Combined polyhydroxyalkanoates (PHA) and 1,3-propanediol production from crude glycerol: Selective conversion of volatile fatty acids into PHA by mixed microbial consortia

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Abstract: Crude glycerol is an important by-product of the biodiesel industry, which can be converted into volatile fatty acids (VFA) and/or 1,3-propanediol (1,3-PDO) by fermentation. In this study, a selective conversion of VFA to polyhydroxyalkanoates (PHA) was attained while leaving 1,3-PDO in the supernatant by means of mixed microbial consortia selection strategies. The process showed highly reproducible results in terms of PHA yield, 0.99 ± 0.07 Cmol PHA/Cmol S (0.84 g COD PHA/g COD S), PHA content (76 ± 3.1 g PHA/100 g TSS) and 1,3-PDO recovery (99 ± 2.1 %). The combined process had an ultimate yield from crude glycerol of 0.19 g COD PHA and 0.42 g COD 1,3-PDO per g of input COD. The novel enrichment strategy applied for selectively transforming fermentation by-products into a high value product (PHA) demonstrates the significance of the enrichment process for targeting specific bio-transformations and could be potentially proved valuable for other biotechnological applications as well.

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3 **Selective conversion of volatile fatty acids into PHA by mixed microbial consortia**

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30	<i>Nomenclature</i>
31	1,3-PDO: 1,3-propanediol
32	C_{mol} : moles of carbon
33	COD: Chemical Oxygen Demand
34	HB: 3-Hydroxybutyrate
35	HP: 3-Hydroxypropionate
36	HV: 3-Hydroxyvalerate
37	MMC: Mixed Microbial Consortia/Cultures
38	OTU: Operational Taxonomic Unit
39	PHA: Polyhydroxyalkanoates
40	q_p : Specific PHA formation rate ($C_{mol} \text{ PHA} \cdot C_{mol}^{-1} \cdot h^{-1}$)
41	$-q_s$: Specific substrate uptake rate ($C_{mol} \text{ S} \cdot C_{mol}^{-1} \cdot h^{-1}$)
42	r_p : PHA formation rate ($C_{mM} \text{ PHA/h}$ or $g \text{ PHA} \cdot L^{-1} \cdot h^{-1}$)
43	$-r_s$: Substrate uptake rate ($C_{mM} \text{ S/h}$)
44	SBR: Sequential Batch Reactor
45	TSS: Total Suspended Solids
46	VFA: Volatile fatty acids
47	X: Cells excluding polymers (PHA and Glycogen)
48	$Y_{P/S}$: Yield PHA/substrate ($C_{mol} \text{ PHA} / C_{mol} \text{ S}$ or $g \text{ COD PHA} / g \text{ COD S}$)
49	$Y_{gly/S}$: Yield Glycogen/substrate ($C_{mol} \text{ Gly} / C_{mol} \text{ S}$)
50	$Y_{X/S}$: Yield biomass/substrate ($C_{mol} \text{ X} / C_{mol} \text{ S}$)
51	$Y_{X/Polymer}$: Yield Biomass/PHA+Glycogen ($C_{mol} \text{ X} / C_{mol} \text{ Gly+PHA}$)
52	μ : Specific biomass growth rate (h^{-1})

1 Introduction

Crude glycerol is a by-product of the transesterification of fats and oils in the biodiesel industry, with an average glycerol content of 70% (Zhu et al., 2013). Its world annual production is in the magnitude of millions of tons, which has stimulated the interest for finding high-value applications such as the production of polyhydroxyalkanoates (PHA) (da Silva et al., 2009). PHA are biopolymers produced in microbial cells under conditions of unbalanced growth (Anderson and Dawes, 1990). Their properties largely resemble some commonly used petroleum derived plastics such as polyethylene and polypropylene, for what PHA have been explored as possible renewable and biodegradable alternative during the last decades (Bugnicourt et al., 2014). Nevertheless, the success of PHA in the market is still limited by their high production costs. In this regard, the use of mixed microbial consortia (MMC), which do not require sterilization and generally present a higher tolerance and adaptability to waste substrates, is being studied as a possible alternative (Kourmentza et al., 2017; Valentino et al., 2016).

Only a few studies have attempted the conversion of glycerol into PHA using MMC (Dobroth et al., 2011; Freches and Lemos, 2017; Moita et al., 2014; Moralejo-Garate et al., 2013), with a common observation of co-accumulation of glycogen, which limits the PHA yield. In order to circumvent that, the substrate can first be fermented to volatile fatty acids (VFA), which can then be converted into PHA at high yields and without glycogen production. The resulting three-step process consists of: 1) Fermentation of the substrate into VFA; 2) Enrichment of PHA accumulating MMC; 3) PHA accumulation (Albuquerque et al., 2007; Serafim et al., 2008). However, glycerol fermentation presents another challenge, which is the production of 1,3-propanediol (1,3-PDO) besides VFA. PHA production from 1,3-PDO has recently been demonstrated in MMC subjected to nitrogen limitation during the famine phase of the enrichment step (Burniol-Figols et al., 2018). Conversion of 1,3 PDO to PHA still needs optimization, as the conversion yields obtained so far were lower than the ones generally obtained from VFA and certain glycogen accumulation also occurred.

Given that 1,3-PDO is a high value product with a similar market price as PHA (1.8 €/kg for 1,3-PDO (Gargalo et al., 2016) and 1.5-5 €/kg for PHA (Chanprateep, 2010)), an alternative approach could be to avoid the consumption of 1,3-PDO while converting the VFA into PHA. This would result in an increase in the purity of 1,3-PDO in the supernatant while converting VFA into a high value product.

The presence of substrates which favor the channeling of carbon towards microbial growth instead of PHA accumulation is a general obstacle, as it limits the PHA content of the cells (Marang et al., 2014; Tamis et al., 2014). In a recent study, the selective consumption of VFA over methanol was achieved in an MMC enriched from aerobic sludge using a strategy based on the selective settling capacity of PHA-containing cells (Korkakaki et al., 2016). By reducing the presence of bacteria utilizing methanol, the maximum PHA storage capacity of the culture increased from 48 to 70 wt% PHA. Along the same line, a selective consumption of VFA over 1,3-PDO has recently been reported using defined co-cultured strains. In the latter case, the outcome was achieved by testing PHA producing strains for their inability to consume 1,3-PDO (Pan et al., 2016).

