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Mixed culture polyhydroxyalkanoates production from sugar molasses: The use of a 2-stage CSTR system for culture selection

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ABSTRACT

Polyhydroxyalkanoates (PHAs) are promising biodegradable polymers. The use of mixed microbial cultures (MMC) and low cost feedstocks have a positive impact on the cost-effectiveness of the process. It has typically been carried out in Sequencing Batch Reactors (SBR). In this study, a 2-stage CSTR system (under Feast and Famine conditions) was used to effectively select for PHA-storing organisms using fermented molasses as feedstock. The effect of influent substrate concentration (60–120 Cmmol VFA/L) and HRT ratio between the reactors (0.2–0.5 h/h) on the system's selection efficiency was assessed. It was shown that Feast reactor residual substrate concentration impacted on the selective pressure for PHA storage (due to substrate-dependent kinetic limitation). Moreover, a residual substrate concentration coming from the Feast to the Famine reactor did not jeopardize the physiological adaptation required for enhanced PHA storage. The culture reached a maximum PHA content of 61%. This success opens new perspectives to the use of wastewater treatment infrastructure for PHA production, thus valorizing either excess sludge or wastewaters.

1. Introduction

Polyhydroxyalkanoates (PHAs) are a group of bio-based microbially produced polyesters which are fully biodegradable, biocompatible and present thermoplastic properties similar to those of a number of conventional polyolefins (e.g. PP, PE), making them very promising bulk materials for a significant number of industrial applications (Cranc and Pattel, 2005). However, to this day, PHAs have not yet entered bulk materials markets due to high production costs (Choi and Lee, 1997).

In recent years, research has focused on the development of alternative cost-effective processes for PHA production, including the use of low value substrates (agricultural or industrial waste or surplus feedstocks) (Kim, 2000; Khanna and Srivastava, 2005; Lee et al., 2008; Reddy et al., 2003) and mixed microbial cultures, MMC (reviewed by Dias et al. (2006) and Serafim et al. (2008)). The combination of the two allows decreasing operating costs by reducing the cost of substrate and that of the energy used (since no sterilization is required).

The main challenge in PHA MMC processes relates to selection of a culture with high PHA-storing capacity. Enrichment in PHA-accumulating organisms is generally carried out by subjecting mixed cultures to transient conditions of carbon supply, designated as Aerobic Dynamic Feeding (ADF) or Feast and Famine (*FF*). This process configuration originates periods of excess (Feast) and lack (Famine) of external carbon substrate resulting in the selection of microbial populations with an enhanced capacity to store PHA (van Loosdrecht et al., 1997; Majone et al., 1996).

Most MMC PHA production processes are operated as side stream processes in which culture selection is carried out separately from subsequent PHA accumulation (Albuquerque et al., 2007, 2010; Beccari et al., 1998, 2009; Beun et al., 2002; Dionisi et al., 2004, 2005, 2006; Johnson et al., 2009a; Lemos et al., 2006; Serafim et al., 2004). The selection step is generally carried out in Sequencing Batch Reactors (SBR), whereas PHA accumulation using the enriched culture is carried out in batch mode, thus allowing the two reactor systems to be operated independently (different optimum conditions – namely different nutrient concentrations – were shown to favour each step).

Until recently, most studies on MMC PHA production processes used chemically defined media supplemented with synthetic organic acids. More recently, agro-industrial wastes and by-products have also been tested as feedstock for PHA production by several authors (municipal wastewaters (Coats et al., 2007); olive oil mill effluents (Beccari et al., 2009; Dionisi et al., 2005); sugar molasses (Albuquerque et al., 2007, 2010); paper mill effluent (Bengtsson et al., 2008)) in order to effectively contribute to the reduction of

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PHA production costs. However, unlike most pure cultures, mixed cultures submitted to ADF conditions do not store carbohydrates as PHA but rather as glycogen (Carta et al., 2001; Dircks et al., 2001). Therefore, PHA production from carbohydrate-rich raw materials requires a previous acidogenic fermentation step in order to transform the sugars in the wastes/surplus streams into organic acids, such as VFA, which can be effectively stored as PHA by mixed microbial cultures. The use of MMC and fermented feedstocks allows for the production of co-polymers with a broad range of monomer compositions (thus offering the possibility of producing PHA with a wide range of thermal and mechanical properties) without the need for added co-substrates as is the case for pure culture fermentations (that produce solely PHB from sugar based compounds).

In order to improve the efficiency of MMC PHA production, a number of authors have attempted to maximize PHA storage efficiency in the final production stage through optimization of the culture enrichment stage (e.g. Albuquerque et al., 2007, 2010; Bengtsson et al., 2008; Beun et al., 2002; Dionisi et al., 2004, 2006; Johnson et al., 2009a; Jiang et al., 2009; Serafim et al., 2004). Such optimization is based on the selection principles resulting from the different operating conditions imposed on the culture enrichment system and has been designated as eco-biotechnology (Johnson et al., 2009a). Using this type of approach, Johnson et al. (2009a) and Albuquerque et al. (2010) obtained very high PHA performances in the final batch production stage (reaching 89% and 74% PHA content in batch studies using acetate and fermented molasses, respectively) by optimizing the selection efficiency of the respective culture selection SBR systems.

