## From waste to biopolymers: sustainable valorization of secondary sludge via hydrothermal carbonization, acidogenic digestion and aerobic fermentation

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The increasing generation of industrial wastewater sludge poses a significant environmental challenge, but it also presents an opportunity for sustainable resource recovery. This study explores an innovative multi-step process, developed by B-Plas, an pioneering start-up, to convert secondary sludge from an industrial wastewater treatment plant into polyhydroxyalkanoates (PHA), a family of biobased and biodegradable polymers.

The developed process includes hydrothermal carbonization (HTC) as a pretreatment to improve sludge dewaterability and enhance the solubilization of organic matter, thus improving its bioavailability for subsequent biological processes. Experimental results indicate that HTC at 200 °C for 30 minutes leads to approximately 50% solubilization of the total COD and a 65% reduction in total suspended solids (TSS), which facilitating the further step of acidogenic digestion (Figure 1).

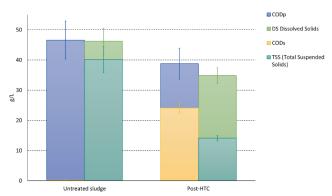


Figure 1. Composition of untreated thickened sludge and HTC-treated sludge in terms of total COD (CODt) and total solids (TS). CODt is the sum of particulate COD (CODp) and soluble COD (CODs), while TS consists of dissolved solids (DS) and total suspended solids (TSS). The HTC process increases soluble COD (CODs) and dissolved solids (DS), enhancing the availability of organic matter.

In the acidogenic digestion phase, performed in a Continuous Stirred Tank Reactor (CSTR), microorganisms convert the solubilized organic matter into volatile fatty acids (VFAs), which serve as building blocks for PHA synthesis. At an organic loading rate (OLR) of  $5.3 \pm 0.8$  gCODs/L\*d and a hydraulic retention time (HRT) of  $\sim 5$  days, VFA production reached a yield of  $2.0 \pm 0.2$  gCOD/L\*d, with a conversion efficiency of  $38.5\% \pm 0.1\%$ . The main VFAs were  $\sim 40\%$  acetic acid,  $\sim 25\%$  propionic acid,  $\sim 15\%$  isobutyric and butyric acids, and  $\sim 20\%$  isovaleric acid. (Figure 2)

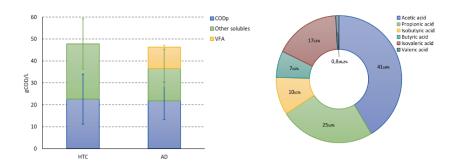


Figure 2. Results of the acidogenic digestion (AD) process. The bar chart shows the COD distribution in the liquid phase. The sum of the "other soluble" fraction and volatile fatty acids (VFAs) represents the soluble COD (CODs). The sum of particulate COD (CODp) and CODs represents the total COD (CODt) before (HTC-treated sludge) and after acidogenic digestion. The pie chart illustrates the composition of VFAs produced, showing the relative distribution of different acids.

Following acidogenic digestion, a solid-liquid separation process using a filter press yielded a **cake** with a **dry** matter content of  $45.3 \pm 1.6\%$ , effectively halving the input sludge volume.

The liquid fraction obtained from the solid-liquid separation was aerobically fermented for PHA synthesis by PHA-accumulating bacteria. An innovative continuous system was employed, combining the *feast and famine strategy* with oxygen limitation to selectively enrich bacterial strains capable of high PHA accumulation. (Figure 3).

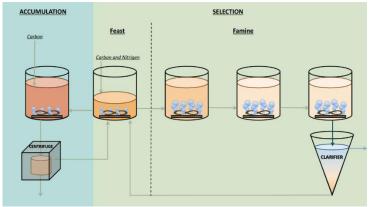


Figure 3. Schematic representation of the continuous process for the selection of PHA-accumulating bacteria. The system operates under a feast-and-famine regime to promote the enrichment of specialized microbial strains, followed by an accumulation step to maximize PHA storage.

For the selection phase, an organic loading rate (OLR) of 10 gCOD/L\*d, a HRT of 4.7 days, and selection cycles of 8 hours, all under a feast-and-famine regime with a feast-to-famine ratio of approximately 20% were applied. Once the optimal bacterial strains were selected, the system continuously shifted to the accumulation phase. In this stage, the bacteria were fed only with VFAs, without any additional nutrients, to stimulate PHA storage. During this phase, the OLR was set to 11 gCOD/L\*d, with feeding cycles maintained at 12 hours, ensuring continuous PHA production. After the accumulation phase, a continuous centrifugation system was employed to efficiently separate the biomass enriched with PHA. This biomass can then undergo further processing for PHA extraction or be used for the production of other valuable molecules, depending on the intended application.

Preliminary data from a synthetic feed mimicking the real substrate composition showed a PHA yield of approximately 50-60% of cell dry weight, demonstrating the potential of this approach. Further tests with the real acidogenic broth will be conducted to validate these results under actual operating conditions.

The B-Plas process integrates hydrothermal and biological technologies to recover energy and valuable resources from wastewater sludge. This approach demonstrates the potential to significantly reduce sludge volumes by up to 80% while upcycling it into multiple by-products. These innovations align with circular economy principles and offer sustainable solutions for sludge management challenges.

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