In the present study, the selective consumption of VFA over 1,3-PDO in the accumulation phase was attained in MMC by not supplying 1,3-PDO during the enrichment phase. More precisely, the enrichment culture was fed with a synthetic medium supplemented with VFA and crude glycerol (Fig. 1). Different substrate concentrations in the accumulation phase were tested in order to evaluate possible inhibition phenomena. Furthermore, the study assessed the stability of the culture and the reproducibility of the process, which are of vital interest and sometimes put under concern in MMC based open-culture processes.

2 Materials and methods

2.1 Crude glycerol fermentation effluent

Crude glycerol continuous fermentation was performed with an influent concentration of 10 g/L of glycerol (14.7 g/L of crude glycerol) as described elsewhere (Burniol-Figols et al., 2018), based on the results obtained in Varrone et al., (2017). The distribution of metabolites in the effluent utilized during the PHA accumulation experiments was the following (g/L): 1,3-PDO: 4.43 ± 0.16 , butyrate: 1.59 ± 0.13 , propionate: 0.80 ± 0.15 , acetate: 0.19 ± 0.04 , glycerol: < 0.01 , TSS: 0.89 ± 0.21 . Measured soluble metabolites accounted for an average of 11.6 ± 0.4 g COD/L, while the total soluble COD was around 14 g COD_{total}/L. Soluble nitrogen in the form of NH_4^+ was 2.56 ± 1.74 mg/L. The effluent was kept at 4°C and at -20°C for short and long term storage, respectively, and was centrifuged (15 min at 4000 g) and filtered (1µm) before being used in the PHA accumulation phase.

2.2 Enrichment (SBR)

The enrichment in PHA producers was carried out in a 2.5 L Minifors reactor (INFORS HT) operating as Sequential Batch Reactor (SBR) with a working volume of 1.7 L. The reactor was operated in cycles of 12 h. At the end of each cycle, half of the volume of the reactor was replaced with fresh medium resulting in a Hydraulic Retention Time (HRT) and Solids Retention Time (SRT) of 1 day. The inoculum (30% v/v in the first cycle) was a mixture of equal parts of anaerobic sludge from the Lundtofte wastewater treatment plant and aerobic sludge from the Daka Biodiesel wastewater treatment plant (Denmark). Air was supplied at a rate of 1 L/min and agitation was set at 500 rpm. pH and temperature were maintained at 8 and 30°C, respectively. The reactor was emptied and cleaned along with the tubing once a week to avoid excessive biofilm formation.

The medium consisted of BA Medium (Varrone et al., 2015) supplemented with VFA and crude glycerol to a carbon concentration of 90 mM of Carbon (C_{mM}) (45 C_{mM} at the beginning of the cycle). Half of the carbon corresponded to glycerol (45 C_{mM}), which was added in the form of crude glycerol (1.88 g/L). The crude glycerol source and characteristics can be found elsewhere (Varrone et al., 2015). The other half of the carbon was in the form of VFA (45 C_{mM}), which were added to the same proportions as in the crude glycerol fermentation effluent: (acetate 2.38 C_{mM} , propionate 12.10 C_{mM} , butyrate 30.52 C_{mM}). C:N:P molar ratio in the medium was fixed at of 100:12:1.5 with NH_4Cl and K_2HPO_4 . Allylthiourea (5mg/L) and Antifoam 204 (Sigma) (85 $\mu L/L$) were supplied to inhibit nitrification and foaming, respectively. The resulting organic loading rate of the SBR was 3.7 g $COD_{total} \cdot L^{-1} \cdot day^{-1}$.

The oxygen saturation (pO_2) was monitored online and used as an indicator of the duration of the feast phase. This parameter was used, together with the PHA content at the end of the feast phase, to assess the stability of the reactor. Moreover, a full cycle was periodically characterized by offline samples (see analytical methods).

2.3 PHA accumulation

Batch tests were performed using the culture from the enrichment at the end of the cycle (12h). 0.85 L of cell culture were allowed to settle in a volumetric cylinder for 30 minutes, followed by removal of 0.65 L of supernatant. At time 0 of the batch experiment, the remaining 0.2 L of settled cells were added to 1.5 L of medium consisting of clarified crude glycerol fermentation effluent diluted with distilled water to reach different VFA concentrations (45 C_{mM} , 60 C_{mM} , 75 C_{mM} and 90 C_{mM}) at the beginning of the batch, corresponding to 5.6, 7, 9.5 and 12 g COD_{total}/L . The correspondent cycle of the SBR for each batch is indicated in Table 1. Allylthiourea and antifoam were supplied to the concentrations described above for the SBR. Aeration, agitation and pH control were also carried out

like described above for the SBR. Likewise, phosphorous was added to a final C:P ratio of 100:1.5. No nitrogen was supplemented in order to provide nitrogen limiting conditions.

After the initial assays at increasing VFA concentrations, the PHA accumulation batch at 90 C_{MM} was repeated 11 times to assess the reproducibility of the results during a period of 2 months (Table 1). The batches were stopped after butyrate depletion, indicated by a raise in the oxygen saturation. The reproducibility of the tests was assessed by the PHA content of the cells at the end of the batch. Moreover, in five of the batches (indicated in bold in Table 1), the rates and yields of the process where characterized by frequent offline sampling. Linear correlations of variables were assessed by means of the Pearson correlation coefficient and 2-tailed test of significance using the software OriginPro v 9.0.0.

2.4 Analytical methods

Offline samples of the reactors were characterized as follows. Total suspended solids (TSS) were measured in fresh samples using Whatman filters GF/F and according to standard protocols (APHA et al., 2005). Samples were thereafter centrifuged (at 4000 g for 10 min) and the pellets were washed with PBS solution (phosphate-buffered saline) and freeze dried. PHA and glycogen in lyophilized cell pellets were measured in duplicate according to the protocols described in Burniol-Figols et al., (2018), and expressed as a weight percentage of the TSS. The PHA and glycogen content was subtracted from the TSS to obtain the concentration of active biomass (cells excl. polymers or X). The supernatant was filtered (0.45µm) and kept at -20°C prior to analysis of soluble nitrogen (N-NH₃) (Hach-Lange kits LCK 305/303), COD (Hach-Lange kits LCK 914/514), as well as VFA and alcohols concentrations. VFA (acetate, propionate, iso and *n*-butyrate, lactate, iso and *n*-valerate and hexanoate) and alcohols (1,3-PDO, butanol and glycerol) in the supernatant were analyzed by HPLC in a Shimadzu HPLC system equipped with an RI detector and an Aminex HPX-87H column (BioRad). Yields and rates were calculated as previously reported (Burniol-Figols et al., 2018). Parameters reported in terms of C_{mol} were based on the compounds measured by HPLC, while parameters reported in terms of COD_{total} were based on the measurements of the soluble COD in the supernatants. The dilution effect of acid and base addition for the pH control, as well as the effect of the sampling, were taken into account for all calculations.

