Long-term validation of integrated polyhydroxyalkanoates (PHAs) production from the sidestream of municipal WWTP

V. Conca*, C. Da Ros*, N. Frison*, A. L. Eusebi**, Francesco Fatone**

*Department of Biotechnology, University of Verona, Strada Le Grazie 15, I-37134, Verona – Italy; vincenzo.conca@univr.it, cinzia.daros@univr.it, nicola.frison@univr.it ** Department of Science and Environmental Engineering (SIMAU), Marche Polytechnic University, Brecce Bianche St., 60131, Ancona, Italy; a.l.eusebi@staff.univpm.it, f.fatone@staff.univpm.it

Abstract: This work reports the results derived from the long-term operation of a novel technology integrating polyhydroxyalkanoates production from the sidestream of municipal wastewater treatment plant at pilot scale. On one hand, the system consisted on the production of volatile fatty acids (VFAs) by mesophilic (37°C) acidogenic fermentation from cellulosic primary sludge by mean of raw wastewater sieving at 350 μ m. On the other hand, the process accomplished the partial ammonia oxidation of anaerobic reject water at relatively high nitrogen loading rate (vNLR 1.6 kgN/m³ d) and a PHA-driven denitritation by means of aerobic-feast and anoxic-famine regime. The overall nitrogen removal efficiency was 80-85% while the selected biomass was able to accumulate PHA with yields between 0.58-0.66 g COD_{PHA}/g COD_{VFA}, which resulted in an overall production of 1.0-1.2 kgPHA/capita per year.

Keywords: Polyhydroxyalkanoates (PHAs); Volatile Fatty Acids (VFAs); Horizon 2020-SMART-Plant

The world population growth leads to inevitable increase of wastewater being produced with increasingly energy consumption and Greenhouse Gases (GHGs) emitted for its treatment and reclamation (Cakir & Stenstrom, 2005). According with the new strategies of the EU to deliver circular bioeconomy, the new challenges for the water sector represents the sustainable conversion of the current wastewater treatment plant (WWTPs) into water resource recovery facilities (WRRFs). In recent years, great interest has been given to polyhydroxyalkanoates (PHAs) production due to their wide application as environmental-friendly bioplastics (Tu et al., 2019). To boost the driver for WRRFs development, an integrated PHA recovery from the sidestream of WWTPs has been reported by Frison et al. (2015). Within the H2020-"Smart-Plant" project framework, the aim of this study was the long-term validation of the PHAs production combined with the via-nitrite nitrogen removal from the anaerobic reject water at pilot scale in Carbonera WWTP (Treviso, Italy). The main steps involved in the process were:

1) Pre-concentration of municipal wastewater through the sieving at 350 μ m and recovery of cellulosic primary sludge (CPS) accomplished by a rotating belt dynamic filter (RBDF) (type SF1000 Salsnes Filter, Norway); 2) Controlled fermentation of CPS at 37°C in a sequencing batch fermentation reactor (SBFR) with a working volume 2.6 m³ to produce VFAs. The fermentation liquid (FL) containing VFAs was recovered by a solid/liquid separation using a ceramic membrane; 3) Struvite crystallizer with a working volume of 50 litres where Mg(OH)₂ is added to enhance P-salts precipitation; 4) Ammonia oxidation via-nitrite from the anaerobic supernatant in a 1.2 m³ sequencing batch reactor (N-SBR). The SRT was maintained at 20-25 days; 5) Selection of PHA storing biomass by means of (aerobic) feast and (anoxic) famine regime in a 2.8 m³ SBR (S-SBR). The applied SRT was maintained at around 5-7 days; 6) PHA accumulation in the biomass-cells was carried out in a 1 m³ fed-batch reactor, where up to 5-8 pulse-addition of VFAs were accomplished in 5-6 hours.

The fermentation liquid (FL) had a VFAs concentration of 8-12 gCOD_{VFA}/L where ammonia and phosphate were released up to 283 mg NH₄-N/L and 68 mg PO₄-P/L respectively. The via-nitrite pathway in the N-SBR was fully achieved operating at around 25°C, vNLR of 1.6 kgN/m³d and 22 days of SRT. The resulted specific ammonium utilization rate (sAUR) was 26 mgN/gMLVSS h. By reducing the SRT at 15 days, the sAUR increased up to 31 mgN/ gMLVSS h, but the treatment capacity of the system decreased to 1.2 kgN/m³d with less than 79% of ammonia oxidation efficiency.



Figure 1.1a) SF1000 Salsnes Filter; b) Fermentation unit; c) Membrane, crystallizer, Nitritation SBR, Selection SBR, Accumulation SBR)

The S-SBR operated at SRT of 5-7 days, the vNLR ranged between 0.60 - 0.63 kgN/m³ d, while the denitritation efficiency was higher than 85%. VFAs were added at the beginning of the feast phase, according with a COD/NO₂-N ratio of 2.5-2.8 gCOD/gN. The carbon source was initially composed by acetate solution to have a benchmark, while then sole FL was used as substrate. The accumulation tests with acetate as carbon source determined higher PHA storage yields (up to 0.66 gCOD_{PHA}/gCOD_{VFA}) and the polymer content in the biomass reached the 46 \pm 5% of volatile fraction. On the other hand, the use of FL resulted in a slightly decreased PHA storage yield of 0.58-0.62 g COD_{PHA}/g COD_{VFA}. However, in this case PHA was constituted by a co-polymer of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV), increasing the elastomeric properties of the biopolymer. The average performances of accumulation batches were reported in Table 1.1.

| Carbon source | PHA content % PHA/VSS | Storage yield (gCOD _{PHA} /gCOD _{VFA}) | PHA productivity (mgPHA/L h) | % HV (wt) |
|------------------|-----------------------------|--|--|--------------|
| Acetate | $46\pm5~\%$ | 0.60 ± 0.06 | 269 ± 34 | nd. |
| FL | 42 ± 6 % | 0.58 ± 0.05 | 234 ± 37 | 24-42 % |

According with the findings obtained during the long-term period, the potential PHA production capacity of the process was assessed as 1.0-1.2 kgPHA/capita per year.

REFERENCES

Cakir, F.Y., Stenstrom, M.K., 2005. Greenhouse gas production: a comparison between aerobic and anaerobic wastewater treatment technology. *Water Res.* **39**, 4197-4203

Frison, N., Katsou, E., Malamis, S., Oehmen, A., & Fatone, F. (2015). Development of a novel process integrating the treatment of sludge reject water and the production of polyhydroxyalkanoates (PHAs). *Environ. Sci. Technol*, **49**, 10877-10885.

Tu, W., Zhang, D., Wang, H. & Lin, Z. (2018). Polyhydroxyalkanoates (PHA) production from fermented thermal-hydrolyzed sludge by PHA-storing denitrifiers integrating PHA accumulation with nitrate removal, *Bioresour. Technol.* **292**, 121895