## Sustainable anaerobic conversion of mixed sludge and food waste into marketable carboxylic acids and bioenergy towards green urban biorefineries

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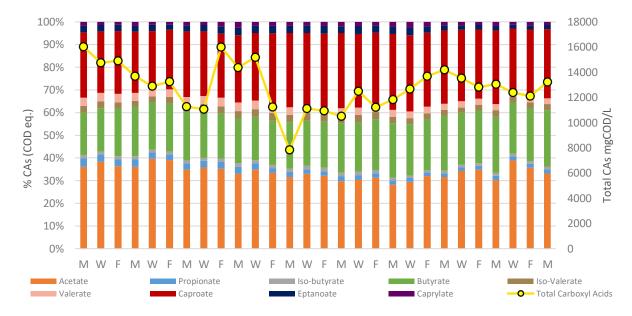
**Introduction.** Living in a world with finite resources, with an increase of population and its living standards, waste management rethinking should open new opportunities and should make possible taking advantage of what nowadays is only considered as a disposal cost. Urban organic waste represents a significant untapped biological resource which can be valorized through biorefinery platforms, promoting a more sustainable waste management and circularity. At now, the hide value of organic waste is used to obtain only few products with low economic value and small range of applications, e.g., biogas, biomethane and compost (Vidal-Antich et al., 2022).

Fermentation seems to be a key biotechnology in most microbial-driven biorefineries due to its capability to transform organic waste into various organic compounds such as lactic acid, alcohols and carboxylic acids (CAs), providing valuable "building blocks" for a subsequent conversion into marketable products (Battista et al., 2022; Garcia-Aguirre et al., 2019). Several studies on mono-fermentation of single organic waste have reported good results in terms of CAs production, but co-fermentation is emerging as a better strategy to increase yields and valorize different type of wastes, by increasing organic loading rate and buffer capacity, and balancing macro-and micro-nutrients (Peces et al., 2020; Perez-Esteban et al., 2022). Researches on co-fermentation have widely focused on the combination of waste activated sludge (WAS) and food waste (FW), produced in large amounts in densely populated metropolitan areas (Perez-Esteban et al., 2024). However, acidogenic fermentation an chain elongation (CE) process require a high demand of electron donors (EDs), as lactate or ethanol, and a strict pH control at 5-5.5 (Zhu et al., 2022). Therefore, this study aimed to set up a stable co-fermentation process for CAs production, particularly caproic acid, by exploiting the high fermentation potential of FW with WAS, avoiding external EDs and other buffering agent addition, without disregarding bioenergy production.

Materials and Methods. Food Waste (FW) was collected from the cafeteria of the National Research Council of Rome and consisted of a high fraction of fruit and vegetable scraps (70%), uneaten bread and pasta (15%), and cheese residues (15%). FW was minced by a lab scale knife and store a -20° C to maintain the same characteristics throughout the study. Waste activated sludge (WAS) was sampled from the Roma Nord WWTP (Italy) and was dynamically thickened with a lab-scale centrifuge. A thermal pretreatment of the feedstock was carried on in a bench scale autoclave Laboklav 25b (T<sub>max</sub> 134 °C, p<sub>max</sub> 3 bar). A 5.5 Liter mesophilic bioreactor, inoculated with anaerobic fermentative biomass, was operated in semi-continuous mode at OLR 10 g VS/L for each feeding and with an HRT of 12 days. A pretreated mix composed by FW/WAS=60/40 (on VS basis) was used for feeding the reactor three times per week, on Monday, Wednesday and Friday. Alkalinity, Carboxylic acids (C2-C8), lactic acid, ethanol, soluble COD, soluble carbohydrates and proteins were monitored throughout the test while the pH of the fermentation broth was monitored 24/7. Fermentate was anaerobically digested in 5.5 L reactors with HRT 15 d and OLR 1.9±0.01 gVS/Ld.

Results and Discussion. The long-term acidogenic fermentation test (~ 3 months) revealed a lactate-based CE pathway, since *in situ* lactate production took place within the first hours after feeding, through the primary fermentation of the soluble sugars of FW, and it was rapidly used as ED to elongate carboxylic acids. Sludge's proteins solubilization was promoted by feedstock thermal pretreatment, helping in buffering the system and stabilizing pH, without controlling agents addition. The feeding regime coupled with the characteristics of the feedstock allowed to carry on a stable single-stage process, which includes fermentation and CE, producing a high amount of carboxylic acids. In particular, caproic acid was found in soluble (60% in mass) and adsorbed (40%) form (total yield: 49 g<sub>C6</sub>/kg VS<sub>fed</sub>), resulting the most abundant acid (>30% of C2-C8, in CODeq).

The subsequent anaerobic digestion step, using the fermentation leftover, rich in unconverted particulate carbon and CAs, assured the further conversion of the residual organic content into methane with a stable yield of  $0.4~Nm^3/kgVS_{fed}$ .



*Figure 1:* Amount of CAs (%CODeq), compared to their total concentration (mgCOD/L), at feeding days (Monday, Wednesday, Friday), during the fermentation test.

**Conclusions.** This public-private research study explores the possibility of a eco-friendly mixed waste management, proposing a zero-chemicals strategy of co-fermentation, chain elongation and a subsequent leftover exploitation for high-value compounds recovery coupled with bioenergy production.

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