PHA were extracted in chloroform and analyzed by Nuclear Magnetic Resonance spectroscopy (¹H-NMR and ¹³C-NMR) as described in Burniol-Figols et al., (2018). The molar mass of the polymer was determined by Size-Exclusion Liquid Chromatography (SEC) in a Shimadzu HPLC system using

two columns in series (SDV 5 μ m 8x300mm 1000Å and 10000Å, PSS Polymer Standards Service GmbH). The oven temperature was set at 31°C. Chloroform was used as eluent at a flow rate of 1mL/min. The system was calibrated with low polydispersity polystyrene standards (Polymer Standards Service GmbH).

Microbial analyses were performed in samples corresponding to time 0 of the SBR cycles indicated in Table 1. Sampling, DNA extraction, 16S rRNA gene amplicon sequencing and data processing were performed as described in Burniol-Figols et al., (2018). Sequencing reads have been deposited to the Sequence read archive of NCBI under the BioProject ID: PRJNA398362.

3 Results and discussion

3.1 Enrichment of PHA-accumulating MMC in absence of 1,3-PDO

The present study was based on the hypothesis that an MMC subjected to a feast and famine regime using medium supplied with VFA, but not 1,3-PDO, would be enriched in bacteria adapted to accumulate PHA predominantly from VFA. Consequently, a culture selected in this manner would not consume 1,3-PDO when supplied with real fermentation effluent containing both substrates in the following PHA accumulation phase (Fig. 1). Therefore, the PHA enrichment was performed in an SBR fed with a synthetic VFA medium. Nevertheless, in order to avoid any inhibition phenomena when the culture faced the real waste effluent in the PHA accumulation, the VFA synthetic medium was supplemented with crude (non-fermented) glycerol.

During the first cycles, VFA were consumed faster than glycerol (Fig. 2 A). Nonetheless, from cycle 50 onwards, the depletion of either substrate was not distinguishable any more from the pO₂ profile, as exemplified by a typical cycle of the stabilized reactor in Fig. 3 A and B. Complete substrate consumption occurred within 65 min, representing a feast/famine ratio of 0.1 (Fig. 3 B). Substrate consumption coincided with a PHA production up to 37.3 wt%, detecting an increment in both HB (3-hydroxybutyrate) and HV (3-hydroxyvalerate) monomers (Fig. 3 C and D). Glycogen was also formed during the feast phase, probably from glycerol, given the tendency of the latter to lead co-accumulation of glycogen and PHA (Moralejo-Gárate et al., 2011). The PHA yield was 0.68 C_{mol} PHA/C_{mol} substrate and the glycogen yield represented 0.12 C_{mol} glycogen/C_{mol} substrate (Table 2). During the famine phase, these polymers were transformed into biomass (Fig. 3 C) at a yield of 0.62 C_{mol} biomass/C_{mol} polymers using the nitrogen present in the medium (Table 2).

The SBR proved to be very stable both in terms of duration of the feast phase and the PHA content of the cells at the end of the feast phase (Fig. 2 A and B). Table 2 displays the yields and rates obtained in two different cycles separated for more than 3 months, which presented practically the same values.

3.2 Microbial community analysis

The culture in the SBR was dominated by OTUs (Operational Taxonomical Units) classified within the genera *Amaricoccus* (class Alphaproteobacteria) and *Thauera* (class Betaproteobacteria), which together accounted for 56.3-72.4 % of the sequences at different cycles of the SBR (Fig. 4). The other identified OTUs rarely presented abundances higher than 5%, suggesting a highly diverse flanking population.

Both dominant genera, *Thauera* and *Amaricoccus* have largely been reported to be prevalent genera in PHA enrichment reactors operated under feast/famine aerobic regime, especially in the case of *Thauera* (Morgan-Sagastume, 2016). Moreover, both genera have been directly correlated with PHA accumulation by means of Nile Blue staining (Lemos et al., 2008).

In regard to substrate utilization, different substrate preferences have been reported for these genera. Members of the *Amaricoccus* genus were described to become dominant over *Thauera* when the substrate of the SBR changed from acetate to propionate (Lemos et al., 2008). However, Yang et al., (2013) identified *Thauera* to be the main genus in a propionate-fed SBR. On the other hand, bacteria from the genus *Thauera* were described to primarily use butyrate as a carbon source when submitted to a mixture of VFA (Albuquerque et al., 2013). To our knowledge, neither of the two genera has been previously described in glycerol-fed systems.

Fluctuations of the relative abundance of the main genera, as well of the sum of the two, were observed in samples taken at different points, despite a steady behavior of the SBR (Fig. 4). This is very well exemplified in the two SBR cycles presented in Table 2 (cycle 298 and 76). The yields and rates of these cycles were very similar despite substantial variations in the microbial population identified at the beginning of the cycles (Fig. 4). The percentage of OTUs classified within the genus *Thauera* was much higher in cycle 298 (29.1 vs. 18.9), and concurrent with a clear drop in the genus *Amaricoccus* (37.9 vs 27.2). Nonetheless, the sum of the two main genera was virtually the same. Stable operation of SBRs with variations in the relative composition of the microbial population has been previously reported (Bengtsson, 2009; Serafim et al., 2006; Valentino et al., 2014). Albuquerque et al., (2013) demonstrated that microbial strains with PHA production capacity can compete for the

same substrate. Thus, small variations in the microbial composition do not necessary imply a change in the overall performance. From the results obtained here, a relative abundance of the two main genera over 50% seemed to be a common denominator for a stable operation of the SBR.

3.3 PHA accumulation with 1,3-PDO recovery

Using the enriched inoculum from the SBR, a set of experiments of PHA accumulation with crude glycerol fermentation effluent was performed at different initial VFA concentrations: 45 C_{mM}, 60 C_{mM}, 75 C_{mM} and 90 C_{mM}, with the last corresponding to undiluted effluent (Fig. 5 and Table 3).

For all the concentrations tested, butyrate was used up before acetate and propionate, even though the initial concentrations of these VFA were lower (Fig. 5 C). Acetate and propionate concentrations decreased right from the beginning, but their consumption rates increased once butyrate was depleted (Table 3, Fig. 5 C). This fact was translated into two different phases of PHA accumulation, which could be clearly distinguished by the pO₂ saturation profile (Fig. 5 A).

