From sewage sludge to polyhydroxyalkanoates: a case study at pilot scale level

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The urgency of the sewage sludge problem is related to the large produced amounts and the necessity to develop recovery options in compliance with the current European and national legislation and with the forthcoming transition towards the principles of a circular economy. The annual sludge production into the EU-28 is in the order of 9.0 million tons of dry solids (Eurostat Website. Available online: https://ec.europa.eu/eurostat/web/products-datasets/-/ten00030). This sludge has four main destinations: agriculture 49.2%; incineration 24.9%; recultivation and land reclamation 12.4%; landfill 8.7%; and other destinations for the remaining amount (4.9%). The use of sewage sludge as a fertilizer in agriculture is the preferred option due to its content of organic substances and nutrients; dewatered sewage sludge contains 50–70% organic matter and 30–50% mineral components (including 1–4% of inorganic carbon), 3.4–4.0% nitrogen (N), 0.5–2.5% phosphorus (P), and significant amounts of other nutrients, including micronutrients (Campo et al., 2021).

As an alternative and innovative option, in the frame of the new national directive related to the discipline of sewage sludge management and the implementation of the 86/278/EEC directive concerning the protection of the environment, a pilot scale process has been set up for the synthesis of polyhydroxyalkanoates (PHA) from this carbon source, available in the full-scale municipal wastewater treatment plant of Treviso (northeast Italy).

Sewage sludge utilised in this study was recovered from the static thickener of the full-scale plant and it was characterized by the following characteristics: total solids (TS) 30 ± 1 g/kg; volatile solids (VS) 22 ± 1 g/kg; soluble chemical oxygen demand (COD_{SOL}) 0.25 ± 0.04 g/L; pH 6.6 ± 0.2; total Kjeldahl nitrogen (TKN) 42 ± 3 g N/kg TS; phosphorus 15 ± 1 g P/kg TS. The adopted methodology consisted in the recovery of volatile fatty acid (VFA) through acidogenic fermentation of sludge. VFA are the precursors for PHA synthesis; hence, their production is the first step of the developed biorefinery. Based on the low COD_{SOL} available from the thickened sludge, a comprehensive pre-treatment evaluation has been assessed, to solubilize the solids organic matter. The effect of temperature (T; 30-70°C) and pH (9-11) was evaluated separately, and then synergistically, under fixed time (24 h). The maximum value obtained was 10.3 ± 0.4 g COD_{SOL}/L at 70°C under neutral pH. The simultaneous thermal and alkaline treatment did not show improvements on the organic matter solubilisation.

The following sewage sludge fermentation was conducted on thermally pre-treated sludge. As first screening, lab-scale batch tests (1.0 L; pH 5-11; T 20-70°C) were performed to find the best option in terms of acidification performances. The thermophilic fermentation (55°C) at acidic (pH 5.0-5.5) condition showed higher VFA concentration (8.1 ± 0.3 g COD_{VFA}/L) and acidification yield (0.47 COD_{VFA}/g VS_0). Alkaline (pH 9.0) condition gave comparable results; however, its replicability at pilot scale (380 L) was difficult for pH control.
In the pilot Continuous Stirred Tank Reactor (CSTR), the thermophilic fermentation of thickened and thermally pre-treated sludge was conducted routinely in batch mode. This step furnished a VFA-rich stream available for the aerobic MMC-PHA production process, characterized by a sufficiently high COD_{VFA}/COD_{SOL} ratio (0.72) and by a VFA profile dominated by acetic (45% COD basin) and butyric (25%) acid, followed by propionic and valeric acid (18% and 12% respectively).

A sequencing batch reactor (SBR) reactor (V 100L) for mixed microbial culture (MMC) selection/enrichment was operated and the so called feast-famine condition at low-medium and medium-high organic loading rate (OLR), respectively equal to 2.0-2.2 (OLR_1) and 4.5-5.0 (OLR_2) kg COD_{SOL}/(m^3 d). The feast-famine approach is widely known to be the best option for the selection of a microbial community with high PHA-storage ability (Valentino et al., 2017). The T was maintained in the range 25-27°C by an immersion heater. Short hydraulic retention time (HRT; 2 days) was maintained for the whole experimentation, as well as the cycle length (12 h) composed by the following phases: feeding (3 min), mid reaction (700 min), withdrawal (2 min) and final reaction (15 min). The culture selection was successful, being the feast phase between 6-7% and 13-16% of the total cycle length respectively in OLR_1 and OLR_2. Such values were calculated based on the oxygen profile in each SBR cycle, daily monitored by the installed online system composed by pH and O_2 probes. The first condition (OLR_1) appears ideal for the MMC selection with VFA-rich stream coming from sewage sludge. The biomass was able to accumulate up to 57% g PHA/g VSS; lower performance was obtained in the accumulations performed with biomass selected in the run OLR_2 (38% g PHA/g VSS). The following Table 1 summarizes the performances (with average data and standard deviations) obtained in the SBR under the two investigated OLR.

Table 1. Main parameters and performances obtained in the two runs performed at different OLR

Selection/Enrichment Reactor (SBR)			
Parameter	Unit	OLR_1	OLR_2
TSS	mg/L	2109 ± 215	2807 ± 201
VSS	mg/L	1927 ± 230	2638 ± 170
feast/cycle length ratio	h/h	0.065 ± 0.004	0.14 ± 0.02
PHA (end of feast)	mg/L	761 ± 142	261 ± 142
PHA (end of cycle)	mg/L	138 ± 60	78 ± 60
HV fraction	%	10 ± 1	12 ± 4
Specific Storage Rate	mgCOD/(gCOD h)	489 ± 68	277 ± 54
Storage Yield ($Y_{P/S}^{SBR}$)	COD/COD	0.40 ± 0.03	0.29 ± 0.06
Observed Yield (Y_{OBS}^{SBR})	COD/COD	0.56 ± 0.02	0.54 ± 0.02
Accumulation Reactor (fed-batch)			
PHA content in the biomass	% (gPHA/gVSS)	52 ± 5	38 ± 4
Storage Yield ($Y_{P/S}^{batch}$)	COD/COD	0.55 ± 0.02	0.40 ± 0.03

At the end of the accumulation tests, PHA-rich biomass was collected, acidified until pH 2 by adding H_2SO_4 and left settle under gravity; the thickened slurry was centrifuged (Heraeus Megafuge 40; Thermo Fisher Scientific) for 15 min at 4500 rpm and the wet pellet was stored in the fridge at 4 °C until extraction. Acidified PHA-rich biomass has been treated by two standard methods for recovering PHA, such as solvent extraction with chloroform (benchmark) and the NaClO oxidation. The two protocols gave comparable results: the extracted polymer was characterized by a chemical composition of 90-91.5% w/w 3-hydroxybutyrate (3HB) and 8.5-10% w/w 3-hydroxyvalerate (3HV); relatively high molecular weight (M_W) 360-410 kDa; degradation temperature (Td^{max}) and melting temperature (T_m) of 265-280°C and 156-161°C respectively; crystallinity (Xc) of 45.8%.

Compared to a wide variety of organic wastes affected by seasonality effects, sludge originated from municipality is a fermentable substrate with stable physical-chemical features during a whole year. In turn, these fundamental characteristics may ensure stability in PHA production process, with a final product having constant and reproducible characteristics too.

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References

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