Valorizing Organic Waste Via Arrested Anaerobic Digestion (AAD): Production of Volatile Fatty Acids (VFAs)

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With the global population growth rate (registered to be around 0.9% per year), the overall consumptions in terms of energy and materials are projected to rapidly increase. Currently, in the so-called *petroleum era*, most of the anthropogenic needs are covered by fossil fuels (oil, coal, and natural gas) that result to be the most employed raw materials not only in the energetic area but also in the material one. Nevertheless, fossil fuels are limited resources and, therefore, intrinsically not renewable (their generation time exceeds the human time windows by several orders of magnitude) and they are the results of natural degradation and conversion processes dating back millions of years. Following the reports, the depletion time of these resources is estimated to be about 100 years (Mikael Höök et al, 2013). Moreover, the utilization of fossil fuels involves substantial environmental impacts that have been linked to the observable climate change phenomena and, consequently, there is an urgent need to reduce their application.

Volatile fatty acids (VFAs) are short-chained organic fatty acids comprising C2-C6 carbon atoms. These acids have a high market value due to their wide range of applications from food to chemicals, textiles, pharmaceuticals, energy, and materials. Due to their versatility, they are in high demand, and their market is projected to rise annually by 3% (Anthony T. Giduthuri et al., 2022). At present, commercial production of VFAs is mostly accomplished by chemical routes, involving fossil fuels as the main feedstock. Therefore, switching to more sustainable and less environmentally impactful production pathways is mandatory. In this regard, biological routes represent valuable candidates. In particular, the present work sheds light on of the arrested anaerobic digestion (AAD).

Anaerobic digestion (AD) is a series of biological processes in which microorganisms convert biodegradable material in the absence of oxygen giving, because of their metabolism, high-value products (i.e., biogas). In the AAD, the methanogenic step is suppressed to inhibit VFAs conversion to biogas, making VFAs themselves the main products of AAD. Regarding feedstock, organic wastes, and agricultural residues (due to their high carbon content) are great candidates to produce VFAs. Moreover, employing this kind of substrate results in proper waste management, which is a non-negligible issue due to the massive waste generation resulting from the increasing global population growth. Valorizing wastes implies applying the circular economy principles (Wee Shen Lee et al., 2013).

Although the concept behind AAD is widely known, its commercialization has been hindered by low VFAs titers and productivity, and a lack of cost-effective separation methods for recovering VFAs themselves (Haoran Wu et al.,2021). Therefore, the aim of this study is envisaging AAD as a candidate for VFAs production, testing different substrates, inocula, and processing conditions.

Nevertheless, to move towards a sustainable socio-techno-economic system, analyzing and improving only the production process itself is not sufficient. Indeed, envisaging the full supply chain is gaining relevance for the current climate change mitigation goals. Shedding light on the environmental impacts and resources used throughout a product's life cycle, i.e., from raw material acquisition, production, and use phases, to end-of-life management, can reveal important environmental footprints to be addressed. For this purpose, *Life Cycle Assessment* (LCA) is employed. LCA is a standardized (ISO 14040-44) tool that identifies and quantifies the used energy and materials, as well as flows released to the environment, and their potential impacts throughout the whole life cycle providing insights into the environmental sustainability of products. Hence, the novelty of the present study is assessing, among the AAD experimental configurations tested (conducted in duplicate) and resulted to be technically feasible, the less environmentally impactful ones, exploring both the production stage and the associated supply chain. The importance of this environmental sustainability analysis is highlighting, and possibly improving, any bottlenecks of the full supply chain. Finally, the results obtained for AAD are compared to the traditional fossil-based production pathways in order to emphasize any advantages or drawbacks of each solution.

The inoculum used in this study involves a complex mixed consortium of microorganisms such as *Bacillaceae*, *Bifidobacteria*, *Streptococci*, and *Enterobacteriaceae* (Flora-21® Kulturen Komplex Kapseln). Methanogenesis is arrested by pre-treating the inoculum with acid shock and heat treatment. The pretreatment involves subjecting the inoculum to phosphoric acid at pH 2 and a temperature of 80 to 90 °C for 2 h under helium-induced agitation. On the other hand, the substrates employed include organic wastes and, in particular, sugar wastewater.

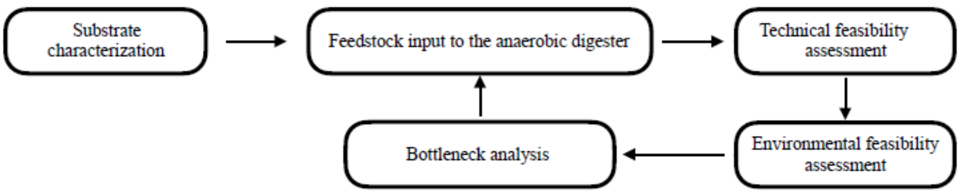
The experiments are conducted in a batch mode. The operating conditions are listed in Table 1 and each experiment is conducted in duplicate (Haoran Wu et al.,2021). The batch digestion experiments are performed to investigate the effects of operating conditions (temperature, pH, and substrate concentration) on digester performance. All batch digesters are initially established with substrate, inoculum, and digestion media (consisting of distilled water, mixed minerals, and buffer solution). Each digester is equipped with a multi-layer foil gas collection bag for gas sampling. Before digestion, nitrogen gas is spared to establish anaerobic conditions in each digester. To investigate the impacts of digestion temperature and substrate concentration, the digester operations are conducted in GL-45 glass bottles with tri-port caps. Ports allow for sampling and reagent addition. Digesters are placed in a benchtop incubating shaker to investigate the temperature effect. The gas bags are sampled to measure volume and composition, and digester pH is measured and manually adjusted. Digesters are operated until total carboxylic acid concentrations (VFAs plus lactic acids) reach their plateaus. Samples are collected periodically and stored at 4 °C for analysis. VFAs and lactic acid concentrations are analyzed using high-performance liquid chromatography (HPLC).

Table 1. Batch digestion operating conditions.

	pH-6	pH-7-S	pH-7-R
Temperature (°C)	40	40	40
Working volume (L)	0.5	0.5	0.5
Inoculum (L)	0.01	0.04	0.04
Substrate concentration (g COD/L)	107	80	80
Frequency of pH adjustment	Once every 24 h	Continuous	Once every 24 h

The environmental impact of AAD is evaluated through the LCA technique. In particular, the inventory phase is carried out by combining primary and secondary data (obtained from the *Ecoinvent v.3.9 cutoff* database) As mentioned, once the production step is investigated, the analysis is extended to the full supply chain, comparing the currently available options for producing VFAs. For this purpose, dedicated datasets are generated using the Activity Browser of Python *brightway2* framework, and 1 kilogram of VFAs is used as the functional unit (FU). The LCA impact assessment phase is conducted using the CED and CML v4.8 2016 methods. Fig. 1 shows a basic overview of the general methodology applied in this study. The experiments are still ongoing.

Figure 1. Schematization of the methodology applied in this study.



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