Valorisation of ovine cheese whey through PHA production

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Abstract

In the present study, PHA production from ovine cheese whey (oCW) through a 3-step process (dark fermentation, biomass selection and PHA accumulation process) was investigated. Different operating pHs were adopted during the fermentation step and no external nutrients were supplied at any step of the process. Results showed that the production of PHA from oCW is a promising valorisation approach.

Keywords: ovine cheese whey; PHA; mixed microbial cultures

1. Introduction

Cheese whey is the main biowaste product of dairy industry and, at present, few, partial and sometimes unprofitable options for its proper management are available. An environmentally-sound management of residues should be based preferably on the recovery of high added value compounds and energy, which should be optimized both quantitively and qualitatively. As far as biodegradable wastes are concerned, such ambitious valorisation target is fully included in the concept of waste-biorefinery. In this framework, the aim of the present study was to investigate the use of ovine cheese whey (oCW) to obtain a specific kind of biopolymer, named polyhydroxyalkanoates (PHA), known to be produced from various carbon sources and accumulated under the form of granules by different bacterial genera. In the proposed process scheme the high organic content of oCW is firstly converted through dark fermentation (DF) (step 1) into a mix of organic acids (OA) with simultaneous recovery of appreciable amounts of biohydrogen. The obtained OA-rich stream is used either for selection and enrichment of PHA-storing microorganisms (from mixed microbial cultures, MMC) (step 2), or for feeding the previously selected biomass in an PHA accumulation reactor (step 3). Framed in a more general project, the activity presented here focused on understanding the effect of the oCW protein content on both steps 2 and 3, with the purpose of assessing whether the peculiar characteristics of oCW permit to avoid the addition of nutrients during the selection process and, at the same time, do not affect the PHA accumulation step, which requires low contents of N with respect to the available C. Furthermore, the influence of the operating pH adopted during the fermentation phase was assessed in terms of onset of specific metabolic pathways, type of acid produced and, in turn, PHA composition and accumulation yield.

2. Experimental Set-Up

The step 1 was performed on raw oCW (TOC=27 g L^{-1}) in a 2-L batch reactor operated for 7 days under anaerobic mesophilic (39°C) conditions, without any addition of inoculum nor any substrate pre-treatment. Two operating pHs (6.0 and 7.5) were adopted in this step. The step 2 was carried out using an aerobic sequencing batch reactor (4 L, 25°C, HRT=1 d, SRT=4 d), operated under a feast and famine (F/F) regime, inoculated by an MMC derived from activated sludge sampled at a full-scale wastewater treatment plant. Sodium acetate (AC) and oCW fermented at different operating pH (FCW-6 and FCW-7.5) were used as substrate for step 2. When AC was fed to the reactor, a synthetic medium was added too (Duque et al., 2013). The organic loading rate was kept in the range of 1.17±0.07 $g_{COD\text{-}OA}$ L^{-1} d^{-1}. The step 3 was performed in an aerobic reactor operating in fed-batch mode (1-L, 25°C), adopting a pulse-wise feeding method controlled by the dissolved oxygen concentration (Duque et al., 2013), using the PHA-storing bacteria previously selected and AC, FCW-6 and FCW-7.5 as substrates.

3. Results

In the step 1, carbohydrates (55 $g_{COD\text{-}sCarbo}$ L^{-1}) were converted through DF to a mix of OA, optimal precursors for PHA synthesis, with a yield ($Y_{OA/sCarbo}$) of 0.84 and 0.71 $g_{COD\text{-}OA}$ $g_{COD\text{-}sCarbo}^{-1}$, for FCW-6 and FCW-7.5, respectively. Both FCWs showed similar total OA content (46.6 and 39.4 $g_{COD\text{-}OA}$ L^{-1}, respectively) but different OA composition, as a consequence of different operating pH adopted and resulting metabolic pathways. FCW-6 contained butyrate, propionate, lactate, acetate and valerate in the proportion of 51/32/13/3/1 (%, COD_{OA} basis), while FCW-7.5 contained propionate, acetate, butyrate, lactate in the proportion of 35/32/24/9 (%, COD_{OA} basis). This difference was expected to be mirrored by PHA composition (Duque et al., 2013). The

C/N ratio in the fermentation outflow, calculated considering the soluble organic carbon and nitrogen, the latter deriving from proteins (10 g L^{-1} in oCW) and ammonia, was higher for FCW-6 (C/N=24) than for FCW-7.5 (C/N=12). The differences in terms of C/N values derive from the different TOC and proteins removal efficiencies observed for the different operating pH values. In this respect, the pH confirms to be the pivotal operating parameter in fermentation processes. Indeed, the test conducted at pH 6 showed a greater TOC and protein removal (24% and 61%) associated with higher TOC conversion in OAs. The attention given to the C/N observed for the fermentation outflows is deserved in relation to the effects that it exerts on the following phases.

