CHEMICAL ENGINEERING TRANSACTIONS Volume 20, 2010
Editors Enrico Bardone, Aurelio Viglia
 ISBN 978-88-95608-12-9 ISSN 1974-9791
DOI: 10.3303/CET1020032

Effect of hydraulic and organic loads in Sequencing Batch Reactor on microbial ecology of activated sludge and storage of polyhydroxyalkanoates

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In this research the production of polyhydroxyalkanoates (PHA) by activated sludge enriched in a sequencing batch reactor (SBR) at high organic load, has been investigated. The SBR was operated at four different organic load rates (OLR), in the range 8.5 ÷ 40.8 gCOD/L/day, and hydraulic retention times (HRT) in the range 1 ÷ 0.21 day, both being simultaneously varied by changing the cycle length from 2 h to 0.42 h. Both parameters affected the establishment of the feast and famine conditions needed for the selection of PHA-producing microorganisms. The highest observed values of polymer production rates and yields (~ 400 mgCOD/gCOD/h and 0.53 COD/COD, respectively) were obtained at the lowest OLR investigated (8.5 gCOD/L/day), with a corresponding HRT of 1 day. Microbial community analysis, based on denaturing gradient gel electrophoresis (DGGE), revealed that hydraulic and organic loads also played a main role on the microbial speciation within the SBR.

1. Introduction

Polyhydroxyalkanoates (PHA) are a group of polyesters which represents an interesting alternative to oil-based plastics because they are fully biodegradable and can be produced from renewable resources. On the other hand, the high costs of production have till now hampered their wider diffusion (Reddy et al., 2003). Indeed, most production processes are based on the use of pure cultures (e.g., *Ralstonia eutropha*) grown on well-defined nutrient-deficient synthetic media (Lee, 1996). The use of activated sludge from wastewater treatment plants has been proposed as a promising alternative to pure culture (Beccari et al., 1998; Chua et al., 2003). However, substrate consumption rate and storage rate of activated sludge are usually very low because the biomass is not very active when grown at a high sludge age (i.e., low organic load rate, OLR). A strategy to enrich activated sludge in microorganisms with high ability to store PHA is to expose it to alternating conditions of excess and lack of external substrates (the "feast and famine" regime) (Van Loosdrecht et al., 1997). Indeed, these conditions

Please cite this article as: Villano M., Lampis S., Valentino F., Vallini G., Majone M. and Beccari M., (2010), Effect of hydraulic and organic loads in sequencing batch reactor on microbial ecology of activated sludge and storage of polyhydroxyalkanoates, Chemical Engineering Transactions, 20, 187-192; DOI: 10.3303/CET1020032

provide a selective advantage to those microorganisms which are able both to remove quickly the external substrate by storing it as PHA during the feast phase, and to use the stored polymer as an internal carbon and energy source for growth in the famine phase. The employment of mixed microbial cultures enriched from activated sludge offers the opportunity to use organic wastes as feedstock for PHA production. Based on these considerations a three-stage process has been proposed (Albuquerque et al., 2007; Dionisi et al., 2004). In the first anaerobic stage, a high-concentration biodegradable waste is fermented to produce an effluent rich in volatile fatty acids, which are the most direct substrates for PHA storage. This effluent is fed to a second aerobic stage, operated in a sequencing batch reactor (SBR) to easily establish the required feast and famine conditions. The storage response of the produced sludge is then exploited in a third aerobic stage, operated in batch, which maximizes the amount of produced polymer.

In order to select microbial cultures with high storage response, the SBR has to be operated at OLR higher than in traditional activated sludge systems. On the other hand, if the OLR is too high, the "strength" of the feast and famine regime is diminished, suggesting the existence of an optimal OLR to be found. Along this line, in this research we investigated the effect of hydraulic and organic loads, both being simultaneously controlled by variation of the cycle length, on the enrichment of an activated sludge in the SBR and on the storage ability of the selected biomass.

2. Materials and methods

2.1 SBR operation (second stage of the process)

The aerobic enrichment of the PHA-producing biomass was operated in a 1 L working volume SBR, inoculated with activated sludge from the 'Roma Nord' full-scale wastewater treatment plant. The reactor was fed with a synthetic mixture of acetic and propionic acid (85% and 15% on a COD basis, respectively), at an overall concentration of 8.5 gCOD/L. No settling phase was performed, therefore the hydraulic retention time (HRT) was equal to the sludge retention time. Four SBR runs were carried out at OLR varied in the range 8.5 ÷ 40.8 gCOD/L/day by changing the length of the SBR cycle (i.e., increasing the number of cycles per day) from 2 h to 1 h, 0.67 h, and 0.42 h, at a fixed volume exchange ratio (0.083 L per cycle) and feed concentration. Accordingly, the resulting HRT was 1 day, 0.5 day, 0.33 day, and 0.21 day. The reactor was stirred by a mechanical impeller and aerated by means of membrane compressors. The dissolved oxygen (DO) concentration was continuously recorded and utilized to identify the length of the feast and famine phases during each cycle: DO concentration was low in correspondence of high bacterial metabolic activity (feast phase), and high in correspondence of reduced metabolic activity (famine phase). SBR cycles were also characterized by measurements of biomass concentration, as volatile suspended solids (VSS, at the end of the feast phase), and of PHA (at the end of the feast phase and at the end of the cycle). For PHA determination, the sludge was treated immediately after sampling with a NaClO solution (7% of active Cl_2). PHA was extracted, hydrolyzed and esterified to 3-hydroxyacyl methyl esters, and determined by gas-chromatography (Braunegg et al., 1978). Storage rates and yields were expressed in terms of COD units, and calculated as described elsewhere (Beccari et al., 2009). Specific rates were

