesion and biofilm formation studies suggest that the hydrodynamic conditions established in the reactors and the geometry of the supports played a crucial role in biofilm formation. Biofilm growth was favoured in the supports that presented a higher internal surface area that protected biofilms from erosion and abrasion detachment mechanisms.

According to the operation phase of the SBBR, there are two relevant mechanisms in the removal of acetate in the SBBR: (1) use of the acetate simultaneously with the growth of biomass and in the metabolism of PHB storage (feeding phase); and (2) direct storage of the acetate as PHB which is later on used in the growth of biomass (aeration phase). The consumption of acetate and its storage as PHB is mostly carried out by the biomass in suspension. Although the biomass in suspension represented only 20 % of the total biomass

present in the reactor, 79 % of the total PHB formed was stored in the biomass in suspension. This result is due to the lower mass limitations of the biomass in suspension in comparison with the biofilm.

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