# Overall process of using valerate-dominant sludge hydrolysate to produce high-quality polyhydroxyalkanoates (PHAs) by mixed culture

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Polyhydroxyalkanoates (PHAs) are biopolyesters synthesized by a wide range of bacteria that can functionally replace conventional petroleum-based plastics. Currently there are two primary obstacles hindering the widespread application of PHAs, high production costs and weak mechanical properties. To reduce the production costs, lots of efforts have been devoted to applying mixed microbial cultures (MMCs) to synthesize PHAs with inexpensive waste substrates, such as fermented hydrolysate from excess sludge, food waste or paper mill wastewater(Jiang Y, 2012).

When apply MMCs to PHAs synthesis with fermented hydrolysate as feedstock, the quality of product is affected by composition of volatile fatty acids (VFAs) and non-VFAs components in the hydrolysate. Previous results have identified that the structurally related propionate and valerate can facilitate 3HV formation and therefore improve polymer properties, through the studies employing simple substrate, such as sole VFA or synthetic VFAs mixture (Jia Q, 2013). However, the uptake rates of these odd acids by microbes are much slower than those of even acids, such as acetate and butyrate, which was believed to be highly related to the metabolic pathways presented in microbes. On the other hand, the presence of non-VFAs components in hydrolysate which is favorable to the growth of microbes under the circumstance of high N and P level, hinder synthesis of PHAs (Johnson K, 2010). Some studies and ours (Hao JX, 2015) have reported a thermophilic ferementation method to produce hydrolysate with high content of valerate, which allow us to investigate the feasibility of synthesis high quality PHAs with the valerate-domninant hydrolysate, and the effect of even acids and non-VFAs components in valerate-dominant hydrolysate on PHAs synthesis. So far, the utilization of complex feedstock for PHAs production, such as the fermented waste streams dominated by propionate and valerate, is much less refined.

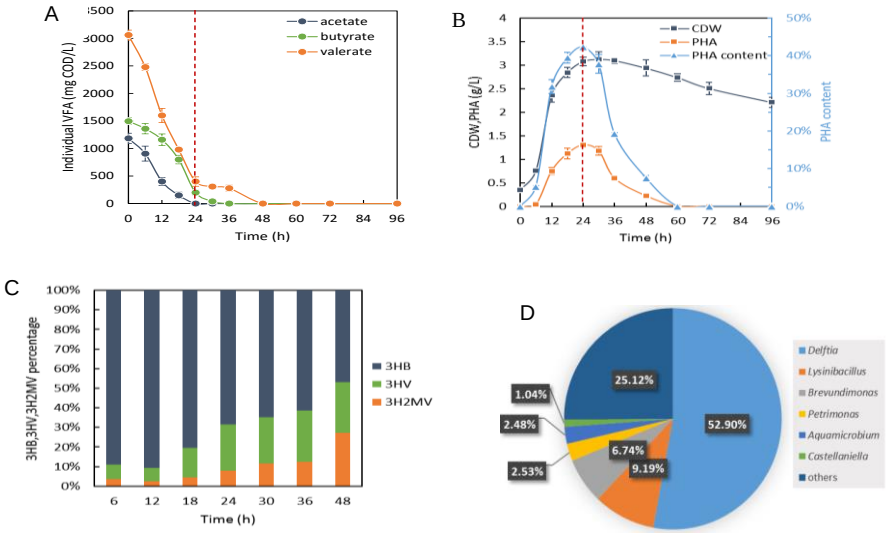


Fig. A. Utilization of individual VFAs by enriched MMC; Fig. C and D. Dynamics of PHAs synthesis, monomer proportion during one cycle in the SBR; Fig.D. The relative abundance levels of genera in the microbial community of the enriched MMC. The genera with abundance less than 1% are classified into 'others'.

In the present study, the overall process of polyhydroxyalkanoates (PHAs) production from a mixed culture with thermophilic fermented sludge hydrolysate featuring high-valerate content (52% in VFAs) was investigated. The experiments were carried out with SBR operated at feast-famine mode. The characteristics of substrate utilization, microbial growth and PHAs synthesis, as well as the microbial composition of the enriched culture, were investigated to reveal the feasibility and efficiency of valerate-dominant hydrolysate as feedstock for PHAs production. A PHAs-producing mixed culture was enriched by using sludge hydrolysate as substrate and step-wise increasing feeding substrate concentration. The enriched culture obtained strong capacity to

produce PHAs under feast-famine conditions, with no need to remove nutrients from the hydrolysate or dilute to low substrate concentration. Valerate in the feedstock was preferentially utilized than acetate and butyrate (Fig. A). The maximum PHAs content (42.31%) and highest PHAs concentration (1.30 g/L) were acquired at the end of feast phase, with polymer composition of 68.4 mmol C% 3-hydroxybutyrate (3HB), 23.7 mmol C% 3-hydroxyvalerate (3HV) and 7.9 mmol C% 3-hydroxy-2-methylvalerate (3H2MV) (Fig.B and C). Microbial community analysis revealed that valerate-dominant feedstock could shape Delftia (53%) to become the prevailing group over other PHAs-producing bacteria ( Fig.D). Moreover, Changing feast-famine ratios showed that moderately shortening famine phase (from feast/famine ration of 1:3 to 1:2) was favorable in terms of increasing PHAs productivity and optimizing PHAs properties. The whole process exhibited a potential to produce 3.4 g PHAs from 1 kg excess sludge. These findings indicate that the valerate-dominant sludge fermented hydrolysate can be used to efficiently and stably produce PHAs containing high 3HV and 3H2MV with simplified operations.

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