Towards closed-loop production of biodegradable plastics from municipal sewage sludge: using multi-meta-omics and quantum computing analyses to improve VFA to PHA production pathways

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Keywords: Polyhydroxyalkanoates, sewage sludge, resource recovery, quantum computing
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Solid waste produced from wastewater treatment plants presents opportunities to recover value-added resources, particularly for the production of bio-based goods. Among these products, polyhydroxylalkanoates, or PHAs, stand out as a group of polymers characterized by thermochemical properties similar to petrochemical-based plastics, but are instead bio-based in origin and biodegradable (Sehgal & Gupta, 2020). PHAs are naturally synthesized by over 300 species of bacteria as intracellular energy reserves using a wide variety of carbon-based substrates (Poltronieri & Kumar, 2019; Liu et al., 2020). Closed-loop waste management systems for PHA production rely on mixed microbial cultures that can utilize wastewater and solid residuals, such as sewage sludge, as the primary carbon source (Morgan-Sagastume et al., 2014). This method, in comparison to pure culture PHA production, may reduce operating costs and thus allow for greater commercial scale-up of sustainable solid waste management technology that also produces value-added products (Nguyenhuynh et al., 2021). Moreover, by diverting an organic portion of wastewater that would otherwise produce low-value-added biogas through anaerobic digestion, the incorporation of high-value-added PHA production in the treatment process may achieve greater carbon credits from the overall WWTP process (Morgan-Sagastume et al., 2016).

Sewage sludge is a by-product of physical and biological wastewater treatment; it is comprised of various materials, including organic matter, microbes, inorganic elements, as well as pathogens and pollutants (Raheem et al., 2018). Thus, sludge requires stabilization to reduce health risks and is often a large portion of total operating costs (20% to 60%) for treatment plants (Crutchik et al., 2020). However, sludge also represents a reservoir of resources that can be transformed for reuse. Currently, anaerobic digestion is the common technique used to transform organic solids in sludge to biogas. While a mature technology, diversifying the end-products from this technique may prove economically advantageous and necessary to replace conventional products, including petrochemical plastics. Volatile fatty acids (VFAs), an intermediate product of anaerobic digestion, are an attractive alternative product, as they are used as chemical building blocks across the food and pharmaceutical industries (Agnihotri et al., 2022). VFAs are of high interest as precursors for PHA production in particular due to their efficient conversion into PHAs by bacteria and their influence on the final PHA monomer composition (Szacherska et al., 2021). The coupled sludge management and PHA production treatment train typically involves a series of batch reactors that first breakdown the solid waste into simpler forms for the bacteria to consume, followed by a PHA-accumulating reactor where the polymer is harvested. However, the key bottleneck for mixed microbial PHA production from VFAs is low PHA yields (~30% dry cell weight) (Nguyenhuynh et al., 2021).

To address this issue, we have developed novel genomics analysis and bioinformatics pipelines to understand the PHA-producing bacterial consortia and their key metabolic pathways, as well as how these pathways evolve over the course of the reactor operation. In doing so, we aim to optimize operating conditions for maximum PHA yield. The bioinformatics analysis encompasses 1) 16S rRNA amplicons to survey the microbial communities, 2) shotgun metagenomics to explore their metabolic pathway potentials, and 3) meta-transcriptomic pipelines to access their active levels of each metabolic pathways identified (Leng et al., 2019; Yang et al., 2019). PHA production is fundamentally rooted in the gene expression machinery of bacterial cells, however it does not closely model classical kinetics. Thus, we employed cutting-edge quantum computing simulations to more fully describe the intricacies of this metabolic process. Classical computing methods often struggle to capture the full spectrum of interactions and dynamics within the gene regulatory network due to their limitations in handling the immense computational demands of these complex systems. However, quantum computing, with its unique capacity to feature entanglement and superposition, offers a promising avenue for a more comprehensive and accurate understanding of gene expression in the context of PHA production (Weidner et al., 2023; Li et al., 2018). As such, these simulations enabled us to create highly refined models for predicting gene regulation. We applied this method to a mixed culture system from a pilot scale batch reactor operated at the University of South Wales from a nearby treatment plant, in which the sludge digestate undergoes acidogenic fermentation to produce three different species of VFAs as the substrate for the PHA producing bacteria. We complete multi-meta-omics analysis and gene regulation models for each reactor at different time-points: before treatment, during the microbial transition, and steady-state. In doing so, we aimed to pinpoint disparities in gene regulation and discern how these variations along the reactor course translated into the final PHA yields and the composition of PHA monomers. This analysis contributes to our non-classical understanding of the fundamental metabolism, gene expression, and community dynamics of PHA producers in mixed cultures, which can better inform the enrichment strategies used to optimize PHA production from mixed microbial cultures.

The production of PHAs from VFAs offers opportunities to transform diverse waste streams into value-added products and potentially reduce greenhouse gas emissions from WWTPs. By using cutting-edge computational techniques that elucidate the fundamental biology underlying biotechnological processes, we present a novel method by which sustainable bio-based production and solid waste management systems can be coupled and optimized. Identifying non-classical operating strategies to not only achieve unprecedented PHA yields from mixed microbial cultures, but also optimize a closed-loop solid waste management technology, may support the industrial application and thus impact of this technology.

Acknowledgement

This work was supported by IDRIC Wave 2 CA and the President's PhD Scholarships at Imperial College London.

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