During the first phase, coinciding with butyrate consumption, a steep increase in the PHA concentration and polymer content was observed. HB was the main monomer produced, but a notable production of HV also occurred (Fig. 5 D and E). A small increment in 3-hydroxypropionate (HP) was detected as well, but never reaching values over 1 wt%. PHA yields during this phase (hereafter referred to as butyrate phase) were higher than 0.9 C_{mol} PHA/ C_{mol} S in all experiments (Table 3). Around 80 % of the substrate was consumed during this phase.

During the second phase, when only acetate and propionate were available, only a slight increase in PHA concentration was observed (Fig. 5 D). The PHA yields were much lower than during the butyrate phase and a considerable increase in the carbon derived to growth was observed for all batches (Table 3). As a result, the overall PHA yields for the entire batch presented always a lower value than during the butyrate phase (Table 3).

Similar observations regarding the preference of butyrate over acetate and propionate have been reported previously in cultures dominated by different microbial genera (Albuquerque et al., 2013; Jiang et al., 2012; Marang et al., 2013). As a matter of fact, this phenomenon has been observed even in cultures that were not exposed to butyrate during the enrichment (Dionisi et al., 2005). In a study with only butyrate and acetate as a substrates (Marang et al., 2013), butyrate depletion before acetate led to the same phases observed here, where specific substrate carbon consumption rates (in C_{mol} S.C_{mol} X⁻¹.h⁻¹) decreased during the second phase. Nonetheless, the specific substrate consumption rate in terms of mol S. C_{mol} X⁻¹.h⁻¹ remained constant throughout the two phases. This fact

indicated that the substrate uptake and activation was the rate-limiting process and that butyrate and acetate shared the same transporter, but it had preference for butyrate. The same phenomenon was observed in the present study: a decrease in the specific substrate carbon uptake rate (in $C_{mol} S. C_{mol} X^{-1}.h^{-1}$) was observed after butyrate exhaustion, but the specific substrate rate in terms of substrate mols was maintained (except for the batch at 75 C_{mM} , Table 3). Nevertheless, this condition was only met when propionate was considered, which, following the same reasoning as in Marang et al., (2013), would indicate that propionate is also uptaken through the same regulation system.

The lower PHA yields obtained during the second phase could be related to various factors. Higher PHA yields have been associated to butyrate compared to acetate, and attributed to the lower ATP needs of the transformation to PHB, thus a reducing carbon flux to the TCA cycle (Marang et al., 2013). Propionate has also usually been reported to present lower PHA yields and higher growth yields compared to acetate (Dias et al., 2008; Jiang et al., 2011). In this case, a lower PHA yield would not be justified by higher energy requirements, but partially because of the loss of carbon during HV production (Pardelha et al., 2012), which justifies in the following argument. HV arises from the condensation of acetyl-CoA and propionyl-CoA. When acetyl-coA is available from the transformation of other substrates (such as butyrate), all the propionate can be converted to propionyl-CoA and incorporated into HV. Nonetheless, when acetyl-coA is not available, propionyl-coA has to be decarboxylated to acetyl-coA, thus lowering the maximum theoretical PHA yield to 0.67 $C_{mol} PHA/C_{mol} S$. In the present case, propionyl-coA from propionate could be combined with acetyl-coA originated from the other VFA during the butyrate phase, and as a matter of fact, over 70% of propionate consumed was recovered as HV (mol basis). During the second phase, the little acetate present in the medium could not provide enough acetyl-coA molecules, so decarboxylation of propionyl-coA to acetyl-coA was needed. During this phase, under 30% of the propionate consumed was recovered as HV (mol basis). This behaviour, which might partially explain the lower yields, is very well illustrated in Fig. 5, where despite the propionate consumption rate increased during the second phase of the batch (Fig. 5 C), the HV monomers continued increasing at the same rate as earlier (Fig. 5 D).

Overall, the lower PHA yields combined with lower substrate uptake rates (in C_{mol}) led to a reduction of the PHA production rate during the second phase. Moreover, a reduction of the apparent PHA production rate could have occurred due to a simultaneous degradation of the PHB produced during the butyrate phase. Nonetheless, the last hypothesis could not be confirmed from the data obtained here.

Measurements of the COD in the supernatant always resulted in higher values than calculated from the sum of identified compounds in the HPLC. Partial consumption of this undefined COD, probably crude glycerol impurities, was observed (Fig. 5 B). Whether these compounds contributed to the observed PHA production could not be ruled out with the analyses performed here. Thus, the PHA yields calculated from the overall COD consumed are also reported in Table 3. It is noteworthy to mention that, despite these yields were lower, they presented the same tendencies as the ones calculated on VFA basis.

Another very important general observation in all experiments was the negligible consumption of 1,3-PDO, which was recovered to a minimum of 95% (Fig. 5 B and Table 3). This value was much higher than the recovery obtained when a similar strategy was tested by selecting defined PHA strains for their incapacity to consume 1,3-PDO (Pan et al., 2016). That strategy resulted in a 1,3-PDO recovery of 80% and a PHA content of around 20 wt% after 28h of incubation. The higher selectivity for VFA obtained in the present study using MMC might be due to the high responsiveness to substrate addition developed by cultures submitted to feast/famine regimes.

The presence of VFA as by-products is common in biological processes such as lactic acid, ethanol or butanol fermentations. The strategy presented here could be tested in other fermentation effluents in order to increase the purity of the desired product while producing PHA as an additional high value product.

3.4 Effect of increasing substrate concentration on the PHA accumulation

The PHA accumulation experiments performed at different VFA concentrations also exhibited some differences in the followed parameters, which are summarized in Fig. 6. Given the different phases observed (described in the previous section) the results are presented both for the overall VFA consumption and the butyrate consumption phase. The polymer percentage inside the cells incremented linearly with the initial substrate concentration until 75 C_{mM} , where 70 wt% PHA was reached (Fig. 6 A). No further increase was observed at 90 C_{mM} initial concentration. Nevertheless, a higher PHA concentration was obtained at 90 C_{mM} , derived from the higher TSS obtained at this concentration. As a matter of fact, there was a linear increase in the maximum PHA concentration obtained at the end of the feast phase (Fig. 6 B), reaching up to 1.79 g/L of PHA at the highest concentration.

