Title: Improving biomass waste upcycling by process intensification in two-stage anaerobic digestion

Abstract

The depletion of fossil fuels and the growing demand for renewable energy have intensified interest in sustainable technologies for organic waste exploitation. Two-stage anaerobic digestion (TSAD) represents an alternative strategy for simultaneously producing energy carriers and value-added products from digestate.

In this study, TSAD was applied to different types of organic substrates, including fruit and vegetable waste (FVW), jam wastewater (JWW), sugar-rich industrial effluents, and animal-based biomasses, to evaluate process performance, limitations, and scalability.

Thermal pretreatment effectively promoted hydrogen-producing microorganisms during the dark fermentation stage, enabling hydrogen production without the addition of external culture media and supporting process scalability. JWW was successfully processed both individually and in combination with FVW. Under optimized conditions, DF of FVW and JWW produced hydrogen yield of 99.5 mL/g_{VS} and led to improved methane yields compared to conventional single-stage anaerobic digestion and demonstrating its potential as a low-cost alternative to commercial sugars within a circular economy framework. Additional experiments showed that biochar addition did not significantly affect dark fermentation performance but increased methane concentration in biogas by approximately 50 % during the second digestion stage, proving benefits for biogas quality rather than overall yield.

Conversely, TSAD applied to sugar-rich wastewater and animal-derived substrates resulted in limited hydrogen production, with yield of approximately 60 and 8 mL/g_{VS}, respectively. This poor performance was mainly attributed inoculum variability and the persistence of methanogenic populations that outcompeted hydrogen-producing bacteria.

For animal-based biomasses, single-stage continuous anaerobic digestion was investigated as an alternative, with intermittent feeding strategies significantly enhancing methane conversion rates and process stability. Post-digestion hydrothermal treatment and bioaugmentation with the hyperthermophilic microorganism *Caldicellulosiruptor bescii* provided short-term improvements in-lignocellulose degradation, corresponding to an increase in cellulose and hemicellulose removal of 18-20 %. Still, they revealed long-term limitations related to microbial washout, kinetic constraints, and system instability.

Preliminary techno-economic and life cycle assessments of an industrial TSAD plant fed with FVW and JWW (50000 t/y) and integrated with humic acid (HA) extraction indicated that, despite its valorization potential the process is currently constrained by low hydrogen yields and high energy demand. Even when assuming selling prices of 7.67 €/kg for H_2, 0.75 €/m^3 for biomethane, 0.08 €/kg for CO_2, and 709 €/t for HA, the economic performance of the process remains strongly affected by energy-intensive operations, particularly those associated with gas purification and humic acid processing. In parallel, the LCA results indicate a relatively high environmental burden, mainly driven by electricity and heat consumption, leading to a climate change impact of 256 kgCO_2eq per ton of substrate treated. Overall, the results highlight the need to further optimize dark fermentation, energy efficiency, substrate selection, and scale-up strategies to enhance the economic and environmental sustainability of TSAD systems. While TSAD coupled with digestate conversion into agro-value products shows promise as an integrated waste-to-resource system, improvements are required before industrial implementation can be realized.