Effects of the applied organic loading rate on the selection of a PHA-storing biomass in a Sequencing Batch Reactor with uncoupled Carbon and Nitrogen feeding

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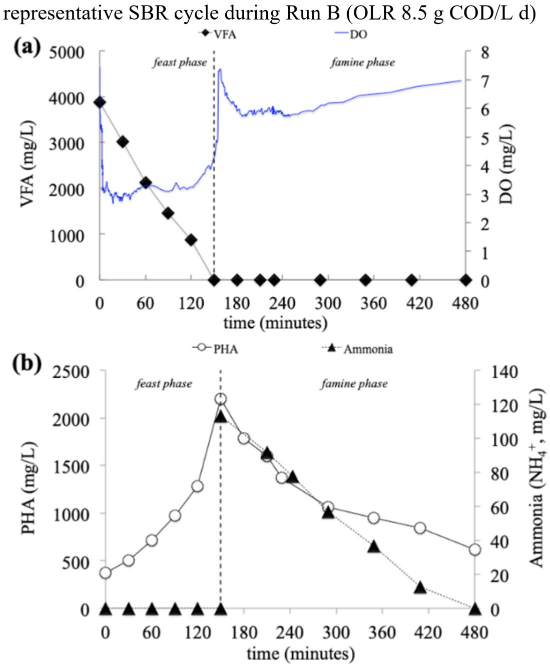
Polyhydroxyalkanoates (PHA) are polyesters of hydroxyalkanoic acids, naturally produced as storage carbon source by different species of PHA-producing microorganisms. They are completely biodegradable and can be produced from renewable resources and waste material, showing thermoplastic properties comparable with traditional plastics. In this sense, mixed microbial cultures (MMCs) were proposed as a cost effective method to produce PHA from renewable resources (i.e. activated sludge and organic wastes) through the selection of PHA-storing microorganisms, obtained applying alternate dynamic feeding conditions (Valentino et al. 2018). High selective pressure for the PHA-storing bacteria in activated sludge is obtained by setting periodic alternating feast (carbon feeding) and famine (absence of carbon sources) conditions. A sequencing batch reactor (SBR) is generally used for the selection of the PHA-accumulating biomass, because it is easy to apply the required dynamic feeding strategy. The following step is the PHA production, usually conducted in an accumulation batch reactor inoculated with the selected PHA-producing biomass. Renewable and fermentable feedstocks may contain varying levels of nutrients (nitrogen and phosphorus) and it has been demonstrated that nitrogen limitation during the accumulation step can substantially increase the production performances in terms of PHA final content. In previous studies (Silva et al. 2016), nitrogen and carbon feeding have been uncoupled, stimulating PHA storage response during the feast phase (in absence of nitrogen) and microbial growth on stored polymer in the famine phase (by adding nitrogen). The impact of nitrogen feeding regulation on the process has been evaluated in a lab-scale SBR with a cycle length of 6h under an applied organic load rate (OLR) equal to 8.5 g COD/L d. Two SBR runs were performed with the carbon source fed at the beginning of the SBR cycle simultaneously to the nitrogen source (coupled feeding strategy) or with the latter fed at the end of the feast phase (uncoupled feeding strategy). As a main result, it was found that PHA content at the end of the feast phase was doubled in the uncoupled feeding case. According to these preliminary results, in the present study the effect of the applied OLR has been investigated maintaining the uncoupled carbon (C) and nitrogen (N) feeding strategy, with a fixed C/N ratio. More in detail, the SBR has been operated with a 12 h cycle length and at three OLRs (A 4.25, B 8.5 and C 12.75 COD/L d), by using a synthetic mixture of acetic and propionic acids as carbon feeding solution.

Figure 1 Typical profiles of volatile fatty acids (VFA) and dissolved oxygen (DO) (a); ammonia and PHA (b) in a representative SBR cycle during Run B (OLR 8.5 g COD/L d)



The trend of the main parameters during a typical SBR cycle was reported in the following figure 1, for Run B (8.5 gCOD/L d). The profiles of VFA, dissolved oxygen (DO), PHA and ammonia in a typical cycle are reported. As long as VFA were available (feast phase) they were consumed along with oxygen; this led to a decrease of DO level (roughly from 8.0 to 5.0 mg/L) in the culture medium. The exhaustion of the C-source corresponded to the end of the feast phase, when the DO concentration showed a fast inversion of its trend, up to values similar to those recorded at the beginning of the cycle. In this case the PHA concentration achieved higher value, according to the higher applied OLR; at the end of the feast phase, PHA was 2199 mg/L, more than double with respect to the value measured in Run A. At the beginning of the famine phase, ammonia was added and it was fairly quickly consumed in concomitance to PHA consumption (figure 1-b). The further increase of the OLR (12.75 g COD/L d) led to an increase of the feast phase length. As shown in Figure 2, the feast phase/cycle length ratio (h/h) in Run C was 27.1 ± 0.9 %, higher than those values measured in Run A (21.0 ± 0.6 %) and B (20.7 ± 0.3 %), but still lower than that of Silva et al. 2016 (6 h cycle length). Table 1 summarizes all the main parameters monitored over the course of the three performed SBR runs. The recent data reported in Silva et al. (2016) are also included, being the most comparable with data from this study, since the