The overall PHA yield and the one obtained during the butyrate phase decreased from 45 to 60 C_{mM} (Fig. 6 C), but remained approximately constant after that. From these data points, one could

suggest a decreasing trend of the PHA yield (considering the data points at 60 C_{mM} as outliers). Alternatively, no trends would be perceived if the batch at 45 C_{mM} was considered an outlier. Looking at the specific substrate uptake rates (q_s) and the specific product formation rates (q_p) (Fig. 6 D and E), no important differences could be observed. Both parameters seemed to be independent of the substrate concentration. Thus, substrate inhibition could not be concluded from the data. Noteworthy to mention, this lack of inhibition occurred despite increasing concentrations of 1,3-PDO.

Overall, increasing the VFA initial concentration up to 90 C_{mM} did not have an apparent effect on the main outcome of the PHA accumulation, the specific substrate concentration rate (q_p). At this concentration, a higher PHA concentration was obtained, which would benefit the downstream costs of the process. Given the different phases observed, it would be reasonable to stop the process after butyrate depletion in order to maintain a high productivity. At this point, the PHA would be produced at a yield of 0.91 C_{mol} PHA/ C_{mol} S with an specific rate of 0.87 $C_{mol} \cdot C_{mol} X^{-1} \cdot h^{-1}$ (Table 3). The 1,3-PDO would be recovered at 97% and would represent 80% of the COD of the supernatant. This figure would increase up to 85% if the rest of the VFA were allowed to be depleted. Nevertheless, the yield would decrease to an overall yield of 0.81 C_{mol} PHA/ C_{mol} S and the productivity would drop to 0.5 C_{mol} PHA. $C_{mol} X^{-1} \cdot h^{-1}$ (Table 3). Consequently, different strategies could be formulated based on a general economic evaluation, taking into account the cost of the further purification of 1,3-PDO depending on the purity of the effluent and the repercussion of the decrease of productivity.

3.5 Reproducibility of the PHA accumulation

The PHA batch accumulation at the highest initial VFA concentration (90 C_{mM}) was repeated 11 times over a period of 2 months to study the stability of the culture (Table 1). The batches were stopped after butyrate depletion, indicated by as sudden increase in the oxygen saturation (Fig. 5 A). The average PHA content obtained at the end of the 11 batches was 76 ± 3.1 wt% (Table 4) implying a relative error of just 4%. The HB: HV ratio was also reproducible, with the average value being 84:16 (± 2.0 :1.8). In the same line, 1,3-PDO was always completely recovered from the fermentation broth, with an average recovery of 99 ± 2.1 %.

In 5 of those batches, the yields and rates of the process were also evaluated (Table 5). Highly reproducible values were obtained in terms of PHA yield and maximum PHA concentration, with average values of 0.99 ± 0.07 C_{mol} PHA/ C_{mol} S (0.84 g COD PHA/g COD S) and 1.48 ± 0.14 g PHA/L, respectively.

A slightly higher degree of variability was observed in terms of rates (Table 5). The substrate uptake rate (r_s) and PHA production rate (r_p) presented relative standard deviations of 15% and 12%, respectively. These standard deviations were mainly caused by a decline of the rates in batches 9 and 10, in which the duration of the batches (defined by the depletion of butyrate and thus by the rate of butyrate consumption ($r_{s\text{ but}}$)) increased from 3 h to 5 h and 4 h respectively. In those batches, the $r_{s\text{ but}}$ presented values of around 13 C_{mM}/h , much lower than obtained in the initial test (21.6 C_{mM}/h). Noteworthy, the decrease in $r_{s\text{ but}}$ was not accompanied with a decrease in the specific substrate consumption for butyrate ($q_{s\text{ but}}$), which, on the contrary, increased considerably in all the repeats compared to the initial PHA accumulation test. Nonetheless, an important decrease of the initial biomass concentration (TSS_{ini}) was observed (from 0.77 g/L in the initial test to an average of 0.46 g/L in the repetitions). A linear correlation was observed between the initial TSS concentration and r_s , $r_{s\text{ but}}$ and r_p (significance level (α) < 0.05). No significant correlation was obtained between the TSS and specific substrate consumption rates (q_s and $q_{s\text{ but}}$).

Remarkably, the lower initial TSS was not caused by a decreased biomass at the end of the SBR cycle, which even slightly increased in later cycles of the SBR (Table 5), but was due to a loss of biomass in the settling step between the SBR and the PHA accumulation. The absolute TSS lost in the clarified fraction was quantified to be 8% of the inoculum in the initial test (B90), while around 50% in the batches 9, 10 and 12 (Table 5).

Correlations were also investigated between the observed parameters and the composition of the microbial population used as inoculum (Fig. 4). No significant correlations ($\alpha < 1$) were obtained between any of the rates (r_s , r_p , $r_{s\text{ but}}$, q_s and q_p) and the relative abundance of *Thauera*, *Amaricoccus* or the sum of the two. However, in terms of concentration of those genera (g/L instead of relative abundance), a significant correlation was obtained between $r_{s\text{ but}}$ and r_p with the sum of *Amaricoccus* and *Thauera* ($\alpha < 0.05$). Thus, giving further evidence that the performance of the systems seemed to be governed by the initial cells concentration (TSS), and consequently of the main genera, rather than the relative composition of the culture. It is important to notice though, that these correlations were not established with the biomass composition present at the beginning of the PHA accumulation but with the biomass used as inoculum, which was the main interest of the study and the most likely source of variations. Consequently, possible changes in relative percentages occurring during the sedimentation step, which could have hampered the correlations, cannot be excluded.

Insufficient settling of the biomass can be caused by overgrowth of filamentous bacteria (or filamentous bulking) (Martins et al., 2004; Morgan-Sagastume et al., 2015). Reported filamentous

genera (Nielsen et al., 2009) were found in very minor quantities in the analyzed samples, generally below 0.2%. Only OTUs classified in the genera *Acidovorax* (0.3-1.1%), *Acinetobacter* (0.05-3.4%) and *Dyadobacter* (1.2-9.6%), of which some members are described to have filamentous growth (Chelius and Triplett, 2000; Nielsen et al., 2009), reached higher abundances. Nevertheless, no correlations were observed between the TSS and the relative abundance of filamentous genera, neither individually nor as a sum. On the other hand, bulking effect can also be caused by the overproduction of exopolysaccharides, typically attributed to the genus *Zooglea* (Janarthanan et al., 2016; Jenkins, 1992). Members of the genus *Thauera*, closely related to *Zooglea*, have also been reported to be related to such episodes in activated sludge (Allen et al., 2004; Lajoie et al., 2000). In the present study, the relative abundance of OTUs classified as *Thauera* increased in all PHA accumulation repeats compared to the initial test (Fig. 4). Thus, exopolysaccharides production, as well as its potential causes, such as nutrient availability and toxicity factors (Sheng et al., 2010), should be further investigated as possible elements causing differences in the settlement behavior.