calculated with reference to the non-polymer biomass (i.e., the difference between VSS and PHA). During each SBR run, samples of biomass were taken at the end of the cycle at different days for molecular characterization through denaturing gradient gel electrophoresis analyses (DGGE), as described in Beccari et al., 2009.

2.2 Batch tests (third stage of the process)

The batch tests were carried out in a 500 mL reactor, at the same temperature (25 °C) and pH (7.5) of the SBR. A sample of biomass was withdrawn from the SBR at the end of feast phase, diluted to the chosen concentration, and spiked with the same substrates fed to the SBR, but at higher initial concentration (2100-4200 mgCOD/L). The sludge in the batch reactor was sampled at regular intervals for analytical determinations of substrates, PHA and ammonium, analyzed as described elsewhere (Beccari et al., 2009). During the tests, the reactor was maintained under air bubbling at oxygen concentrations in the range 7-8 mg/L. The polymer content in the biomass was calculated by dividing the measured PHA concentration by the overall biomass concentration (both expressed as g COD). The storage yield was given by the ratio between the stored polymer and the removed substrate, all given on a COD basis.

3. Results and discussion

The performance of the SBR was strongly dependent on the establishment of the feast and famine conditions that was in turn dependent on the applied OLR.

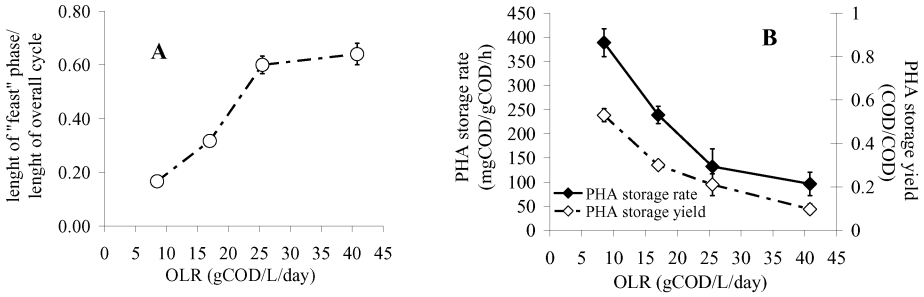

Figure 1. Effect of the applied OLR on the ratio between the length of the feast phase and the overall SBR cycle (A), and on PHA storage rates and yields in the SBR (B).

Figure 1A shows the effect of the applied OLR on the ratio between the length of the feast phase and of the overall SBR cycle: it increased from 0.17 to 0.64 as OLR increased from 8.5 to 40.8 gCOD/L/day. As the fraction of the cycle under feast conditions increased, a progressive loss of the selective pressure required to select microorganisms with high storage response was observed. Indeed, a decrease of both PHA storage rate and yield was observed (figure 1B), the highest values being obtained at the lowest OLR investigated (about 400 mgCOD/gCOD/h and 0.53 COD/COD, respectively at 8.5 gCOD/L/day). On the other hand, biomass productivity in the SBR almost linearly increased at increasing OLR, from ~ 2.5 (at 8.5 gCOD/L/day) to ~ 11 gVSS/L/day (at 40.8 gCOD/L/day).

In order to better investigate the storage ability of the sludge selected in the SBR at the different OLR and cycle lengths, aerobic batch tests were performed by spiking the biomass with higher concentration of substrates (i.e., volatile fatty acids).

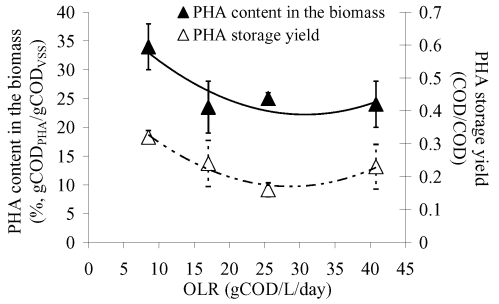

Figure 2. Maximal PHA content in the biomass and the corresponding storage yield, measured in batch accumulation tests.

