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Polyhydroxyalkanoate production by municipal wasted activated sludge: the case study of Marineo (ITALY) wastewater treatment plant

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Abstract: Polyhydroxyalkanoates (PHAs) are biodegradable polymers with promising applications in various industries. The production of PHAs from sewage sludge represents an innovative and sustainable approach to both waste management and biopolymer synthesis. Sewage sludge, a by-product of wastewater treatment, contains a rich organic carbon source, making it an ideal substrate for producing PHA. This microbial synthesis of PHAs from sewage sludge transforms waste into a valuable resource and mitigates the environmental impact of sludge disposal. Additionally, the cost-effectiveness of utilising sewage sludge as a raw material further enhances the economic viability of PHA production. Still, despite the promising developments, the spread of this process needs to be improved by several challenges. The environmental and economic aspects of the process are yet to be proved since the literature has mainly focused on optimising the process at the laboratory scale. In this context, this study presents the preliminary promising results of a PHA production process by sewage sludge. The experiments were carried out at the deviation line built inside the wastewater treatment plant of Marineo, Italy. Direct accumulation was the strategy adopted by controlled pulsate feeding, which was controlled automatically by homemade software. Preliminary results show a consistent PHA production in quantity (22.1 ± 1.5 g PHA/g VSS %) and monomeric composition at low VFA concentration (186.6 ± 13.7 mg COD/L). Future studies will also focus on evaluating direct greenhouse gas emissions in view of assessing the efficiency and environmental impact of the direct PHA accumulation by sewage sludge.

Keywords: Biosolid management; Polyhydroxyalkanoates; Resource recovery.

1. INTRODUCTION

The increased demand for plastic-based products such as plastic packaging, which makes up about 40% of the overall demand for plastic, has become a big problem in the last decade due to the plastic lifecycle being too long for the purpose it is utilised for (Macht et al., 2023). This demand doesn't show any signs of decreasing. On the contrary, the need for those products remains high and is expected to rise steadily. For that reason, it is mandatory to look for other viable solutions. One of them is represented by a group of bio-polymers called polyhydroxyalkanoates (PHAs) which are classified as polyesters. Due to their similar properties, They can replace conventional plastics like polypropylene and low-density polyethene (Mannina et al., 2020). PHAs are produced by microorganisms from renewable resources, such as sugars and lipids, making them a more sustainable option than petroleum-based plastics. Consequently, they can be easily broken down by various microorganisms in different environments, including soil, freshwater, and marine environments, which helps reduce plastic pollution. This biodegradability and renewability make them an ideal sustainable alternative to replace petroleum-based plastics (Kourmentza et al., 2017).

PHAs are naturally produced by various bacteria as intracellular granules, serving as carbon and energy reserves. These bacteria accumulate PHAs when exposed to specific growth-limiting conditions. The production of PHAs is crucial for reducing environmental impact, as they offer a biodegradable alternative to synthetic plastics. Currently, research is focused on reducing these costs by using renewable raw materials, production methods improvements, and biotechnology advancements, including mixed microbial consortia (MMC) and synthetic biology tools (Liu et al., 2023). Still, some challenges must be overcome to introduce PHAs as a comprehensive large-scale solution to the wide use of petroleum-based plastics. High production costs, low yields, and process scalability are the main areas that still require more research. Moreover, the environmental impact of the solution adopted should be taken into account. Feedstock variability is also an issue that needs to be addressed since using waste streams or biomass as feedstocks can introduce variability, which might affect the process consistency and quality of the PHAs produced.

In view of the above, this work is focused on evaluating the direct accumulation of PHA as a sustainable, environmentally friendly, and economic approach to producing PHA from sewage sludge. The experiments were carried out at the deviation line built inside the wastewater treatment plant (WWTP) of Marineo, Italy.

