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Butyrate as preferred substrate for polyhydroxybutyrate production

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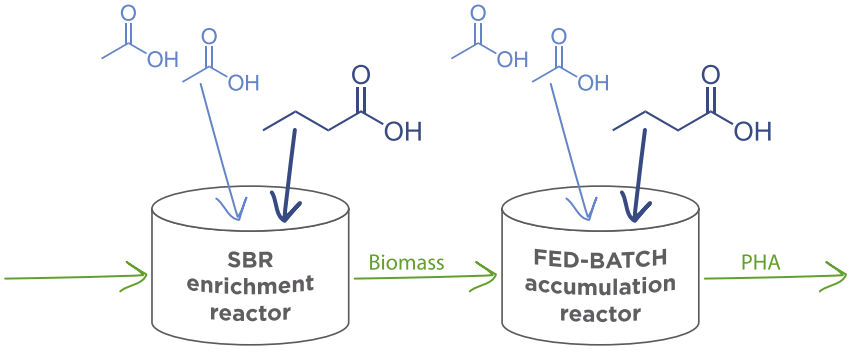
HIGHLIGHTS

- Butyrate is to be preferred over acetate as substrate for PHB production.
- *P. acidivorans* dominates enrichment cultures on both acetate and butyrate.
- Higher PHB production rate and higher PHB yield on butyrate than on acetate.
- Accumulation of 83–85 wt.% PHB in the presence of ammonium.
- To optimize PHB production: optimize butyrate production in pre-fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the suitability of butyrate as substrate for polyhydroxyalkanoate (PHA) production by microbial enrichment cultures was assessed. Two sequencing batch reactors were operated under feast–famine conditions: one fed with butyrate, and another with mixed acetate and butyrate. The obtained results were compared to previous results with acetate as sole substrate. In all three reactors *Plasticicumulans acidivorans* dominated the enrichment culture. The carbon uptake rate and PHA yield were significantly higher on butyrate than on acetate, resulting in a higher PHA production rate. When both substrates were available the bacteria strongly preferred the uptake of butyrate. Only after butyrate depletion acetate was taken up at a high rate. The molar substrate uptake rate remained the same, suggesting that substrate uptake is the rate-limiting step. The results show that for optimized waste-based PHA production the pre-fermentation process should be directed towards butyrate production.

1. Introduction

Polyhydroxyalkanoates (PHAs) are microbial storage polymers accumulated by many different groups of bacteria as an intracellular reserve of energy and carbon (Steinbüchel, 1991). The chemical properties of the polymer are similar to those of petrochemical plastics like polypropylene, and PHA is already commercially available as a fully biodegradable bioplastic. The interest for PHA is broader than just its use as a biopolymer though (Chen, 2009). The methyl esters of its monomers could be used as a biofuel. Moreover, since the polymer is enantiomerically pure, its hydroxy fatty acid monomers could serve as chiral building blocks for the production of all kinds of biochemicals (Chen, 2009).

The use of cheap substrates, such as organic waste, has been investigated by many researchers as a way to reduce the production cost of PHA (Khosravi-Darani et al., 2013; Koller et al., 2010). Pure culture processes require however sterilization of the reactor and liquid streams entering the process. To eliminate this need for axenic conditions – and lower the energy and equipment cost – microbial enrichment cultures can be used (Albuquerque et al., 2010; Bengtsson et al., 2008b; Dionisi et al., 2005b; Jiang et al., 2012). These enrichments of natural bacteria are obtained

Abbreviations: HPLC, high-performance liquid chromatography; HRT, hydraulic retention time; PHA, polyhydroxyalkanoate; PHB, polyhydroxybutyrate; SBR, sequencing batch reactor; SRT, solids retention time; SSqRE, sum of the squared relative error; TSS, total suspended solids; VFA, volatile fatty acid.

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Nomenclature

α	exponent of the PHB inhibition term (–)	m_{ATP}	biomass specific ATP requirement for maintenance (mol/Cmol/h)
δ	efficiency of the oxidative phosphorylation (mol ATP/mol NADH)	q_i	modeled biomass specific production rate of compound i ((C)mol/Cmol/h)
f_{Bu}	fraction butyrate in total substrate uptake (Cmol/Cmol)	q_i^{max}	maximum biomass specific production rate of compound i ((C)mol/Cmol/h)
k	rate constant for PHB degradation ((Cmol/Cmol)$^{1/3}$/h)	$Y_{i,j}$	modeled actual yield of compound i on j ((C)mol/Cmol)
μ	modeled biomass specific growth rate (Cmol/Cmol/h)		
μ^{max}	maximum biomass specific growth rate (Cmol/Cmol/h)		

from activated sludge by subjecting the microbial community to feast–famine conditions, thus generating a competitive advantage for bacteria that store substrate inside their cell as a reserve (Reis et al., 2003). In 2009 Johnson et al. (2009a) reported the enrichment of a culture producing 89 wt.% PHA from acetate in 7.6 h. This still is the highest PHA content reported for a microbial enrichment culture on volatile fatty acids (VFAs). The culture was dominated by *Plasticicumulans acidivorans* – a microorganism with high specific substrate uptake rates for a wide range of VFAs, including butyrate (Jiang et al., 2011d).