In this frame, figure 2 reports the effect of the OLR on the maximum PHA content in the biomass obtained in the batch tests and the corresponding storage yield (i.e., the fraction of the removed substrate converted into polymer in correspondence to the maximum % PHA in the biomass). The highest observed values were obtained at an applied OLR of 8.5 gCOD/L/day (~ 34% gCOD$_{PHA}$/gCOD$_{VSS}$ and 0.32 COD/COD for the maximum polymer content and storage yield, respectively), whereas lower and similar values were obtained at higher OLR values. This result confirmed a decreasing storage ability of the biomass at increasing OLR and indicated that biomass performance in batch was dependent on its previous cultivation conditions in the SBR.
On the other hand, by considering both SBR and batch reactors as sequential steps for PHA production, it was calculated that polymer productivity (i.e., the amount of PHA produced per unit of overall volume of both reactors and per unit of time) increased from ~1.65 to ~2.81 gPHA/L/day, when the OLR increased from 8.5 to 25.5 gCOD/L/day; then decreased to ~ 2.26 gPHA/L/day at 40.8 gCOD/L/day. This indicates that the increased biomass productivity was able to more than counterbalance the decreased storage rate and yield up to 25.5 gCOD/L/day. A clear and stable storage response was maintained at 25.5 gCOD/L/day, higher than in our previous study where the OLR was varied by changing the SBR feed concentration at fixed HRT and cycle length (Dionisi et al., 2006). In that cases, unstable performance with randomic changes between growth and storage response was observed at OLR higher than 20 gCOD/L/day.
Finally, the microbial communities selected in the SBR were characterized through DGGE analyses. The DGGE profiles obtained from sludge collected at different sampling time during the experimentation indicated that the change in ORL strongly affected the bacterial speciation within the reactor. In fact, as shown in figure 3, the increase in ORL values from 8.5 to 25.5 and eventually to 40.8 gCOD/L/day led to an alteration of the dominance of the major bands in the DGGE profiles.

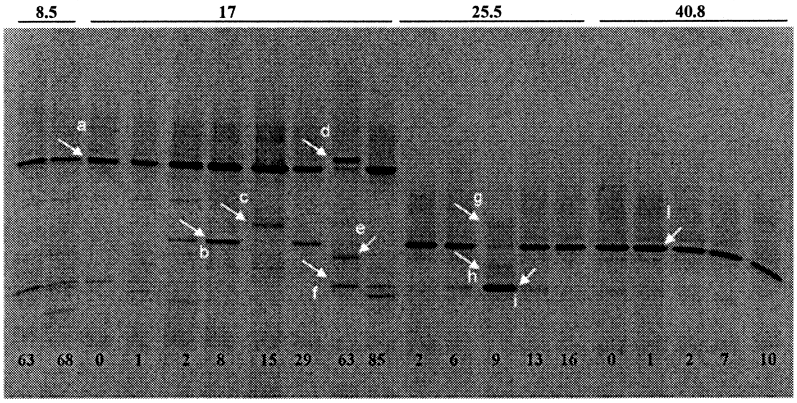

Figure 3. DGGE profiles of SBR runs maintained at increasing OLR values: 8.5, 17, 25.5 and 40.8 gCOD/L/day. Numbers at the bottom of each lane indicate the sampling time in days relative to each run. Arrows and letters indicate bands that have been excised, cloned and sequenced.

In particular, band a, corresponding to the bacterial strain *Lampropedia hyalina* (Table 1), which resulted to be dominant at 8.5 and 17 gCOD/L/day, disappeared at the higher OLR values meanwhile band l, corresponding to *Thauera sp.* strain (Table 1), took place and persisted at 40.8 gCOD/L/day. Both these two bacterial strains are known to be high rate PHB producers (Lemos et al., 2008; Oshiki et al., 2008; Stante et al., 1997) and are currently found associated to wastewater or activated sludge (Oshiki et al., 2008).

Table 1 – Taxonomic characterization of the major bands in the DGGE profile

DGGE band	Phylogenetic group	TAXON	Identity
a	*β -Proteobacteria*	*Lampropedia hyalina*	100%
b	*β- Proteobacteria*	*Thaurea sp.*	100%
c	*α -Proteobacteria*	*Uncultured Rhodobacteraceae bacterium*	100%
d	*β- Proteobacteria*	*Lampropedia hyalina*	96%
e	*Actinobacteridae*	*Leifsonia sp.*	99%
f	*α -Proteobacteria*	*Phyllobacterium sp.*	100%
g		*Uncultured Bacterium*	98%
h	*β- Proteobacteria*	*Achromobacter sp.*	100%
i	*α -Proteobacteria*	*Phyllobacterium sp.*	100%
l	*β- Proteobacteria*	*Thaurea sp.*	100%

4. Conclusions

This study investigated the effect of hydraulic and organic loads applied to a SBR on PHA production from activated sludge. The selection and enrichment of PHA-storing biomass was strongly affected by the SBR operating conditions. The best observed storage response was obtained, both in SBR and batch experiments, at the lowest OLR

investigated (8.5 gCOD/L/day), corresponding to a HRT of 1 day. However, it is noteworthy that a clear and stable storage response was maintained at least up to 25.5 gCOD/L/day, where the polymer productivity was the highest recorded. To our best knowledge, this is the highest OLR never reported where mixed cultures still maintain a storage response under dynamic feeding.

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