SBR are the configuration most commonly used to carry out culture selection in mixed culture PHA production processes (e.g. Albuquerque et al., 2007, 2010; Beccari et al., 2009; Beun et al., 2002; Dionisi et al., 2004, 2005; Dionisi et al., 2006; Johnson et al., 2009a; Serafim et al., 2004). As an alternative to SBR, one study (Bengtsson et al., 2008) reports the use of continuous reactors to select for PHA-accumulating organisms under Feast and Famine conditions. The system, including two sequentially disposed continuous reactors followed by a settler, simulated a wastewater treatment plant (WWTP) configuration. In this type of system, the hydraulic retention times (HRT) of the first and second reactors corresponded to the Feast and Famine phases of the SBR cycle. The culture thus selected reached a maximum PHA content of 53% in nutrient-deficient batch accumulation studies, however, its kinetic performance was relatively low (a maximum PHA storage rate of 0.06 Cmol PHA/Cmol *X* h was reported).

Although the influence of several reactor operating conditions on PHA storage in the enrichment SBRs has been investigated by a number of authors using mostly synthetic media (reviewed by Dias et al. (2006)), the mechanisms which either enhance or impair the selective pressure for PHA-storing organisms are yet to be fully elucidated, particularly when complex substrates are used as feedstock for the culture enrichment step. Moreover, the generalization of the ecological selection principles resulting from operating conditions, so far studied almost exclusively on SBRs, to continuous reactors subjected to *FF* conditions remains to be demonstrated.

In order to determine the impact of reactor operation mode on PHA-accumulating culture selection under *FF* conditions, a 2-stage CSTR system was used to carry out culture enrichment in a 3-stage PHA production process using a sugar molasses feedstock. The effect of different operating parameters such as influent substrate concentration and ratio between the HRT of the two CSTRs on the selected culture's PHA storage and cell growth was assessed. Moreover, the subsequent impact of culture enrichment on the final PHA accumulation stage was evaluated.

2. Methods

2.1. Experimental setup

The experimental setup consisted of three bench-scale reactor systems and a hollow fiber membrane filtration module (Fig. 1). The molasses acidogenic fermentation (step 1) was carried out in a continuous stirred tank reactor (CSTR) operated under anaerobic conditions. The reactor effluent was clarified by microfiltration and the clarified fermented molasses was used as a feedstock for culture selection (step 2) and PHA batch accumulation (step 3). Selection of PHA-accumulating cultures (step 2) was carried out in a 2-stage CSTR system: composed of two continuous reactors, sequentially disposed, followed by a settler. The enrichment reactor system was designed to impose Feast and Famine conditions, by feeding the carbon substrate to the first reactor (Feast reactor), the resulting effluent passing (by overflow) to the second reactor (Famine reactor). The sludge was recirculated to the first reactor following the settling stage, while the exhaust supernatant was discharged by overflow. PHA accumulation (step 3) was carried out in a batch reactor inoculated with sludge from the culture

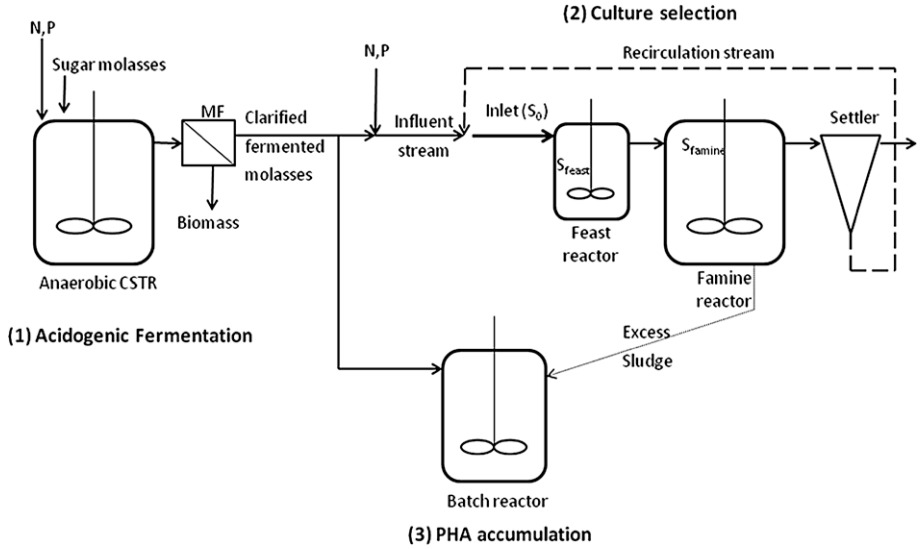

Fig. 1. 3-Stage PHA production process from sugar cane molasses using a system composed of two sequentially disposed continuous reactors operated under Feast and Famine conditions to carry out culture enrichment.