

# Potential of anaerobic co-fermentation in wastewater treatments plants: A review

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## Introduction

The implementation of the circular economy paradigm requires the transition of waste treatment systems from end-of-pipe towards integrated resource recovery. However, the transformation of wastewater treatment plants (WWTP) into resource recovery facilities (RRF) is far from been accomplished.

Fermentation (not anaerobic digestion) is as an essential biotechnology in waste processing biorefineries since it allows converting organic waste into easily assimilable organic compounds such as volatile fatty acids (VFA), lactic acid and alcohols. These compounds can be subsequently used as carbon source to support other biotechnologies such as biological nitrogen removal and recovery, biological phosphorous removal, bioplastics production and chain elongation, among others. However, primary sludge (PS) and waste activated sludge (WAS) is low since no more than 10% of the chemical oxygen demand (COD) contained in the sludge is converted to VFA. Intensification of sludge mono-fermentation can be achieved by mixing other waste together with PS and WAS to increase VFA production, a process known as co-fermentation.

Co-fermentation overcomes the limitations of mono-fermentation by (i) increasing the organic loading rate (OLR), (ii) providing additional buffer capacity (prevents pH drops and alkali consumption), (iii) modifying the organic matter composition, (iv) balancing macronutrients (e.g. C/N ratio), (v) diluting potential inhibitory and toxic compounds, and/or (vi) providing an active fermentative microbial community.

Co-fermentation is a rather new concept with publications devoted to the topic being noticeable from 2013 onwards. Due to the emergence of co-fermentation research, this work aims to present a comprehensive and critical overview of the achievements and perspectives of mixed-culture co-fermentation in RRF. To conduct this review, the authors have read and summarised the 44 publications available in the peer-reviewed literature. Recommendations for the selection and application of different co-substrates as well as the integration with other technologies are discussed. Finally, the review identifies a series of knowledge gaps that require further research.

## Co-fermentation overview

Most co-fermentation publications have used WAS as the main substrate (i.e. the predominant substrate in the mixture), while PS and mixed sewage sludge (SS, i.e. mixture of PS and WAS) are the second and third most used main substrates in co-fermentation publications (Fig. 1). These outcomes show that co-fermentation is expected to have an important role in future WWTP. Other organic-rich wastes such as food waste (FW), pig manure (PM) and agro-industrial waste (AgriW) have been rarely used as the main substrate. However, FW and AgriW are the most common co-substrates, probably due to their fast and high biodegradability.

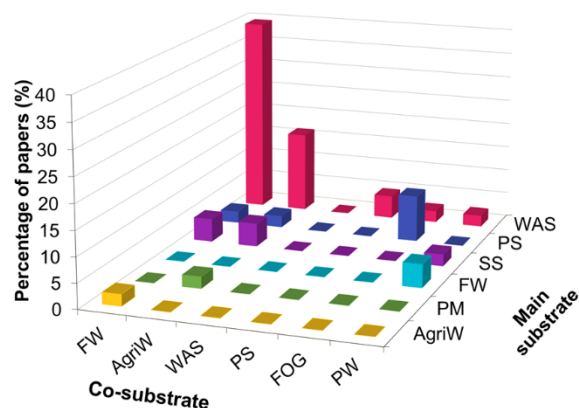


Fig. 1. Main substrates and co-substrates in publications

## Waste activated sludge co-fermentation

The most studied mixture is WAS and FW followed by mixtures between WAS and AgriW (Fig. 1). Mixture selection should rely on the ability to establish strategic and integrated platforms. Therefore, WAS and FW mixtures are attractive for waste-based biorefineries located in populated metropolitan areas while mixtures between WAS and AgriW are interesting for agropoles and agro-processing regions.

The benefit of co-fermenting WAS-FW and WAS-AgriW relies primarily on (i) the WAS buffer capacity to sustain the pH above 5.0 and prevent fermentative bacteria severe inhibition by low pH, and (ii) the high biodegradability of the co-substrate (FW or AgriW) to boost fermentation yields. WAS-FW and WAS-AgriW mixtures have been mainly designed to balance the C/N ratio. However, optimising mixtures based on C/N ratio is an oversimplification since it does not consider the operational parameters of the fermenters (e.g., temperature, pH, HRT or OLR) nor waste pre-treatment. Co-fermentation aims to boost fermentation yields but also to drive the fermentation product profile without incurring into major capital and operating costs.

