Evaluation the use of food waste-derived volatile fatty acids (VFAs) effluent on polyhydroxyalkanoates (PHAs) production by *Cupriavidus necator* DSM 545

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Introduction

Starch and sugar-based substrates has been the main feedstock in polyhydroxyalkanoates (PHAs) production. The use of which, however, accounts for a significant share of up to 30 – 50% in the total production cost that highly hinders the PHA commercialization. Fermentable compounds such as volatile fatty acids (VFAs) have emerged as promising candidates to replace the expensive conventional feedstock in PHA production. VFAs not only engage directly in PHA biosynthesis but also can be produced in a large amount via acidogenic fermentation of various organic waste. Therefore, the aim of this study was to employ food waste-based VFA stream as the economical feedstock for PHA production. The application of VFAs in PHA-bearing microbial cultivation is believed to increase the cost-competitiveness of PHA production and the sufficiency in resource uses, fitting the concept of bioeconomy.

Material and methods

Synthetic VFAs with different loadings were initially employed to determine the feasible VFA concentrations for the growth of *Cupriavidus necator* DSM 545. Afterwards, a mixture of synthetic VFAs mimicked real VFA effluent of 4.45 g/L was employed to evaluate the bacterial growth in VFA mixture. Lastly, the cultivation of *C. necator* was conducted using real VFA effluent and without any nutrient supplement. All experiments were executed in duplicate using a 2-L continuous stirred tank reactor (CSTR) at 32˚C, 120 rpm and pH control at 7 using 2M NaOH and 2M HCl. In prior to each cultivations, the media preparations were performed in sterilized conditions.

The VFA effluents used in this study were produced and recovered from acidogenic fermentation of food waste provided by Wainaina et al. (2020). Cell density, substrate concentration, biomass and PHA accumulation were measured at different interval times to assess the fermentation performance.

Result and discussion

As presented in Figure 1b, acetic and butyric acid were found to be favourable carbon sources for the cultivation of *C. necator*, the concentrations of which were almost depleted after 36h. Whilst the assimilation of propionic and isobutyric acid was slower at the same concentration of 5 g/L. The concentration of valeric, isovaleric and caproic acids, on the other hand, was diluted to 2.5 g/L to achieve the feasible bacterial growth. The changes in cell density were measured throughout the fermentation, showing the similar pattern corresponding to the VFA consumption (Figure 1a). Regardless the potential in replacing conventional substrates in PHA production, the use of VFAs could pose a drawback of substrate inhibition at excessive concentration of above 5 g/L (Agustín Martinez et al. 2015; Yun et al. 2013).

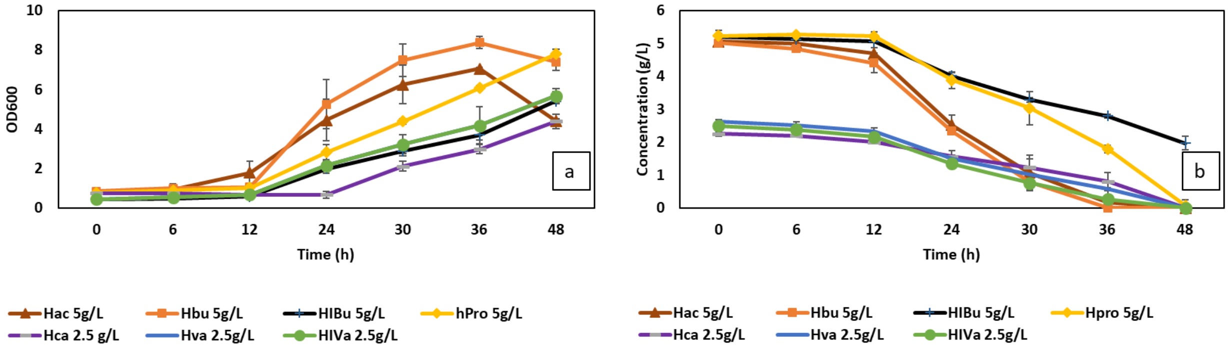

Figure 1. Changes in cell density (**a**) and volatile fatty acid (**b**) concentration during the fermentation in a bioreactor using individual VFAs at different concentrations.

A significant change in metabolic behaviour was observed as the bacteria was cultivated in a synthetic VFA mixture (Figure 2a). In this regard, butyrate acid became the most preferable component which was consumed the first at an accelerating rate among the blend of VFAs. The consumption of acetic acid, on the other hand, was only conducted after the depletion of other VFAs. The similar phenomenon was observed in the case of food waste-based VFAs as the substrates were assimilated in the same pattern but at a faster uptake rate (Figure 3a). However, the biomass and PHA yield obtained in synthetic VFAs were higher than that of acquired in real VFA effluent, 2.8 and 1.49 g/L compared to 2.26 and 1.02 g/L, respectively. Generally, the PHA yield obtained in this study was notably higher than those reported in other studies by Cerrone et al. (2014), Tajima et al. (2003), (Agustín Martinez et al. 2015).

