Continuous process for Polyhydroxyalkanoates production with mixed microbial cultures

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Nowadays, we are facing with the constant increase of organic wastes in the worldwide certainly due the human activities, this negatively affects the environment and strategies to dispose of these wastes possibly valorizing them are required. As an example, the production of bioplastics through a biorefinery process is one of the possible solutions for waste valorization. Indeed, most of the plastic material present in the market came from the petroleum industry and are typically not biodegradable thus they can remain for a long period of time in the environment causing serious concerns. Nevertheless, there is a group of biopolymers known as PHA (Polyhydroxyalkanoates) that represent a family of polyesters with characteristics able to substitute conventional plastics (PE, PP, PS etc.) in a wide range of applications ranging from packaging to the medical sector. PHA are generally obtained during the cultivation of pure cultures of specific microorganisms (*Escherichia coli* recombinant, *Cupriavidus necator* etc.) that can convert the carbon source in PHA granules inside the cell by the synthesis of specific enzymes. The use of pure cultures presents the advantage to reach up to 75% (w/w) of the intracellular PHA content (Kedia et al., 2014), however it requires the need of sterile conditions to prevent the contamination, strongly affecting the final PHA cost (higher than 4 €/kg). However, to reduce the production cost an alternative consists in the use of mixed microbial cultures (MMC) which to not require sterile conditions and can be fed with waste feedstocks. The process that involves MMC for the PHA production involves different stages that include the acidogenic fermentation of the feedstock to produce a stream rich of VFA that are direct substrates for PHA production with MMC (Valentino et al., 2018). Also, a selection stage is required to enrich an activated sludge in PHA – storing microorganisms, as well as PHA accumulation stage to maximize the intracellular PHA content of the selected culture. Among these three stages an important role is played by the microbial selection stage. Conventionally, the selection process occurs in a SBR (sequencing batch reactor) in which a particular growth condition, referred to as feast and famine regime, is performed. This regime consists in the alternance of presence (feast) and absence (famine) of the external carbon source. The latter is stored during the feast phase to be used as intracellular carbon source for microbial growth during the famine phase (Lorini et al., 2020). An SBR is operated through a sequence of cycles that are characterized by different phases, such as the C feed, the biomass withdrawal, the N feed, etc. Hence, the utilization of large pumps and oversized liquids/solids separation instruments are required, that could rise the productions costs at industrial plant applications.

This work deals with the development of an innovative continuous process for the selection of PHA-storing microorganisms, as shown in **figure 1**. In this process a spatial selection in two separated reactors was applied. The first one was a reactor with a plug flow (PF) configuration with 1L working volume in which the feast condition was applied whereas the second one was a Continuous Stirred Tank Reactor (CSTR) with 5L working volume in which the famine condition was performed. The feeding solution was only fed to the Feast reactor and the effluent of this reactor was in part discharged, and in part recirculated into the Famine reactor, from which by overflow the liquid phase was recirculated again to the Feast reactor. The process was operated in continuous without any interruption for all the duration of the experiment (in total around 200 days). This configuration was adopted since the PF reactor theoretically allows maintaining a gradient of substrate concentration from the inlet (corresponding to the maximum concentration) to the outlet (minimum concentration), hence ensuring a low or no presence of organic substrates entering the famine CSTR reactor. A coupled feeding of C and N – source was adopted and as C – substrate a synthetic mixture of 65 % (of the overall chemical oxygen demand, COD) of acetic acid and 35 % of propionic acid was used. In order to identify the optimal operating conditions of the system, in terms of PHA production, different parameters have been studied such as the Recirculation Factor (R_C), identified as the ratio between the recirculation flow rate (Q_R) and the feeding flow rate (Q_E), and the applied organic load rate (OLR). In particular, four different values of R_C (from 1 to 8) and two ORL values (between 2,12 and 8,50 gCOD/Ld) have been investigated. Also, with the biomass selected at all investigated conditions at least three accumulations trials in a Batch reactor (fed Batch modality) were performed to maximize the intracellular

PHA content. As a main result, it was found that at a fixed OLR of 2.12 gCOD/Ld the intracellular PHA content in the feast reactor increased to over 30 % (w/w) by increasing the R_C up to 4. As for the HV content in the stored copolymer, this parameter was not significantly affected by the applied operating conditions. Overall, the obtained results indicate that the continuous configuration is promising for PHA production with MMC, also taking into account that the process can be operated without the utilization of oversize equipment's minimizing the production costs.

