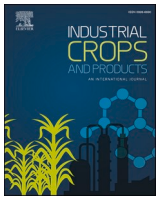
Industrial Crops & Products 220 (2024) 119206

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



# Optimising butyric and lactic acid yield from xylose by adjusting pH

J. Iglesias-Riobó *, M. Mauricio-Iglesias, M. Carballa

*CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain*

ARTICLE INFO

*Keywords:*
Carbohydrates
Carboxylate biorefinery
Medium-chain fatty acid precursors
Mixed-culture anaerobic fermentation
Volatile fatty acids

ABSTRACT

This work studies the pH effect in xylose mixed culture fermentation, focusing on lactic and butyric acid production. Batch tests showed a complete substrate consumption at slightly acidic (pH 6) and alkaline conditions (pH 8), but a great inhibition of xylose conversion was observed at pH 4. Acetic acid was the only product at pH 8, while at pH 6, butyric and propionic acids were quantified as well with a large proportion of unidentified compounds. Continuous reactor operation showed that changes within the acidic pH range (pH 4.5 to pH 6) affect the acidification degree but not the product distribution, reaching the maximum butyric acid yield (0.38 Cmol/Cmol-s) at pH 5, while lactic acid was only detected when xylose conversion or butyric acid concentration dropped. Batch tests demonstrated that butyric acid was not inhibitory for xylose or lactic acid conversion and that the main product of lactic acid fermentation was butyric acid. Although pH is an essential variable steering the fermentation yield and selectivity, this study demonstrates that it must be combined with other operational conditions in order to obtain a stable production of lactic and butyric acids.

## 1. Introduction

In recent years, research has focused on harnessing several types of waste to shift the current linear economy model to a circular economy approach (Okolie et al., 2022). Within this framework, the biorefinery concept is gaining importance, since diverse organic wastes can be converted into added-value compounds, leading to the reduction of the use of other resources (Agler et al., 2011). For organic residues and high-strength wastewaters, anaerobic digestion has become the standard technology to produce biogas, mostly for energetic purposes (Appels et al., 2008). However, other options within the biorefinery model may entail higher profitability, such as polyhydroxyalkanoates, which have the potential to replace conventional plastics (Saavedra del Oso et al., 2023), or medium-chain fatty acids (MCFA), which can be used as pharmaceutical of food additives, besides being precursors in the production of biofuels (Scarborough et al., 2018; Cavalcante et al., 2017). These high added-value compounds have in common that are based on the carboxylate platform, in which volatile fatty acids (VFA) are produced and further used as building blocks (Agler et al., 2011).

VFA are obtained through anaerobic fermentation, whereby an organic substrate, mainly composed of carbohydrates, lipids and proteins, is firstly hydrolysed into simpler molecules (monosaccharides, amino acids, fatty acids), which are subsequently converted into VFA. Focusing on carbohydrate fermentation, the most common monomer is glucose, which is converted via glycolysis to pyruvate and then to VFA (C2-C4) and other typical fermentative products (lactic acid, formic acid, succinic acid, ethanol, etc.) (González-Cabaleiro et al., 2015). Moreover, other products can be produced derived from this primary fermentation, such as MCFA.

One of the most common MCFA is caproic acid (six carbon atoms), which can be obtained through reverse β-oxidation, where butyric acid is elongated to caproic acid via an electron donor, such as lactic acid, which provides the reducing equivalents necessary for the process (Spirito et al., 2014). An alternative pathway to reverse β-oxidation is the fatty acid biosynthesis, in which the carbon donor is the malonyl-ACP instead of acetyl-CoA (Han et al., 2018).

To target caproic acid production from carbohydrates fermentation in a single stage, acidic pH (pH $<$ 5.5) and low organic loading rates (OLR, 1.1–2.0 g COD/L·d) are required (Qian et al., 2020; Wang et al., 2023). Due to the low OLR needed, a two-stage process firstly targeting the medium-chain fatty acid precursors, such as butyric acid and lactic acid or ethanol, followed by a subsequent stage targeting caproic acid seems to be the optimal strategy to maximize MCFA production.

Glucose mixed-culture fermentation has been deeply analysed, concluding that glucose is always consumed at pH between 4 and 8.5, but the product distribution is highly dependent on pH (Rafay et al., 2022; Temudo et al., 2007). Specifically, butyric acid yield is optimised at acidic pH range (4 – 5.5) as well as lactic acid yield, which is also

* Corresponding author.
*E-mail address:* juaniglesias.riobo@usc.es (J. Iglesias-Riobó).

https://doi.org/10.1016/j.indcrop.2024.119206
Received 26 March 2024; Received in revised form 9 July 2024; Accepted 10 July 2024
Available online 17 July 2024

enhanced by high organic loading rates due to the high growth rates of lactic acid bacteria (Rombouts et al., 2020). In contrast, literature about producing butyric and lactic acid production from xylose is limited, and in some cases inconsistent, despite being the second most abundant sugar in the earth. For example, Temudo et al. (2009) observed that xylose is fully converted at alkaline conditions (pH 8), with butyric acid, acetic acid and formic acid as the main products (lactic acid was not detected). In contrast, Rombouts et al. (2018), operating under the same conditions as Temudo et al. (2009), noticed a lower butyric acid production in favour of ethanol formation, which was attributed to the difference in the $N_2$ supplied.