During the entire step 2, the feast to famine ratios (calculated as the ratio between the lengths in hours of the two phases, F/F) resulted to be in the range 0.16-0.24. As stated by Valentino et al. (2014), a F/F ratio should not be higher than 0.33 to obtain a correct selection of a PHA-storing biomass. The PHA-accumulation performance parameters, calculated as indicated by Duque et al. (2013), are presented in Table 1. The tests performed using AC as substrate show values in line with those reported by Valentino et al. (2017), confirming the possibility to select a PHA-producing biomass characterised by good storage capacity from MMC under the conditions adopted. The produced polymers consisted exclusively of 3-hydrobutyrate, as expected.

The accumulation tests performed using FCW-6 exhibited lower performance compared to AC, as expected when a real waste feedstock is processed. Nevertheless, the obtained results in terms of PHA content and $Y_{PHA/OA}$ look promising and suggest that a biomass with good-storage capacity could be selected using FCW-6 even if no external nutrient was added during the selection step. This result is not trivial; in fact, the availability of the nitrogen contained in proteins-rich substrates such as CW is questionable and matter of discussion since it may be limited by the culture's metabolizing capacity (Oliveira et al., 2018); this is precisely the reason why the addition of an easily bioavailable nitrogen source during the selection of PHA-accumulating cultures is a practice widely reported in literature. In this study, the selected bacteria were able to exploit the high oCW protein content for their growth, as confirmed by a specific protein consumption ($-q_{PROTE}$) of 0.7 mg_{PROTE} mg_X^{-1} h^{-1}.

On the other hand, the nitrogen made available by the protein degradation somehow affected the accumulation step which, as mentioned before, requires nutrient starvation conditions to minimise the onset of active biomass growth. The accumulation tests performed using FCW-7.5 showed a lower performance in terms of final PHA content (183 vs. 351 g_{PHA}/kg_{VSS}) with respect to FCW-6, probably due to the lower C/N ratio. Furthermore, the pulse-wise feeding operational mode, leading to protein accumulation in the medium, could represent a further limiting factor, since it increases the risk of growth-response overtaking storage-response.

Regarding polymer composition, the co-polymer produced from FCW-6 was composed of 3-hydrobutyrate (HB, 66%) and 3-hydroxyvalerate (HV, 34%), whilst a co-polymer composed mainly of HB (76%) and having a lower content of HV (24%) was obtained using FCW-7.5. The possibility to influence the presence of HV by controlling, through the proper choice of the operating pH, the fermentative metabolic pathways and, in turn, the type of OA to be fed to the PHA storing biomass, is interesting in light of the benefits achievable in terms of polymers physical characteristics such as crystallinity, brittleness and flexibility.

Table 1. Accumulation tests performance parameters.

Parameters	AC	FCW 6	FCW 7.5
PHA content (g_{PHA} kg_{VSS}^{-1})	492±68	317±47	183±64
Polymer Composition (ΔHB:ΔHV) (%, W/W)	100:0	66:34	76:24
$-q_{OA}$ (g_{COD-OA} g_{COD-X} h^{-1})	0.61±0.01	0.53±0.08	0.26±0.08
q_{PHA} ($g_{COD-PHA}$ g_{COD-X} h^{-1})	0.42±0.02	0.21±0.01	0.09±0.03
$Y_{PHA/OA}$ ($g_{COD-PHA}$ g_{COD-OA}^{-1})	0.67	0.40	0.33
PHA productivity (g_{PHA} g_{VSS}^{-1} h^{-1})	0.34±0.04	0.16±0.04	0.10±0.01
$-q_{PROTE}$	n.a.	0.05±0.02	0.06±0.01

4. Conclusions

Results obtained in the present study show that PHA can be efficiently produced from oCW through the proposed 3-step process. By setting and controlling the operating pH value of the fermentation step, a mix of different organic acids can be produced as PHA precursors, influencing the final composition of the polymer. Moreover, the experimental data demonstrated that, while the fermented oCW protein content provides enough nitrogen to avoid the need for external addition during the biomass selection step, it can also limit the nitrogen-starving conditions required for achieving high PHA saturation levels. Therefore, further tests aimed at optimizing the operating conditions of the fermentation and accumulation phases are currently underway.

References

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