WWTP biorefinery for polyhydroxyalkanoates (PHAs) recovery from cellulosic primary sludge

Vincenzo Conca*, Cinzia Da Ros*, Nicola Frison*, Anna Laura Eusebi, Francesco Fatone****
*Department of Biotechnology, University of Verona, Strada Le Grazie 15, I-37134, Verona – Italy (vincenzo.conca@univr.it)
**Department of Science and Environmental Engineering (SIMAU), Marche Polytechnic University, Brecce Bianche St., 60131, Ancona, Italy (VE) – Italy.

Abstract: This work reports the results obtained by the long-term operation of the Short-Cut Enhance Phosphorus and PHA Recovery (SCEPPHAR) system at demonstration scale. The system consisted on the production of volatile fatty acids (VFAs) by mesophilic (37°C) acidogenic fermentation from cellulosic primary sludge by means of raw wastewater sieving at 350 µm. On the other hand, the process accomplished the partial ammonia oxidation of anaerobic reject water at relatively high nitrogen loading rate (vNLR 1.5 kgN/m^3 d) and a PHA-driven denitritation by means of aerobic-feast and anoxic-famine regime. The overall nitrogen removal achieved was 85% while the selected biomass was able to accumulate up to 56% of PHA, which resulted to an overall production of 1-1.2 kgPHA/capita per year.
Keywords: Short-Cut Enhanced Phosphorus and PHA Recovery (SCEPPHAR), PHA, biological nitrogen removal; external carbon source; Horizon 2020 – Smart-Plant

The world population growth leads to inevitable increase of wastewater being produced with increasingly energy consumption and Greenhouse Gas (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 biorefineries for bio-based resource recovery. Recent studies demonstrated the potential benefits of combined recovery of added value products such as cellulose (Ghasimi et al., 2016), nutrients, organic building blocks (like, Short Chain Fatty Acids, SCFA) (Crutchik et al., 2018, Longo et al., 2015) and polyhydroxyalkanoates (PHAs) from the wastewater treatment (Frison et al., 2015). On the other hand, the Innovation Action Horizon 2020 – Smart-Plant aimed the scale-up of the PHAs production combined with the short-cut nitrogen removal from reject water at demonstration scale in Carbonera WWTP (Treviso, Italy). The main steps involved in the SCEPPHAR system were:

1) Pre-concentration of municipal wastewater through the sieving at 350 µm 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 m^3 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 the crystallization;
4) Ammonia oxidation via-nitrite from the anaerobic supernatant in a 1.5 m^3 sequencing batch reactor (N-SBR). The SRT was maintained at 15-20 days;
5) Selection of PHA storing biomass by feast/famine regime in a 2.9 m^3 Sequencing Batch Reactor (S-SBR) under aerobic/anoxic conditions. The applied SRT was maintained at around 5 days;

6) PHA accumulation in the biomass-cells was carried out in a 1 m^3 SBR, where up to 5-8 pulse-addition of VFAs were accomplished in 5-6 hours.

The fermentation liquid (FL) had a SCFA concentration of 8-12 gCOD/L where ammonia and phosphate were released up to 283 mgN-NH_4/L and 68 mgP-PO_4/L respectively. The via-nitrite pathway in the N-SBR was fully achieved operating at around 25°C, vNLR of 1.5 kgN/m^3d and 25 days of SRT. The resulted specific ammonium oxidation rate (sAUR) was 20 mgN/gMLVSS h. By shorten the SRT at 15 days, the sAUR was improved (up to 25 mgN/ gMLVSS h) but the treatment capacity of the system decreased to 1.2 kgN/m^3d with 85% of ammonia oxidation efficiency.

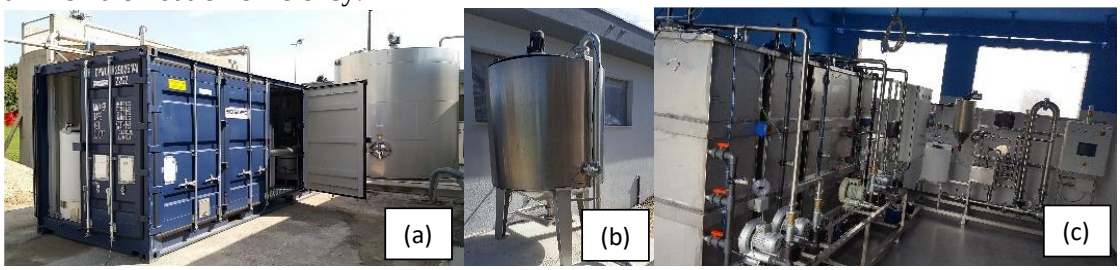

Figure 1 a) SF1000 Salsnes Filter; b) Fermentation unit; c) Membrane, crystallizer, Nitritation SBR, Selection SBR, Accumulation SBR)

The S-SBR operated at SRT of 5-7 days, vNLR ranged between 0.50 - 0.54 kgN/m^3 d, while the denitritation efficiency was higher than 85%. VFAs were added at the beginning of the cycle according with a COD/N ratio of 2.5-3.0 gCOD/gN. The carbon source was initially acetate solution and later the FL was used.

The higher actual COD/N resulted by the acetate during the accumulation (around 50 gCOD/gN) determined higher PHA storage yield (up to 0.60 $gPHA_{COD}/gVFA_{COD}$) and polymer content in the biomass reached the 56 ± 6% of volatile fraction. When FL was replaced the PHA storage yield reduced to 0.41-0.62 $gPHA_{COD}$/gCOD anyway the PHA was constituted by a co-polymer of hydroxybutyrate (HB) and hydroxyvalerate (HV). The average performances of accumulation batches were reported in Table 1.

Table 1 Performances of PHA accumulation batches (n° batches performed 8)

Carbon source	**PHA content** % PHA/VSS	**Storage yield** ($gPHA_{COD}/gCOD$)	**PHA productivity** ($kgPHA/m^3_{reactor}$)	**HV %** (HV/PHA w/w)
Acetate	56 ± 6 %	0.51 ± 0.13	1.3 ± 0.2	nd.
FL	42 ± 19 %	0.49 ±0.11	0.8 ± 0.2	42 ± 19 %

According with the findings obtained during the long-term period, the potential PHA production capacity of the SCEPPHAR process accounted for 1-1.2 kgPHA/capita per year.

REFERENCES

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

Crutchik, D., Frison, N., Eusebi, A.L.& Fatone, F., 2018. Biorefinery of cellulosic primary sludge towards targeted Short Chain Fatty Acids, phosphorus and methane recovery. *Water Res*. **136**, 112–119.

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). Environmental science & technology, 49(18), 10877-10885.

Ghasimi, D.S.M., de Kreuk, M., Maeng, S.K., Zandvoort, M.H. & van Lier, J.B., 2016. High-rate thermophilic bio-methanation of the fine sieved fraction from Dutch municipal raw sewage: Cost-effective potentials for on-site energy recovery. *Appl. Energy* **165**, 569–582.

Longo, S., Katsou, E., Malamis, S., Frison, N., Renzi, D. & Fatone, F., 2015. Recovery of volatile fatty acids from fermentation of sewage sludge in municipal wastewater treatment plants. *Bioresour. Technol*. **175**, 436–444.