2. MATERIALS AND METHODS

The experimental activities were conducted at the deviation line built inside the Marineo WWTP (Mannina et al., 2022, 2021). The PHA production pilot plant scheme is reported in Figure 1. The excess sludge produced during the wastewater treatment process produces VFA in the fermenter unit (total volume: 210 L). At the end of the fermentation process, VFA are recovered by a membrane bio reactor (MBR) equipped with a hollow fiber membrane. The obtained VFA-rich liquid is a carbon source that produces PHA directly in the accumulation sequencing batch reactor (A-SBR). The PHA accumulation was run continuously by adopting homemade software, as Mineo et al. (2023) reported.

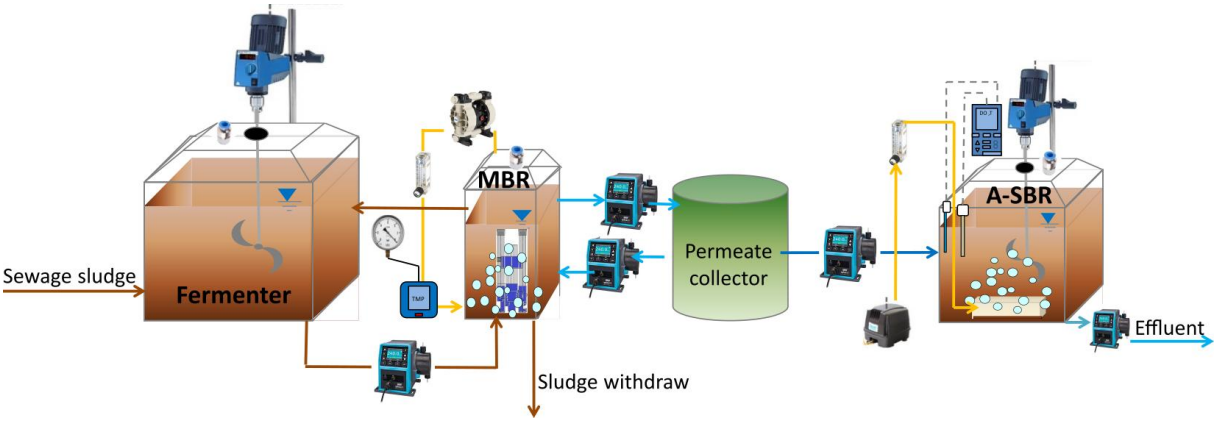

Figure 1. Pilot plant scheme.

Soluble chemical oxygen demand (sCOD), VFA, ammonium (NH_4^+-N) and phosphate (PO_4^{3-}-P) were analysed in the fermentation of mixed liquor, permeate and A-SBR effluent. PHA samples were taken from the A-SBR mixed liquor three times per week and analysed as reported by Mannina et al. (2019). Briefly, PHA concentration was analysed by collecting mixed liquor samples, subsequently mixed with 10 mL of formaldehyde solution (37%) to inhibit the biological activity. After that, samples were centrifuged at 8000 rpm for 30 min, the supernatant

was removed and samples were lyophilised. Butanol and hydrochloric acid were added to weighted lyophilised samples, which were incubated at 100 °C for 8 h. An extraction with hexane and Milli-Q grade water was performed, and the organic phase was collected and filtered (0.22 µm). A gas chromatograph equipped with a flame ionisation detector and a Restek Stabilwax column (30 m × 0.53 mm x 1.00 µm film thickness) was used to determine the polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) monomer concentration. Direct greenhouse gas emissions were monitored as dissolved and gaseous nitrous oxide (N_2O) concentration in the A-SBR.

3. RESULTS AND DISCUSSION

PHA concentration was calculated as the sum of polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) monomers. PHA accumulation runs continuously but is interrupted when there is no appreciable PHA concentration variation in the last 3 days, as reported in Figure 2, between days 12 and 15. At the end of a batch, the PHA's enriched biomass is withdrawn to extract the produced PHA.