Interestingly, the TSS at the beginning of the batch had a linear correlation with the initial PHA content of the cells (Table 5). High PHA contents are known to favor cell settlement, due to increased density (Korkakaki et al., 2016). Nonetheless, differences observed here (from 1.6-4.4 wt%) could not justify important differences in the cell density. Alternatively, the PHA content at the end of the famine phase could be read as an indication of the physiological state of the cells, affecting their settling behavior. Similar observations were reported in the study of Valentino et al., (2015).

All in all, the causes of insufficient settling should be further investigated and avoided in order to consolidate a robust process and to maximize the transfer of biomass from the enrichment to the PHA accumulation. Alternatively, the implementation of flocculation agents or a centrifugation step could be considered.

3.6 Analysis of the polymer

In order to rule out the presence of any other PHA unit besides the ones measured by GC chromatography (HB, HV, HP), NMR analysis of the polymer was performed. This analysis was especially important given the observed partial consumption of undefined COD during the batches, which could have given unexpected PHA monomers. However, NMR results of the chloroform extracted PHA at the end of the butyrate phase confirmed the presence of only HB and HV monomers. Moreover, HB-HV bonds could be identified in the ¹³C-NMR, revealing that the HB and HV units were in the form of a copolymer P(HB-co-HV) (Fig. A.1 and A.2 Supplementary materials).

SEC analysis displayed a unimodal distribution, corroborating that a copolymer was formed from HB and HV monomers during PHA accumulation (Fig. A.3 Supplementary materials). The weight-average molar mass ($\overline{M_w}$) was 529 kg/mol and the polydispersity index 2.37, which are values in the range of those previously reported for MMC, and a priori acceptable for thermoplastic applications (Laycock et al., 2014).

3.7 Overview of the developed process

The average PHA yield ($0.99 \text{ C}_{\text{mol}} \text{ PHA/C}_{\text{mol}} \text{ S}$; 0.84 g COD/g COD), PHA content (76 wt%) and rate ($1.13 \text{ C}_{\text{mol}} \text{ PHA.C}_{\text{mol}} \text{ X}^{-1} \cdot \text{h}^{-1}$ or $0.41 \text{ g PHA.L}^{-1} \cdot \text{h}^{-1}$) obtained in the present study during the PHA accumulation were in the very high range of results previously reported in the literature (Valentino et al., 2016). The highest yield obtained from crude glycerol in MMC is of $0.51 \text{ C}_{\text{mol}} \text{ PHB/C}_{\text{mol}} \text{ glycerol}$ (Freches and Lemos, 2017), which led to 59 wt% PHA. Considering as well the stoichiometry of the fermentation, 1 C_{mol} of glycerol would produce $0.50 \text{ C}_{\text{mol}}$ of 1,3-PDO and $0.23 \text{ C}_{\text{mol}}$ PHA, resulting in an overall carbon recovery of $0.73 \text{ C}_{\text{mol}}/\text{C}_{\text{mol}}$. This value would be higher than producing PHA directly from glycerol, which has a theoretical maximum of $0.67 \text{ C}_{\text{mol}} \text{ PHB/C}_{\text{mol}} \text{ glycerol}$ due to the CO_2 release occurring during the production of acetyl-coA as intermediate (Moralejo-Gárate et al., 2011).

In terms of total COD, and considering the total COD input of the three steps of the process (consumed or not), the overall COD recovered in the products would be $0.61 \text{ g COD/g COD}_{\text{in}}$ ($0.19 \text{ g COD PHA/g COD}_{\text{in}}$ and $0.42 \text{ g COD 1,3-PDO/g COD}_{\text{in}}$) (Fig.7 B). Remarkably, this value would not increase any further by leaving the substrate to be totally consumed. Moreover, it is important to note that the COD provided in the enrichment represented only 12% of the total input, due to the low organic loading rate of this reactor. The total COD recovered as products was much higher than the maximum overall yield reported from PHA production directly from crude glycerol ($0.32 \text{ g COD PHA/g COD}_{\text{in}}$ in Moita et al., 2014). It was also higher than preliminary results obtained through crude glycerol fermentation and conversion of both VFA and 1,3-PDO to PHA ($0.31 \text{ g COD PHA/g COD}_{\text{total}}$ in Burniol-Figols et al., 2018).

Besides the advantages in terms of COD recovery, the process may also present a value in terms of the type of PHA produced. No other monomers besides HB have been described in PHA produced from direct transformation of glycerol (Zhu et al., 2013). In contrast, the PHA produced here from fermented crude glycerol included both HB and HV units. This could represent a further advantage of the suggested three step process, as P(HB-co-HV) copolymers are described to have better thermoplastics properties than PHB (Laycock et al., 2014).

A future development of the process should include the investigation of higher substrate concentrations, observing possible inhibition phenomena from 1,3-PDO or other inhibitory components in crude glycerol. Moreover, avoiding the use of synthetic substrates in the SBR could be evaluated. Despite the input being minor compared to the total COD (Fig. 7), its substitution would probably benefit the economics of the process. The use of other fermentation effluents containing only VFA or crude glycerol alone could be tested.

4 Conclusions

Using fermented crude glycerol and mixed microbial consortia (MMC), the present study assessed the possibility to selectively convert volatile fatty acids (VFA) into polyhydroxyalkanoates (PHA) while leaving 1,3-propanediol (1,3-PDO) in the supernatant. The following conclusions could be extracted:

- Selective consumption of VFA over 1,3-PDO was attained by using microbial biomass enriched in the absence of 1,3-PDO.
- The PHA accumulation led, on average, to 76 ± 3.1 wt% PHA in the form of a copolymer of P(HB-co-HV), with a complete recovery of 1,3-PDO (99 ± 2.1 %).
- The SBR reactor presented a steady behavior despite changes in the relative percentage of the main genera of the MMC: *Thauera* and *Amaricoccus*.
- Highly reproducible values were obtained in terms of PHA yield and maximum PHA concentration, with average values of 0.99 ± 0.07 C_{mol} PHA/C_{mol} S (0.84 g COD PHA/g COD S) and 1.48 ± 0.14 g PHA/L, respectively.
- Small variations in the substrate uptake rates and PHA formation rates during the PHA accumulation were attributed to variations in the biomass settling behavior prior to the PHA accumulation.
- The MMC presented preference for butyrate over acetate and propionate.
- The microbial culture was not inhibited when exposed to up to 90 C_{mm} of VFA and 150 C_{mm} of 1,3-PDO, and presented a high PHA production rate of 0.41 g PHA.L⁻¹.h⁻¹.
- Taking into account the three steps of the process (crude glycerol fermentation, enrichment and PHA accumulation), the overall COD recovered as products was 0.61 g COD/g COD_{in}, where 0.19 g were in the form of PHA and 0.42 g in the form of 1,3-PDO.

