## Investigation of the optimal operating strategy for mixed culture polyhydroxyalkanoate (PHA) production under different nutrient conditions

Waste streams containing volatile fatty acids (VFAs) can be used for polyhydroxyalkanoate (PHA) production by mixed microbial cultures (MMCs) and most of the operating strategy for MMCs PHA production includes moderate organic loading and nitrogen limitation. However, waste streams in reality commonly contain different concentration of carbon and nitrogen (for example COD=24.0 g/L, Ammonia N=6.58 mg/L for fermented paper mill wastewater and COD=5978.17 mg/L, Ammonia N=398.18 mg/L for sludge fermentation liquid). This paper aims to investigate whether there is an optimal strategy for MMCs PHA production that appeal to a wide carbon and nutrient level spectrum.

Three typical sequence batch reactors (SBRs) submitted to aerobic dynamic feeding (ADF) mode were operated under the same C/N ratio, VFAs composition and hydraulic retention time (HRT) but different combination of sludge retention time (SRT), organic load rate (OLR) and cycle length (CL) to enrich PHA accumulating MMCs from municipal activated sludge. The PHA production capacity of SBRs under nutrient excess, limitation and starvation conditions (Cmol/Nmol ratio equals to 8, 40 and ∞, respectively) was evaluated in batch assays. The succession of microbial communities in SBRs and batch assays was analyzed by the method of terminal restriction fragment length polymorphism (T-RFLP). Batch assays of SBR#1 (long SRT, low OLR, long CL), SBR2# (short SRT, high OLR, long CL) and SBR#3 (short SRT, high OLR, short CL) showed similar results under nutrient starvation condition, with PHA content of 46.60 wt% (g PHA/g VSS), 46.46 wt% and 47.12 wt% achieved respectively after 7.5 h reaction, while batch assay of SBR#3 reached the maximum PHA content (54.85 wt%) under nutrient excess condition, compared to that of SBR#1 and SBR#2 of 49.99 wt% and 50.04 wt%, respectively.

Regarding active biomass growth, batch assays of SBR#2 and SBR#3 showed an increase of 20.61% (g Biomass/g Initial Biomass) and 38.92% under nutrient limitation, 19.80% and 24.79% under nutrient excess, respectively, while no apparent growth occurred in batch assay of SBR#1 (8.92% and 4.15% under nutrient limitation and excess respectively). Negative growth were observed under nutrient starvation of all SBRs because of sampling loss. Due to the inhibition of free ammonia under nutrient excess condition, biomass growth was less compared to that under nitrogen limited condition. The results showed that SBR#3 had the best overall PHA production performance considering its relatively high PHA content and productivity in all nutrient conditions, which will guarantee the production performance with adaptability for a wide range of VFA-rich waste streams. Nitrogen has great impact on the biomass yield especially when OLR is high, the presence of nitrogen results in the increase of biomass consequently increases the final PHA productivity that can be calculated from PHA productivity(gPHA/gX0) =

PHA content ×VSS weight initial biomass weight