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Polyhydroxyalkanoates production with mixed microbial cultures: from culture selection to polymer recovery in a high-rate continuous process

Research Paper

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Polyhydroxyalkanoates (PHA) production with mixed microbial cultures (MMC) has been investigated by means of a sequential process involving three different stages, consisting of a lab-scale sequencing batch reactor for MMC selection, a PHA accumulation reactor and a polymer extraction reactor. All stages were performed under continuous operation for at least 4 months to check the overall process robustness as well as the related variability of polymer composition and properties.

By operating both biological stages at high organic loads (8.5 and 29.1 gCOD/L d, respectively) with a synthetic mixture of acetic and propionic acid, it was possible to continuously produce PHA at 1.43 g/L d with stable performance (overall, the storage yield was 0.18 COD/COD). To identify the optimal operating conditions of the extraction reactor, two digestion solutions have been tested, NaOH (1 M) and NaClO (5% active Cl_2). The latter resulted in the best performance both in terms of yield of polymer recovery (around 100%, w/w) and purity (more than 90% of PHA content in the residual solids, on a weight basis). In spite of the stable operating conditions and performance, a large variation was observed for the HV content, ranging between 4 and 20 (%, w/w) for daily samples after accumulation and between 9 and 13 (%, w/w) for weekly average samples after extraction and lyophilization. The molecular weight of the produced polymer ranged between 3.4×10^5 and 5.4×10^5 g/mol with a large polydispersity index. By contrast, TGA and DSC analysis showed that the thermal polymer behavior did not substantially change over time, although it was strongly affected by the extraction agent used (NaClO or NaOH).

Introduction

Polyhydroxyalkanoates (PHA) are biologically synthesized polyesters completely biodegradable to water and carbon dioxide and therefore considered among the most promising substitute of oil-based synthetic polymers [1,2]. Presently, industrial processes for PHA production are based on the use of pure cultures of selected strains (e.g. *Cupriavidus necator*) and of *ad hoc* designed unbalanced growth media (e.g. glucose and propionic acid in a N-poor mineral medium) [3]. Hence, PHA production is expensive, mostly because of the costs of culture maintenance, substrate formulation and both substrate and reactor sterilization [4]. Even though not at the industrial scale yet, the production of PHA by using mixed microbial cultures (MMCs) appears promising because it does not require to maintain sterile conditions and it makes easier the use of low-cost feedstocks, such as agro-industrial waste effluents [5,6]. The MMC-based PHA production process brings the advantage of simultaneously reducing the polluting load of the waste stream and requires different stages which are strictly interconnected (Fig. 1).

Indeed, the physical and mechanical properties of the final PHA also depend on its monomeric composition, which in turn is

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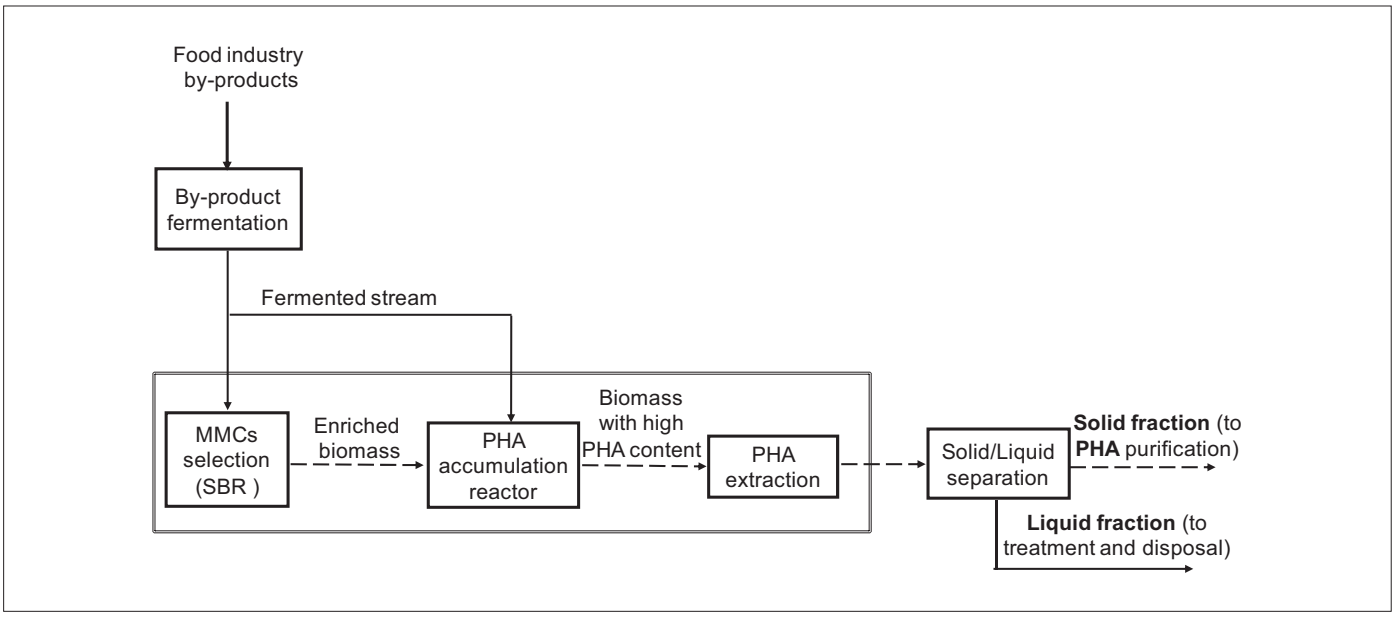