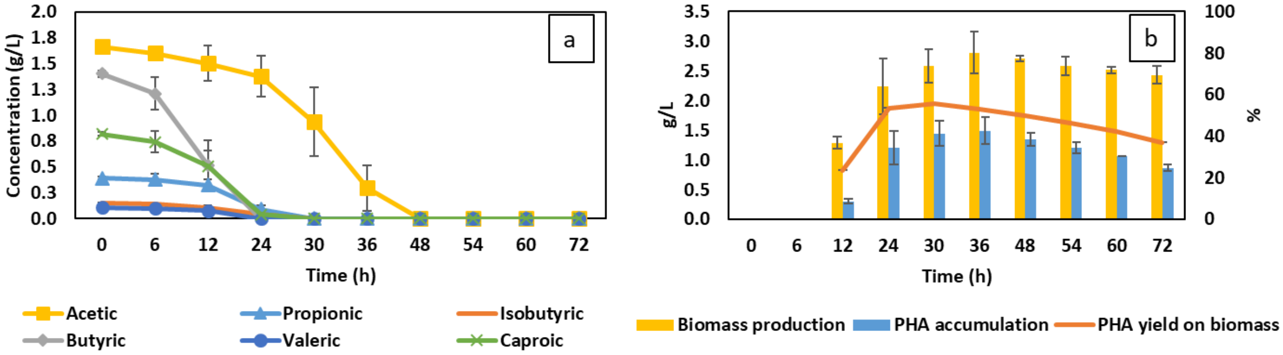

Figure 2. Changes in volatile fatty acid (VFA) concentration (**a**), and biomass and PHA production (**b**) using synthetic mixture VFAs during the fermentation in a bioreactor.

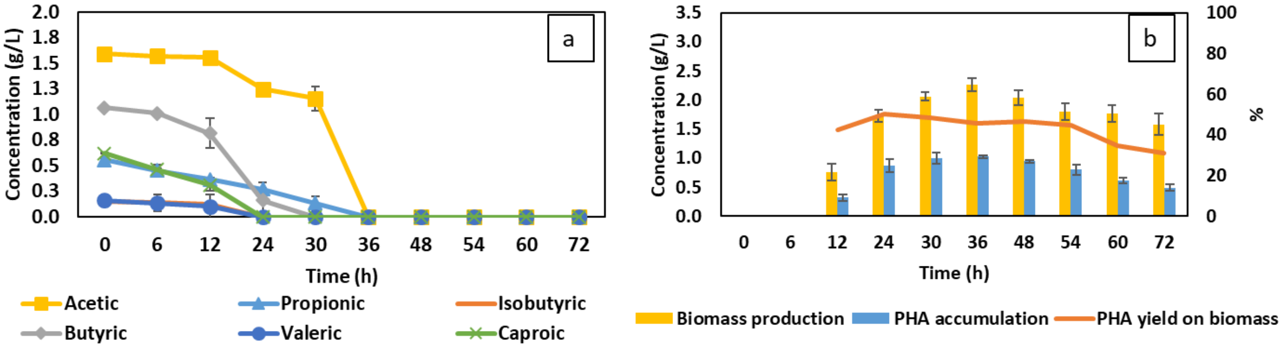

Figure 3. Changes in volatile fatty acid (VFA) concentration (**a**), and biomass and PHA production (**b**) using food waste-based VFA during the fermentation in a bioreactor.

Nuclear Magnetic Resonance (NMR) analysis was used to determine the composition and crystallinity of the accumulated PHAs (Table 1). The PHA obtained was found to be a copolymer of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and the crystallinities of which were in compliance with samples obtained from a study by William et al. (1995).

Table 1. Composition and total crystallinity of the copolymers produced from two different carbon sources.

Sample	**Fraction %**		**Total crystallinity**
	HB	**HV**	
Synthetic mixture VFAs	93.8	6.2	62.2
Food waste-based VFAs	93.5	6.5	61.4

Conclusion

In this study, food waste-derived VFAs can be used directly for the cultivation of PHA-bearing bacteria without the addition of external nutrients. This, in turn, provides a great opportunity to replace the expensive conventional feedstock in PHA production. Bacterial tolerance at high VFA concentration should be considered in future studies to increase the fully exploit the VFA potential.

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