Supplementary data

Appendix A: Supplementary figures of the polymer analysis

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Table 1. SBR cycle and day of the PHA accumulation tests, SBR cycle characterization and microbial samples analysis. The abbreviation BY_x stands for “PHA accumulation batch at Y concentration (C_{mM})” where x is the number of the batch test with 12 batches (including the initial) performed in total. The rates and yields were measured only for the batches indicated in bold.

PHA accumulation test	Cycle SBR	Day SBR	Microbial samples analyzed (time 0)
(SBR cycle characterization)	76	38	x
B45_initial test	86	43	
B60_initial test	88	44	
B75_initial test	120	60	
	126	63	x
B90_initial test	128	64	
B90_2	232	116	
B90_3	244	122	
B90_4	246	123	x
B90_5	248	124	
B90_6	268	134	
B90_7	282	141	
B90_8	284	142	
B90_9	286	143	x
(SBR cycle characterization)	298	149	x
B90_10	300	150	
B90_11	312	156	
B90_12	314	157	x

Table 2. Yields and rates in the SBR cycles 76 and 298.

		Yields					Rates		Specific rates			TSS ^a	PHA ^a
		Y _{P/S}	Y _{P/S}	Y _{Gly/S}	Y _{X/S}	Y _{X/polymers}	-r _s	r _p	-q _s	q _p	μ		
Phase		C _{mol} PHA/ C _{mol} S	g COD PHA/ g COD _{total}	C _{mol} Gly/ C _{mol} S	C _{mol} X/ C _{mol} S	C _{mol} X/ C _{mol} PHA+gly	C _{mM} S/h	C _{mM} PHA/h	C _{mol} S/ (C _{mol} X.h)	C _{mol} PHA/ (C _{mol} X.h)	h ⁻¹	g/L	wt%
Cycle 76	feast	0.68	0.68	0.12	0.04		39.0	26.8	0.98	0.65		1.83	37.3
	famine					0.62		-7.1		-0.19	0.11		
Cycle 298	feast	0.78	0.72	0.14	0.05		34.3	27.1	0.87	0.67		1.88	39.0
	famine					0.88		-8.2		-0.23	0.16		

Y_{P/S}: yield PHA/substrate; Y_{Gly/S}: Yield glycogen/substrate; Y_{X/S}: Yield biomass/substrate; Y_{X/polymers}: Yield biomass/polymers (PHA+glycogen). - r_s: Substrate consumption rate. r_p: PHA formation rate. q_p: Specific PHA formation rate.- q_s: Specific substrate uptake rate. μ: Specific growth rate.

^a TSS concentration and PHA contents (wt%) correspond to the end of the feast phase.

Table 3. Yields and rates from the PHA accumulation experiments with fermentation effluent at increasing initial concentration of VFA.

		Yields			Rates			Specific rates						Recovered
		Y _{P/S}	Y _{P/S}	Y _{X/S}	-r _s	r _p	-q _s	-q _s				q _p	1,3-PDO	
Initial VFA concentration	Phase	C _{mol} PHA/ C _{mol} S	g COD PHA/ g COD _{total}	C _{mol} X/ C _{mol} S	C _{mM} S/h	C _{mM} PHA/h	C _{mol} S/(C _{mol} X.h)	mol S/(C _{mol} X.h)				C _{mol} PHA /(C _{mol} X.h)	%	
								Ac	Prop	But	Ac+But			Total VFA
45 C _{mM}	Overall VFA	1.00	0.82	0.04	21.8	20.2 ^a	0.71						0.57 ^a	100%
	Butyrate	1.07	0.82	-0.03	26.7	28.3	0.73	0.03	0.12	0.63	0.66	0.19	0.89	100%
	Acetate/propionate	0.71	0.84	0.34	9.2	7.7	0.50	0.06	0.22		0.06	0.18	0.10	100%
60 C _{mM}	Overall VFA	0.84	0.82	0.09	19.6	15.9 ^a	0.92						0.50 ^a	98%
	Butyrate	0.96	0.96	0.02	24.6	23.5	0.98	0.02	0.13	0.73	0.75	0.27	0.87	99%
	Acetate/propionate	0.40	0.39	0.33	11.0	4.7	0.79	0.08	0.29		0.08	0.28	-0.05	98%
75 C _{mM}	Overall VFA	0.88	0.64	0.04	19.5	16.7 ^a	0.88						0.57 ^a	96%
	Butyrate	0.97	0.78	-0.04	24.5	23.8	0.72	0.02	0.13	0.73	0.75	0.18	1.00	98%
	Acetate/propionate	0.06	0.28	0.85	9.8	4.3	1.10	0.08	0.29		0.08	0.38	-0.23	96%
90 C _{mM}	Overall VFA	0.81	0.66	0.05	23.2	18.4 ^a	0.85						0.50 ^a	95%
	Butyrate	0.91	0.75	-0.01	29.7	26.8	0.91	0.03	0.13	0.69	0.71	0.25	0.87	97%
	Acetate/propionate	0.22	0.18	0.39	10.3	2.7	0.77	0.04	0.24		0.04	0.26	-0.17	95%

$Y_{P/S}$: Yield PHA/substrate. $Y_{X/S}$: Yield biomass/substrate. $-r_s$: Substrate consumption rate. r_p : PHA formation rate. q_p : Specific PHA formation rate. $-q_s$: Specific substrate uptake rate.

Ac: Acetate. But: Butyrate. Prop: Propionate.

^a Overall PHA rates for the feast phase were not constant given the two distinctive phases. The number in the table is calculated by dividing the increment in the $C_{mM} \text{ PHA}$ or $C_{mM} \text{ PHA} / C_{mM} \text{ X}$ by the time interval and not with the slope as with the other parameters.

Table 4. PHA content (wt%), monomer composition and 1,3-PDO recovery (%) obtained in the PHA accumulation repeats with fermentation effluent at 90 C_{mM} initial concentration of VFA.