FIGURE 1

Multi-stage process for PHA production by food industry by-products with mixed microbial cultures (solid lines refer to liquid fluxes, dotted lines refer to biomass fluxes). The three stages in the box have been investigated in this paper under continuous-flow conditions.

influenced by the type of organic acids obtained during the acidogenic fermentation [7,8]. The VFA-rich fermented stream is fed into the two successive stages aimed at the selection and production of PHA-storing microorganisms and the subsequent polymer accumulation, respectively. Culture selection can be achieved under conditions of feast and famine (FF) regime, usually established in a sequencing batch reactor (SBR) [9]. The ratio between the length of the feast and famine phase (*F*/*F* ratio) is a crucial parameter affecting the performance of the selection stage [10]. In general, low *F*/*F* ratio ensures the selection in the MMC of microorganisms that are most able to store PHA and their physiological adaptation toward PHA synthesis in the feast phase. Another objective of the selection stage is to produce the PHA-storing culture at the highest possible productivity, that is, the amount of biomass produced per unit of volume of reactor and per unit of time. To achieve high biomass volumetric productivities, the SBR needs to be operated at organic load rates (OLR) as high as possible, which corresponds to high influent substrate concentration and/or short hydraulic retention time (HRT). Previous investigation from this research group showed that low *F*/*F* ratio and related good selection of PHA storing microorganisms can be steadily obtained at OLR as high as 8.5 gCOD/L d whereas unstable or poor performance were observed at higher OLR [11].

The successive stage is aimed at PHA accumulation in microbial cells and its efficiency strongly depends on the storage capacity of the selected culture. A highly relevant parameter is the maximum attainable polymer content in the biomass that can decrease the polymer extraction cost. In general, if the accumulation stage is performed under nutrient-limiting conditions the driving force for storage will be kept high until a PHA saturation level is reached. On the contrary, if the biomass is continuously exposed to nutrients and carbon substrates (nutrient-rich conditions) a growth response will progressively increase whereas the storage response will progressively decrease and, as a consequence, the maximum PHA content will be lower than the cells' maximum storage capacity [12]. By contrast, the PHA volumetric productivity could be higher because of simultaneous growth and PHA storage have also been reported to be maintained for some time [13].

As for PHA extraction and recovery, a large array of extracting agents and operating conditions have been tested, mostly using pure cultures [14,15]. Among them, several chemical digestion methods have been adopted, which consist in using a chemical solution to disrupt the cell wall and release the intracellularly accumulated PHA while possibly limiting polymer degradation. However, the chemical solution used strongly affects the quality of the obtained PHA, in terms of purity and molecular weight, which in turns affects the final polymer application [16].

Substantial research efforts are being made at lab-scale level on MMC-based PHA production, mostly focusing on the SBR selection stage; the related performance of the enriched MMC in the following accumulation step is then typically investigated by single batch experiments [17,18]. By contrast, the process scale up requires that each stage of the process be continuously operated (even if with intermittent feeding) with the length of the different stages being appropriately tuned to each other. This issue is also relevant because concerns are often raised about the possible lack of reproducibility of process performance and polymer properties when dealing with MMC-based continuous processes, with respect to pure culture batch processes [19].

Based on these considerations and according to the scheme of Fig. 1, this research investigated a lab-scale process with continuous sequential operation of three key stages, from (a) the selection of PHA-storing microorganisms to (b) PHA accumulation and (c) polymer extraction. Finally, a solid liquid separation was performed and the produced polymer was recovered in the form of a dry powder.

Based on long-term operation of this continuous sequential process, main attention has been paid at evaluating the process