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Polyhydroxybutyrate production by direct use of waste activated sludge in phosphorus-limited fed-batch culture

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HIGHLIGHTS

- PHB production in fed-batch culture with phosphorus growth-limitation is proposed.
- High PHB content (70% in COD) was achieved by direct use of WAS of various origins.
- Dynamics of active bacteria depend on WAS origin and culture conditions.
- The dynamic control of intracellular P is crucial to optimize PHB production.
- PHB production by direct use of WAS could overcome the usual enrichment step.

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ABSTRACT

Polyhydroxybutyrate (PHB) production directly by waste activated sludge (WAS) was investigated in aerobic fed-batch conditions using acetic acid as substrate. PHB production was induced by phosphorus limitation. WAS of different origin were tested with various degrees of phosphorus limitation and PHB contents of up to 70% ($gCOD_{PHB}/gCOD_{particulate}$) were obtained. This strategy showed the importance of maintaining cell growth for PHB production in order to increase PHB concentration and that the degree of phosphorus limitation has a direct impact on the quantity of PHB produced. Pyrosequencing of 16S rRNA transcripts showed changes in the active bacteria of the WAS microbial community as well as the acclimation of populations depending on sludge origin. The monitoring of the process appeared as the key factor for optimal PHB production by WAS. Different strategies are discussed and compared in terms of carbon yield and PHB content with the feast and famine selection process.

1. Introduction

The ever increasing world population produces large quantities of urban and industrial waste. Recycling waste and wastewater to give them a certain added value opens up a real opportunity for reducing both their quantity and their treatment cost. Biological transformations by mixed cultures offer a wide potential for chemical or energy production (Kleerebezem and van Loosdrecht, 2007). In particular, microorganisms present in wastewater are known to store the biopolymer polyhydroxyalkanoate (PHA). This substance is very attractive due to its biodegradability and the fact that its properties are close to those of some petrochemical plastics (polyethylene and polypropylene) (Lee and Choi, 1999).

Currently, industrial production of PHA uses pure microbial cultures and the technical constraints of the process, such as sterility and the need for purified substrates, lead to high production costs (Lee, 1996). Consequently, PHA is not yet competitive compared to conventional plastics. The use of mixed cultures that do not require an axenic environment and that can thus be run as continuous open cultures, together with the use of substrates derived from waste are therefore a very interesting alternative to PHA production based on pure cultures (Dias et al., 2006). In the specialized literature, PHA production by mixed cultures is generally achieved in fed-batch mode by limiting cell growth

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after conducting an enrichment step to increase the fraction of PHA producers in the open culture system. Two main processes have been described for PHA-producer enrichment: the anaerobic–aerobic (AN/AE) process and the feast and famine process (i.e. aerobic dynamic feeding – ADF – system). Detailed reviews of these processes have been published (Dias et al., 2006; Serafim et al., 2008).

The AN/AE system for PHA production includes a culture enrichment step performed in the activated sludge treatment plant (ASTP) in which AN/AE conditions are imposed, followed by an accumulation step for PHA production. This last stage is performed in a reactor fed with fermented substrate containing volatile fatty acids (VFA). The maximum PHA cell content obtained with these cultures is generally low, not exceeding 20% of the dried mass of cells (Satoh et al., 1996), but can be increased by supplying a limited amount of oxygen to the anaerobic zone of an AN/AE system. PHA contents varying from 33% to up to 62% have been reached in this way (Takabatake et al., 2000). Alternatively, by applying a nitrogen (N) limitation in the accumulation stage, an amount of PHA representing 60% of dry cell was produced under aerobic conditions (Bengtsson, 2009).

ADF is a fully aerobic process in which sludge is subjected to consecutive periods of external substrate excess (feast) and starvation (famine). In many cases, the ADF–SBR process is used primarily to select and enrich on PHA-storing organisms. Nowadays, ADF is considered as the most promising strategy for industrial production of PHA because it enables a stable PHA accumulation potential in the fed-batch production stage (Salehizadeh and Van Loosdrecht, 2004), reaching up to 67% without imposing nutrient limitation (Serafim et al., 2004) or up to 89% when N starvation is applied (Johnson et al., 2009) to the accumulation stage. Recently, this strategy has been successfully applied for PHA production with either synthetic or non-synthetic feedstock (Albuquerque et al., 2011). The enrichment step is the key factor for producing PHA at high rates and yields: microorganisms efficiently storing PHA have to be enriched under appropriate conditions, i.e. the process has to be operated with concentrated influent, dynamic feeding, etc. (Beccari et al., 2009). Up to now, only one experiment has shown the capacity of activated sludge to directly store a large quantity of PHA (up to 56.5% of dry cell) under N limitation without acclimation (Mengmeng et al., 2009).