At the end of the first PHA accumulation batch (day 12), PHA accounted for 23.1 g PHA/ g VSS %, with 80 % being PHB (18.4 g PHA/g VSS %). The PHV accounted for 4.7 g PHA/ g VSS %, primarily because of the high shares of propionic and valeric acid produced during the first two fermentation weeks, as reported in Table 1. In the second PHA accumulation batch, from days 15 to 25, a slightly lower amount of PHA was produced (20.8 g PHA/g VSS %). This result is related to the higher nutrient concentrations obtained from the sewage sludge fermentation during weeks 3 and 4, leading to increased biomass growth rather than PHA production. Moreover, PHV accounted for only 1.8 PHA/g VSS % because of the low shares of propionic, butyric, and valeric acids obtained (Table 1).

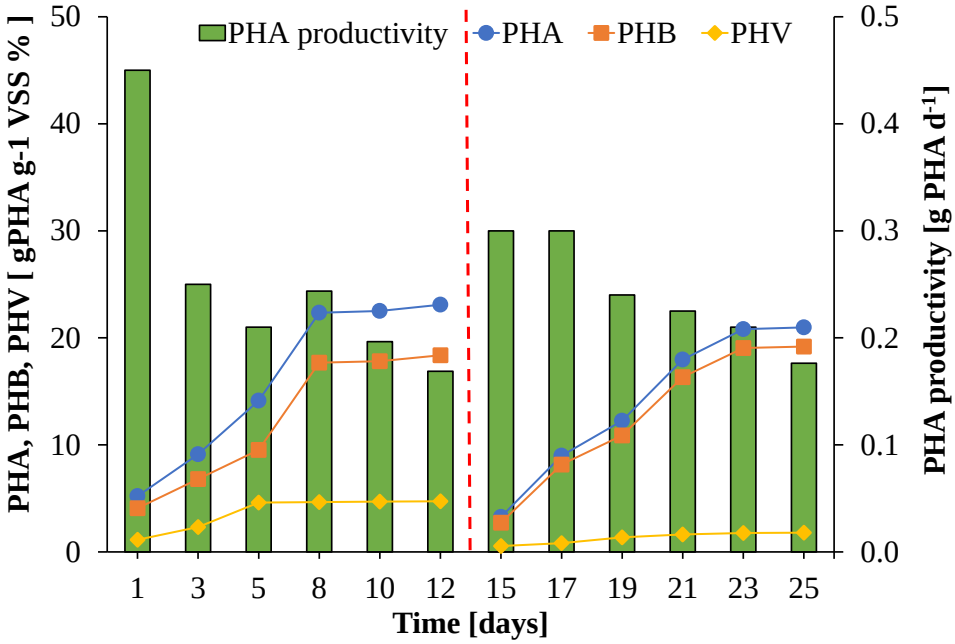

Figure 2. PHA concentration and productivity in the A-SBR.

Table 1. sCOD, VFA, and nutrient concentration were obtained at the end of the fermentation process.

Fermentation week	sCOD (mg/L)	NH_4^+-N (mg N/L)	PO_4^{3-}-P (mg P/L)	VFA (mg COD/L)	Acetic acid %	Propionic acid %	Butyric acid %	Valeric acid %
1	357.6	11.8	3.7	203.8	55.6	21.3	4.9	18.2
2	358.4	10.4	5.6	182.8	47.8	22.9	4.5	24.8
3	362.7	31.2	9.4	177.7	84.9	10.1	2.6	2.4
4	298.5	20.8	10.2	182.1	86.4	9.8	2.1	1.7

CONCLUSIONS

The preliminary results reported in this abstract show the feasibility of producing PHA inside the WWTP operation without performing the enrichment step. Even at the low VFA concentration reported (186.6 ± 13.7 mg COD/L), it was possible to obtain 22.1 ± 1.5 g PHA/g VSS %. Moreover, the limited number of reactors and utilities compared to more complex configurations will reduce the indirect GHG emissions, thus highlighting the potential of this PHA production system. Future studies will also focus on direct greenhouse gas emissions, mainly gaseous and dissolved nitrous oxide. Being able to produce the required amount of PHA in a short time coupled with low environmental impact could lead towards optimising and scaling up this system in view of transforming the WWTP into water resource recovery facilities.

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