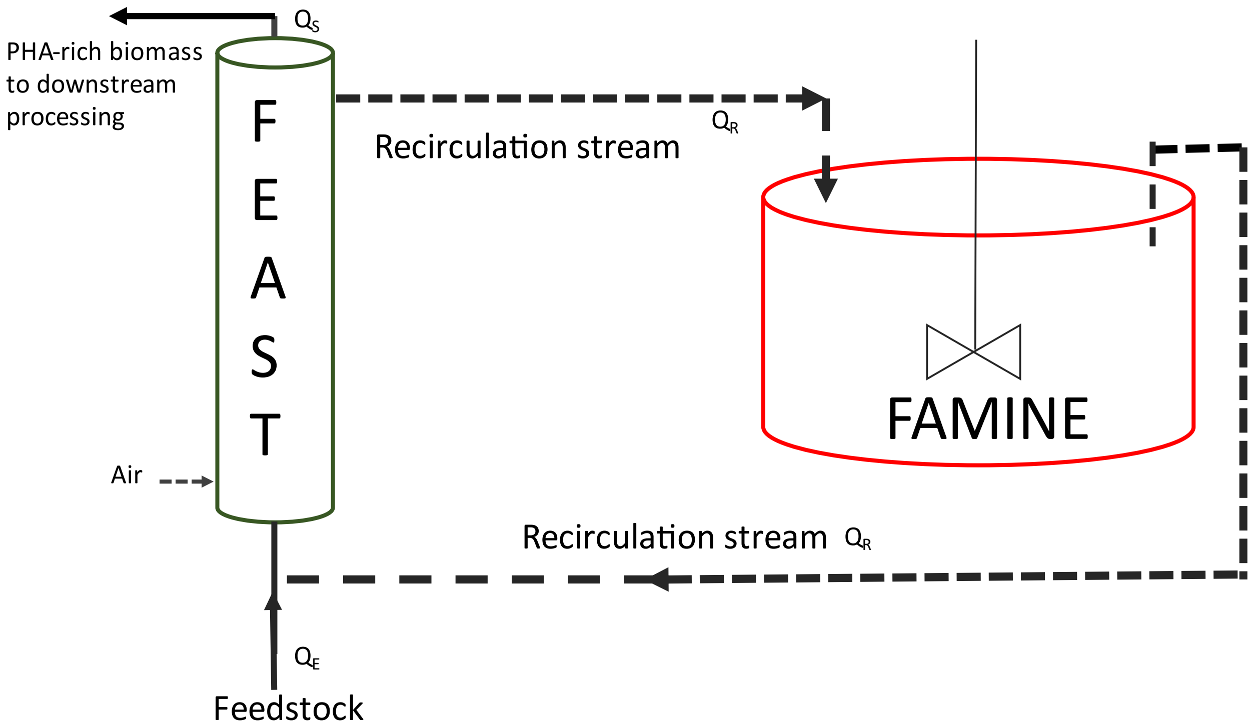

Fig 1: Continuous process for the selection of PHA-storing microorganisms from an activated sludge.

References

Kedia, G., Passanha, P., Dinsdale, R. M., Guwy, A. J., & Esteves, S. R. (2014). Evaluation of Feeding Regimes to Enhance PHA Production Using Acetic and Butyric Acids by a Pure Culture of Cupriavidus necator. *Biotechnology and Bioprocess Engineering*, *19*, 989–995. https://doi.org/10.1007/s12257-014-0144-z

Lorini, L., di Re, F., Majone, M., & Valentino, F. (2020). High rate selection of PHA accumulating mixed cultures in sequencing batch reactors with uncoupled carbon and nitrogen feeding. *New Biotechnology*, *56*, 140–148. https://doi.org/10.1016/J.NBT.2020.01.006

Valentino, F., Gottardo, M., Micolucci, F., Pavan, P., Bolzonella, D., Rossetti, S., & Majone, M. (2018). *Organic Fraction of Municipal Solid Waste Recovery by Conversion into Added-Value Polyhydroxyalkanoates and Biogas*. https://doi.org/10.1021/acssuschemeng.8b03454

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