Under N limitation, protein synthesis decreases and the carbon flow is redirected to PHA synthesis. N limitation impacts cell growth by reducing enzymatic activity. In contrast, phosphorus (P) is supposed to be more transferable than N from one component of the cell to another under P limitation, due to its reorganization in the cells (Paul et al., 2012). The major P pools in cells are RNA, DNA and phospholipids which are subject to high turnover (Ehlers et al., 2010). For example, some bacteria are able to partially replace phospholipids by phosphorus-free lipids during P limitation (Zavaleta-Pastor et al., 2010). Moreover, under P limitation ATP synthase activity decreases and the Krebs cycle is restrained, promoting the conversion of excess carbon into PHA (Marzan and Shimizu, 2011). PHA production by axenic cultures appears to be higher under P limitation than under N limitation (Rhu et al., 2003).

The aims of this paper are: (i) to evaluate the capacity of WAS to efficiently accumulate high polyhydroxybutyrate (PHB) content in a fed-batch culture operated with growth limitation by P, (ii) to assess the reproducibility of this production for WAS sampled at different times in one ASTP or in different ASTPs, (iii) to examine the structure of the active bacterial community in activated sludge under these conditions and (iv) to discuss this production strategy by comparing its performance with those of other processes described in the literature.

2. Methods

2.1. Origins of WAS

The initial inoculum was WAS (WAS_1, 2 and 3) from the second aerobic stage of 3 different ASTPs in France (ASTPs_1, 2 and 3, respectively) treating real municipal wastewaters. WAS_1 was collected at 2 different times in ASTP_1: WAS_1a in February and WAS_1b in May 2010. This ASTP currently treats 5100 m^3/day of a conventional urban wastewater from the southwest of France. Its configuration is closed to a modified Ludzack–Ettinger process. WAS_2 and WAS_3 were collected in November 2010 in ASTP_2 and ASTP_3 treating 96,000 m^3/day of the wastewater of Toulouse city (France) and 34,000 m^3/day of the wastewater of Cergy (near Paris, France), respectively. Before starting the experiments, all WAS were filtered at 400 μm, centrifuged and washed in order to remove the supernatant and thus the dissolved salts. In this operation, most of the dissolved P was removed and only the P contained in the biomass remained (mainly P contained or adsorbed on cells).

2.2. Fed-batch reactor operation and monitoring

PHB production was investigated in fed-batch reactors of 8 L working volume for WAS_1a, 9 L for WAS_1b and 6.5 L for WAS_2 and WAS_3. At the incubation stage, the initial volatile suspended solids (VSS) concentration in the fed-batch reactor was 1 g/L. Stirring was ensured by two Rushton turbines with automatic control of agitation speed to avoid any oxygen limitation for the cells (minimum residual oxygen concentration of 2 mg/L). Air flow was maintained at 2 L/min with mass flow controllers. Temperature was set at 25 ± 0.5 °C. The unregulated pH varied from 6.8 to 7.8 according to the feeding times of AA (acetic acid) and P solutions. The home-made Biosr-grl-vfo software was used for online data acquisition (dissolved oxygen, pH and temperature). Oxygen uptake rate (OUR) was measured using a respirometer connected to the reactors. It consisted of a 300-mL chamber with a DO probe (TriOxmatic, WTW) and a peristaltic pump for circulation of the reactor media to the respirometer. OUR was measured by intermittently stopping the circulation pump and recording the DO slope. The feed solution consisted of 261 g/L of AA and 20.6 gP/L of P solution composed of 180 g/L Na_2HPO_4 $12H_2O$ and 12 g/L KH_2PO_4. The mineral medium was composed of 1.2 g/L $C_6H_8O_7FeNH_3$, 0.2 g/L $CaCl_2$ $2H_2O$, 10 g/L $MgSO_4$ $7H_2O$, 100 g/L $(NH_4)_2SO_4$, 3.8 g/L $C_6H_9NO_6$ and 20 mL/L trace element solution (0.3 g/L H_3BO_3, 0.21 g/L $CoCl_2$ $6H_2O$, 0.11 g/L $ZnSO_4$ $7H_2O$, 0.04 g/L $MnCl_2$ $4H_2O$, 0.03 g/L Na_2MoO_4 $2H_2O$, 0.02 g/L $CuSO_4$ $5H_2O$ and 0.01 g/L $NiCl_2$ $6H_2O$). Allylthiourea (20 mg/L) was also added in order to inhibit nitrification.

2.3. Strategy for PHB production

PHB production by WAS was carried out in fed-batch culture mode with a P-growth limitation strategy to induce the polymer synthesis. To ensure that no further nutritional limitation than P limitation during the experimentations, mineral medium was regularly added to maintain the residual salt concentrations in excess, excepted P, compared to growth requirements. Residual N concentration in the supernatant was always higher than 50 mg NH_4^+–N/L. Only PHB production was targeted in this study. Hence, only AA was used as substrate and conditions were aerobic. At time t_0, reactors were inoculated with each of the WAS. Then, a first phase (around 10 h) was dedicated to the depletion of residual P (adsorbed on the VSS corresponding to about 1 mgP/gVSS). Thus, only AA and mineral medium (if necessary) were fed to the reactor