Figure 2 Feast phase/cycle length ratio as a function of applied OLR and overall cycle length (12 h and 6 h)

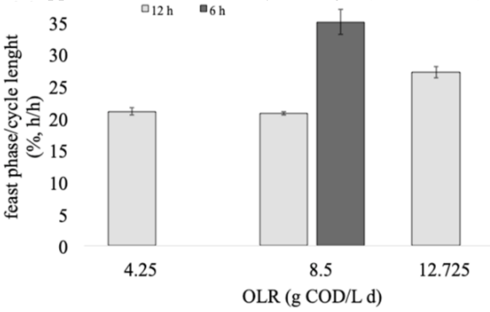
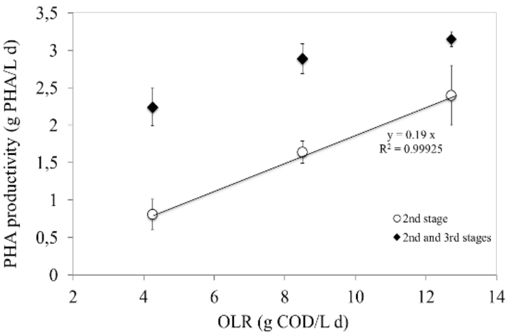


Figure 3 Volumetric PHA productivity of the 2nd stage and 2nd+3rd stages as a function of the applied OLR



same C/N ratio of 33.4 g COD/g N was applied. For all the three runs, the increase of PHA concentration was almost linear up to 4 hours on average from the beginning of the test, as well as the constant rate of VFA consumption. In the last hour of the tests, the microbial activity remarkably dropped since the polymer achieved a considerable intracellular content. The biomass selected in Run A expressed its storage response for longer time (up to 5 hours), even though its accumulation potential was lower, for both yield and maximum PHA content (0.63 ± 0.03 COD_{PHA}/COD and 0.66 ± 0.02 g PHA/g VSS respectively), if compared to both parameters obtained with Run B biomass (0.74 ± 0.03 COD_{PHA}/COD and 0.70 ± 0.02 g PHA/g VSS respectively). On the other hand, the biomass selected in Run C expressed its storage response until the fourth hour, even if both yield and maximum PHA content (0.36 ± 0.03 COD_{PHA}/COD and 0.62 ± 0.02 g PHA/g VSS respectively) were lower than the other parameters from Runs A and B. This was due to a lower selective pressure obtained during the biomass selection in the SBR. In order to consider the process technology as technically and economically viable, other important parameters have to be taken into account such as the overall PHA storage yield and PHA productivities (gPHA/Ld). Then, the overall PHA productivity, including the second and third stages, was calculated by considering the PHA concentration at its maximum intracellular content. PHA productivity linearly increased from 0.81 ± 0.05 to 2.4 ± 0.1 g PHA/L d (Figure 3) (Lorini et al. 2020). In Run C, the productivity after the accumulation step increased up to 3.15 ± 0.01 g PHA/L d thanks to the higher OLR, but not linearly with respect to Runs A and B. This was due to the lower selective pressure obtained during the selection step. This work clearly highlights the possibility to significantly enhance the PHA production in an SBR operating with uncoupled carbon and nitrogen feeding by finding the optimal operating conditions. The applied OLR has been demonstrated to have a significant impact on the aerobic MMC selection/enrichment, and, as a consequence, on the storage performances and productivity. The relatively high PHA content achieved at the end of the feast phase (0.40 - 0.53 g PHA/g VSS) points out the option to simplify the process by withdrawing the biomass (at its maximum PHA content) from the SBR towards downstream processing, with no need for the intermediate accumulation step. On the other hand, PHA productivity could be considerably increased by adding the accumulation step, as well as the final PHA content (2.89 g PHA/L d and 0.70 g PHA/g VSS, respectively) if relatively medium-high OLRs are applied (up to 8.5 g COD/L d). The application of OLRs above 12.75 g COD/L d, together with higher SRT (freed from HRT) may be a possible solution for a further increase of PHA productivity, provided that the maintenance of an efficient selective pressure is ensured.

Table 1 Main parameters with average data and standard deviations monitored and quantified in the SBR runs

Parameters	Run A	Run B	Run C	Silva et al. 2016
OLR (gCOD/Ld)	4.25	8.5	12.75	8.5
Cycle length (h)	12	12	12	6
PHA content at the end of feast (gPHA/gVSS)	0.40 ± 0.02	0.52 ± 0.02	0.53 ± 0.03	0.28 ± 0.02
PHA concentration at the end of feast (mg/L)	807 ± 58	1639 ± 40	2389 ± 145	758 ± 37
PHA productivity (2nd stage)	0.81 ± 0.05	1.64 ± 0.04	2.4 ± 0.1	0.78 ± 0.04

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