Hydraulic retention time as an operational tool for the production of high value-added bioproducts via anaerobic fermentation of carbohydrate-rich organic wastes

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In the European Green Deal recently announced by the European Commission, at least 50 % reduction in green house gasses has been set by 2030 (European Commission, 2021). To attain this target, one of the main initiatives is boosting technologies that enable the substitution of fossil-based fuels and chemicals by renewable and sustainable ones (European Commission, 2019). For such a purpose, the valorization of organic waste streams for the production of high value-added bioproducts is attracting great interest. Anaerobic digestion (AD), which is a robust and well-established technology for the production of biogas, could be applied for producing biochemicals with high value for the chemical industry. Among these biochemicals, short chain fatty acids (SCFAs), lactic acid (HLact) and ethanol (EtOH) are the most frequent. The four main steps constituting conventional AD are hydrolysis, acidogenesis, acetogenesis and methanogenesis. AD can be shortened to the fermentative stage (hydrolysis and acidogenesis), avoiding the growth of methanogenic archaea through the manipulation of process operational conditions. The latter process is commonly known as anaerobic fermentation (AF) (Llamas et al., 2022; Zhou et al. 2018).

Hydraulic retention time (HRT) is a major parameter affecting AF. Since growth rate of microorganisms involved in AF is lower than that of methanogenic archaea, HRT could be used as an operational tool to tune this bioprocess. A suitable HRT that allows the growth of acidogens and hydrolytic bacteria while washing out acetolactic methanogens is crucial to obtain high yields of valuable intermediates products (Greses et al., 2021).

The objective of this investigation is the valorization of carbohydrate-rich organic wastes (*i.e.* molasses) for the production of high value-added intermediates products (*i.e.* SCFAs) via AF. A special focus is given to the effect of HRT on the SCFAs production in a semi-continuous AF at optimum pH for promoting the fermentative stage (between 5.5 and 6.0). For this purpose, three semi-continuous stirred tank reactors (CSTRs) were performed at 25 ºC, slight pH (5.5-6.0) and three different HRTs (*i.e.* 30 days, 15 days, and 8 days), hereafter named as RC_30d_HRT, RC_15d_HRT, and RC_8d_HRT, respectively. The start-up of the experiments was conducted by using a conventional sludge collected from a wastewater treatment plant without any previous pre-treatment.

As control parameters, daily pH levels and biogas production (volume and composition) were monitored. A high biogas production would imply SCFAs consumption by archaea and low acids accumulation. Other parameters related to the organic material degradation and the process efficiency (*i.e.* SCFAs, total and soluble COD, ammonium nitrogen, total and volatile solids, etc.) were analyzed 2 to 3 times a week. Moreover, samples for microbial analysis were collected from the reactors and preserved at -80 ºC to identify the predominant microorganisms involved in the process at each HRT (at steady-state conditions).

Results indicated that for AF of molasses, pH tend to decrease abruptly during the start-up periods with pH levels around 4.5-4.8 and a daily adjustment with an alkaline reagent (NaOH, 5M) was necessary to maintain the pH within the optimum range. Acetic and propionic acids were the predominant SCFAs at the start-up period, while pH stabilization around 4.7-5 led to the predominance of butyric acid. For the reactor RC_15d_HRT and after two times HRT (*i.e.* 30 days of the operational period), pH levels around 5-5.8 were maintained and valeric and caproic acids concentration started increasing. SCFAs concentrations were 10.7 g/L, 6.1 g/L, and 3.4 g/L for reactors RC_15d_HRT, RC_30d_HRT, and RC_8d_HRT, respectively. Table 1 shows the SCFAs concentration in each reactor (expressed as percentages of each individual SCFA over total fatty acidity).

It is worth mentioning that the economic value of SCFAs increases with the increase of carbon number, with valeric acid being the most expensive (4,251 $/ton) compared to caproic acid (3,815 $/ton), butyric acid (,2163 $/ton), propionic acid (2,000 $/ton), and acetic acid (600 $/ton) (Calt, 2015).

Table 1. SCFAa content of each reactor

Reactors	HAc (%)	HPr (%)	HBut(%)	HVal (%)	HCap (%)
RC_30d_HRT	30.73	8.29	41.64	14.56	4.78
RC_15d_HRT	10.12	4.09	33.19	35.93	15.74
RC__8d_HRT	50.24	28.74	19.93	1.08	0.00

*HAc: Acetic acid; HPr: Propionic acid; HBut: Butyric acid; HVal: Valeric acid; HCap: Caproic acid

Methane production was observed at the start-up period as a result of using conventional sludge. However, once acidification occurred, methane was reduced and hydrogen content increased. It should be highlighted that hydrogen is a common co-product of SCFAs when shortening the AD in the AF (Greses et al., 2021). Hydrogen was higher in RC_15d_HRT and RC_8d_HRT (23-24%) compared to RC_30d_HRT (5.8%).

It has been found that HRT is a crucial variable in the AF process of carbohydrates-rich organic wastes, affecting both the concentration and distribution of SCFA, as well as hydrogen production.

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