It is well-known that pH controls the fermentation yield and product profile of mixed-culture fermentation, yet the pH itself is not enough to predict the product profile. For WAS-FW and WAS-AgriW mixtures, the highest fermentation yields have been achieved at circumneutral pH. This is an encouraging outcome since the buffering capacity of WAS can be used to keep the pH around neutrality, hence preventing the inhibition of fermentative bacteria. Most experiments have been performed in batch assays which results are driven by the capabilities of the starting microbial community and do not allow evaluating the microbial acclimation that occurs under continuous conditions. Beyond pH, temperature, hydraulic retention time (HRT) and OLR are variables that can be controlled to optimise the performance of continuous co-fermenters. In fact, co-fermentation operational conditions (and pH as a key operational parameter) need to consider conditions where hydrolytic and fermentation activity is high but also conditions where the proliferation and activity of methanogens are limited.

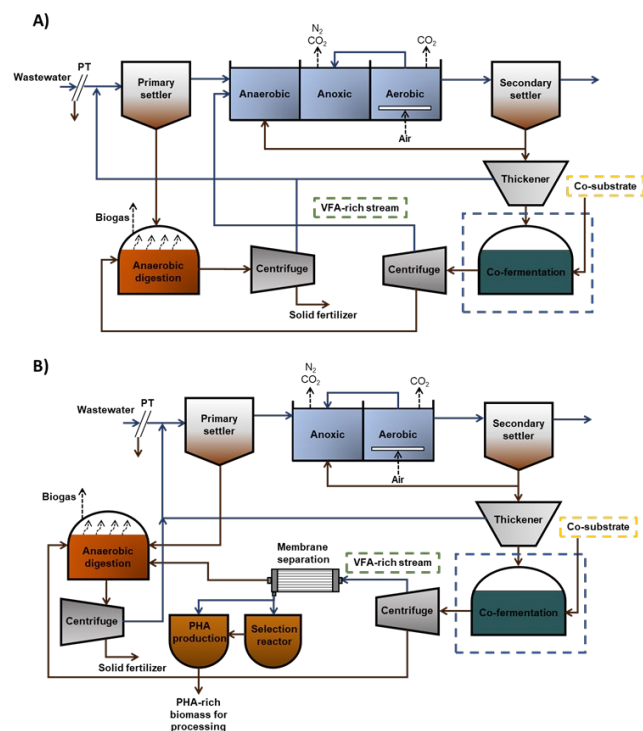
Hong and Haiyun, (2010) evaluated the interaction between different process variables (i.e. pH, WAS-FW ratio, HRT and OLR) by testing 30 different combinations in mesophilic continuous experiments. The results showed that pH had a significant interaction with other variables. The optimum pH increased from acidic (~6.0) to neutral (~7.0) values as the OLR increased from 4 to 12 kgVSS·m<sup>-3</sup>·day<sup>-1</sup> and as the mixture was enriched with FW. These results suggest that a higher pH is needed as the amount of biodegradable organic matter in the system increases, which could be related to the lower product inhibition by undissociated VFAs at higher pH. Hong and Haiyun (2010) also reported a significant interaction between pH and HRT, however, the experimental data did not allow explaining these results. In this regard, Garcia-Aguirre et al. (2019) stated that the combination of pH and HRT is a strategy to limit the growth of methanogenic archaea. Nonetheless, the HRT should be long enough to hydrolyse most of the particulate organic matter that can be later fermented into VFA.

### Integration of co-fermentation in existing WWTP

The integration of co-fermentation in a WWTP depends on the type of sewage sludge used as the main substrate and on the use of the VFAs. Fig. 2 illustrates the two main configurations considered in the literature to integrate co-fermentation in a WWTP: (Fig. 2A) to support biological nutrient removal (Long et al., 2014), and (Fig. 2B) to produce polyhydroxyalkanoates (PHA) (Moretto et al., 2020).

In Fig 2., WAS was considered as main substrate since it is the most studied main substrate in co-fermentation research. However, PS could also be considered as the main substrate. Fig. 2 includes an anaerobic digester to produce biogas from PS and the remaining solid fraction after co-fermentation.

Techno-economic studies are necessary to evaluate under which conditions the implementation of co-fermentation to support these technologies is economically attractive for WWTP operators. Importantly, these studies should consider not only the potential of the co-substrate to improve VFA yield but also the nutrients backload in the VFA-rich stream and the biogas production in the anaerobic digester, among others. These analyses should also consider the capital and operating costs associated with the new infrastructure and products processing.



**Fig. 2.** Configurations to integrate co-fermentation in a WWTP

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