Butyrate is an interesting substrate for PHA production for two reasons. First, it has a high theoretical product yield. The stoichiometric yield of PHA on butyrate is 0.94 Cmol/Cmol, which is 40% higher than the yield on acetate (Shi et al., 1997). Second, and more important, butyrate is produced in large amounts during acidogenic fermentation of organic waste streams. The presence of butyrate in, e.g., fermented sugar cane molasses, olive oil mill effluents, paper mill wastewater, waste activated sludge, or food waste has been reported by various researchers (Albuquerque et al., 2007; Beccari et al., 2009; Bengtsson et al., 2008a, 2008b, 2010; Dionisi et al., 2005b; Jiang et al., 2012; Morgan-Sagastume et al., 2010; Rhu et al., 2003). Nevertheless, PHA production from butyrate has hardly been studied. Long-term experiments on the production of PHA from butyrate are limited to microbial enrichments on fermented waste (Albuquerque et al., 2007, 2010, 2013; Bengtsson et al., 2010; Jiang et al., 2012). Due to the complexity of the substrate and the resulting microbial community, these results are not conclusive. The two reports found on experiments with pure butyrate or defined VFA mixtures, Lemos et al. (2006) and Jiang et al. (2012), report the conversion by non-adapted communities only.

The aim of this study was to assess the suitability of butyrate as substrate for PHA production by microbial enrichment cultures. Detailed knowledge on the impact of butyrate on the composition of the microbial community and the kinetics of PHA production enables the prediction of waste stream suitability and optimization of the PHA production process. To study the production of PHA from butyrate two sequencing batch reactors (SBRs) were operated: one on sole butyrate and one on a 1:1 Cmol mixture of acetate and butyrate. These enrichment cultures were compared to a similar PHA-accumulating enrichment culture on acetate.

2. Methods

2.1. Sequencing batch reactor for enrichment of a PHA-producing culture

A double-jacket glass bioreactor with a working volume of 2 L (Applikon, The Netherlands) was used to enrich and maintain the PHA-producing microbial community. The basic setup and operation of the reactor were the same as described by Johnson et al. (2009a). The reactor was operated as a non-sterile SBR with 12 h cycles, each consisting of a start phase (5 min), feeding phase (10 min), reaction phase (685 min), and effluent phase (20 min). Since there was no settling phase, the solids retention time (SRT) equaled the hydraulic retention time (HRT), and both were 1 day. The air flow rate to the reactor was set to 0.2 L_N/min using a mass flow controller (Brooks Instrument, USA). The total gas flow rate through the reactor was increased to 1.4 L_N/min by partial recirculation of the off-gas. The temperature in the reactor was controlled at 30 ± 1 °C, and the pH was maintained at 7.0 ± 0.1 by the addition of 1 M HCl and 1 M NaOH. Controlling of the pumps, stirrer, airflow, temperature, and pH was done by a biocontroller (Biostat Bplus, Sartorius Stedim Biotech, Germany).

The culture previously enriched on acetate by Johnson et al. (2009a) had been maintained for comparison. Biomass from this enrichment culture, highly dominated by *P. acidivorans* (Fig. 1),

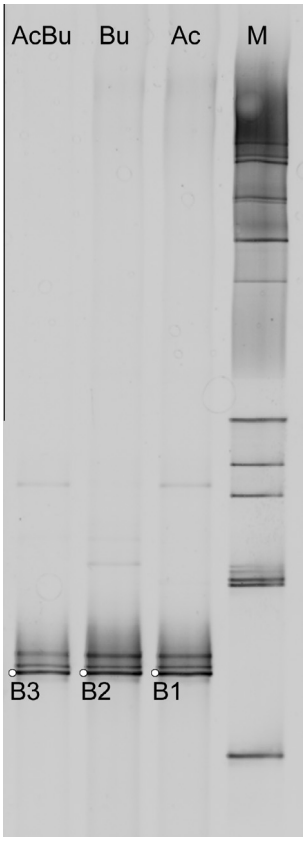

Fig. 1. DGGE gel of PCR-amplified 16S rRNA gene fragments from the enrichment cultures on acetate (lane labeled Ac), butyrate (Bu) and mixed acetate and butyrate (AcBu). SmartLadder (Eurogentec) was loaded in the lane labeled with M. The bands labeled with B1-3 were excised and re-amplified for microbial identification. Previous analyses have shown that the bands just above B1-3 also belong to *P. acidivorans* (Jiang et al., 2011c).