Batch	Total PHA (wt%)	HB (wt%)	HV (wt%)	HB:HV ratio	1,3-PDO recovery (%)
B90_2	70.7	57.1	13.5	80.9 : 19.1	100.0
B90_3	74.8	61.8	13.0	82.6 : 17.4	94.4
B90_4	73.1	60.5	12.6	82.8 : 17.2	100.0
B90_5	75.9	63.7	12.1	84 : 16	98.5
B90_6	74.6	65.8	8.9	88.1 : 11.9	99.1
B90_7	79.4	66.8	12.5	84.1 : 15.7	97.8
B90_8	78.7	66.0	12.6	83.8 : 16	98.7
B90_9	81.8	67.1	14.6	82 : 17.8	97.2
B90_10	78.2	65.4	12.7	83.5 : 16.3	99.2
B90_11	78.2	67.3	12.3	86 : 15.7	107.9 ^a
B90_12	75.7	64.2	11.4	84.8 : 15.1	102.4
Av	76	64	12	84 : 16	99
SD	3.1	3.2	1.4	2.0 : 1.8	2.1

^a Value excluded from the average

Table 5. Yields and rates from the PHA accumulation repeats with fermentation effluent at 90 C_{mM} initial concentration of VFA.

Yields				Rates /productivity				Specific rates				PHA ini ^a	PHA end ^b	PHA end ^b	TSS ini ^a	TSS end SBR ^c	TSS lost ^d
Y _{P/S}				-r _s				r _p	-q _s		q _p						
Batch	Duration (h)	C _{mol} PHA/ C _{mol} S	g COD PHA/g COD _{total}	C _{mM} S/h				C _{mM} PHA/h	C _{mol} S/(C _{mol} X.h)		C _{mol} PHA /(C _{mol} X.h)	wt%	wt%	g/L	g/L	g/L	wt%
				Overall	Ac	Prop	But		Overall	But							
B90_initial test	3	0.91	0.75	29.7	0.86	4.2	21.6	26.8	0.91	0.69	0.87	4.4	69.9	1.73	0.77	1.35	8%
B90_4	3	0.99	0.78	21.7	0.11	2.8	18.5	21.4	0.95	0.83	1.03	2.6	73.1	1.36	0.53		
B90_5	3	0.99	0.88	21.8	0.08	2.7	18.8	21.2	1.00	0.92	1.18	2.3	75.9	1.36	0.49		
B90_9	5	1.02	0.88	16.2	0.35	2.3	13.0	16.3	0.89	0.75	1.08	1.8	81.8	1.69	0.43	1.76	57%
B90_10	4	1.08	0.82	16.6	0.01	2.0	14.0	17.5	0.85	0.83	1.13	1.6	82.4	1.52	0.39	1.46	57%
B90_12	3.5	0.89	0.84	22.5	0.73	3.4	17.4	20.5	1.23	0.94	1.21	1.8	75.7	1.48	0.45	1.73	42%
Average repeats	Av	0.99	0.84	19.8	0.22	2.6	16.3	19.4	0.98	0.85	1.13			1.48	0.46		
	SD	0.07	0.04	3.1	0.33	0.5	2.7	2.3	0.15	0.08	0.07			0.14	0.05		

^a at the beginning of the PHA accumulation; ^b at the end of the butyrate phase in the PHA accumulation; ^c at the end of the SBR used as inoculum; ^d lost during the sedimentation step between the SBR and the PHA accumulation

Y_{P/S}: Yield PHA/substrate..- r_s: Substrate consumption rate. r_p: PHA formation rate. q_p: Specific PHA formation rate.- q_s: Specific substrate uptake rate.

Ac: Acetate. But: Butyrate. Prop: Propionate.

Figure captions

Fig. 1. Strategy for production of PHA from volatile fatty acids and recovery of 1,3-propanediol from fermented crude glycerol. (CSTR: Continuous Stirred Tank Reactor, SBR: Sequential Batch Reactor).

Fig. 2. Evolution of the SBR enrichment reactor. A: Duration of feast phase (determined by pO_2 saturation). B: Polymer and monomer content in the cells (g /100 g TSS) at the end of the feast phase.

Fig. 3. Characterization of cycle 76 of the enrichment reactor (SBR). A: Profile of oxygen saturation. B Concentration of substrates (C_{mM}) and $N-NH_3$ (N_{mM}). C: Concentration of products (C_{mM}). D: Polymer and monomer content in the cells (g /100 g TSS).

Fig. 4. Relative abundance of the predominant bacterial genera identified by 16S rRNA gene sequencing at different cycles of the SBR (>1% in at least one of the samples). Numbers within the bars indicate the relative abundance of the correspondent genus. Numbers on top of the bars indicate the summed abundance of OTUs classified as the genera *Thauera* and *Amaricoccus*.

Fig. 5. PHA accumulation experiments with crude glycerol fermentation effluent at increasing initial concentration of VFA. Each experiment corresponds to a column of graphs. A: Profile of oxygen saturation. B: 1,3-PDO concentration (C_{mM}) and undefined COD (mg/L). C: Concentration of substrates (C_{mM}) and $N-NH_3$ (N_{mM}). D: Concentration of products (C_{mM}). E: Polymer and monomer content in the cells (g /100 g TSS). *Undefined COD refers to the difference between the COD measured in the supernatant and the sum of theoretical COD of metabolites measured by HPLC.

Fig. 6. Comparison of calculated parameters from the PHA accumulation experiments with crude glycerol fermentation effluent at increasing initial concentration of VFA. A: Maximum PHA content inside the cells (g polymer/100 g TSS). B: Maximum PHA (g/L). C: $Y_{P/S}$ (Yield PHA/substrate). D: Specific substrate consumption rate. E: Specific PHA formation rate. For all graphs, values for the overall batch (black circle ●) and for the butyrate phase (green triangle ▲) are presented.

Fig. 7. COD Balance of the process with influents indicated in red and effluents in green. Values next to the compounds indicate the g COD consumed or produced for one PHA batch accumulation (90 C_{mM}) (A) and mass fraction of each component referring to the total COD input (g COD/g COD input) (B). For the PHA and 1,3-PDO streams, two sets of values are indicated separated by a bar, corresponding to values obtained after the butyrate phase or after depletion of all VFA. The term “Others soluble” refers to soluble COD excluding glycerol, 1,3-PDO, butyrate, acetate and propionate, thus contemplating mostly undefined COD present in the crude glycerol.

Figure 1

Figure 2
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Figure 7

Appendix A

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