Poor selectivity is one of the main challenges in resource recovery by anaerobic fermentation. The fermentation stoichiometry is mainly dependent on the acidogenesis step, i.e. transformation of xylose to VFA. Therefore, the mechanisms of this step are crucial to steer the fermentation towards the desired products.

Taking into account that pH modifies the bioenergetics in mixed-culture fermentation and consequently the product spectra, especially under limited substrate conditions (González-Cabaleiro et al., 2015; Kohn et al., 2000; Regueira et al., 2020), this work aims to unravel how pH changes can support the selective production of butyric and lactic acid with the perspective of obtaining MCFA precursors. The strategy consisted firstly of a pH screening in batch mode (pH 4, pH 6 and pH 8) to find out the most interesting pH range. Once the most promising pH range was identified, a continuous reactor was operated at several pH values to achieve a steady lactic acid production without compromising xylose conversion and butyric acid production. An interplay between lactic acid and butyric acid production was observed in several operating periods which led to the formulation of expressly designed batch tests (xylose, xylose + lactic acid and xylose + butyric acid) to understand the role of butyric and lactic acid in xylose fermentation.

## 2. Materials and methods

### 2.1. Feedstock description

The selected substrate for this study was xylose (Iris Biotech GMBH, Germany) as the sole carbon source (12 g COD/L). The feedstock also included a mineral medium which contained macronutrients and micronutrients as described in Suppl. Mat to avoid any microbial growth limitation. During the continuous reactor operation, the feedstock was stored at 4 °C to avoid xylose degradation.

### 2.2. Preliminary batch tests

Three batch experiments were conducted at different pH levels (pH 4, pH 6 and pH 8), controlled via a multiparametric analyser (CHEMITEC, Italy) by the addition of NaOH (3 M) or HCl (3 M). Reactors of 1.0 L of working volume were used, and the experiments were conducted in mesophilic conditions (37 °C) with a constant nitrogen sparging (≈ 10 mL/min) to ensure anaerobiosis and keep the dissolved $H_2$ concentration limited.

The inoculum was collected from a sewage sludge anaerobic digester (pH 7.4; hydraulic retention time (HRT) of 54 d), being their main characteristics summarised in Supp.Mat The experiments were performed with a substrate-to-inoculum ratio of 10 g COD xylose/g VSS, with an initial biomass concentration of 1.2 g VSS/L. Samples were taken at 2–3 h intervals at the beginning of the experiment to identify the intermediate compounds produced, decreasing this frequency progressively once xylose was fully consumed. Total and soluble chemical oxygen demand (tCOD, sCOD), VFA, xylose and soluble microbial products (SMP), such as lactic acid, succinic acid, formic acid and ethanol were determined.

### 2.3. Continuous reactor

A continuous 1.0 L (working volume) stirred tank reactor (CSTR) was inoculated with the same biomass as the batch reactors and it was operated under mesophilic conditions (37 °C), with constant $N_2$ sparging, an HRT of 1.0 d and an OLR of 12 g COD/L·d.

Throughout the experimental period (232 days), pH was slightly modified (from pH 6–4.5 and finally to pH 5) once the steady state was reached at each operational condition.

Reactor operation was monitored via the measurement of the total and soluble chemical oxygen demand (tCOD, sCOD), VFA, SMP and xylose concentrations twice a week, and the total and suspended solids concentration once a week.

### 2.4. Lactic-butyric acid batch tests

Three batch experiments in duplicate with a working volume of 0.2 L were inoculated with biomass from the continuous reactor (day 231) with an initial in-reactor concentration of 0.5 g VSS/L. The reactors were orbitally shaken and operated under mesophilic conditions (37 °C). The pH was set at 6 by phosphate buffer and controlled by NaOH addition. In order to avoid methanogenesis, 2-bromoethanesulphonate (BES) (0.5 g BES/g VSS) was added into the medium, which also contained the same macronutrient and micronutrient concentration as in previous experiments. 8 g COD/L of xylose was the sole carbon source in one experiment (control), while 4 g COD/L of lactic and butyric were also added to experiments 2 and 3, respectively. Samples were taken at the beginning of the experiment and after 24 h, 72 h and 144 h. Total and soluble COD, xylose, SMP and VFA concentrations were determined.

### 2.5. Analytical methods

Conventional physicochemical parameters were determined according to Standard Methods (APHA, 2017) Mixed liquor samples were used to calculate the total (TS and VS) and suspended (TSS, VSS) solids (SM2540 B, D, E) and total COD (SM5220C). Filtered samples were used to measure soluble COD (SM5220C), xylose, SMP and VFA.

VFA and MCFA from C2 to C7 were measured with a gas chromatography Shimadzu UV-1800 with a DB-Wax column from Agilent Technologies (30 m x 0.250 mm×0.25 μm). The injector temperature was set at 200 °C while the detector was set at 300 °C. $N_2$ was used as the carrier gas. Samples were centrifuged and filtered (0.45 μm) and then acidified with 10 μM of concentrated $H_3PO_4$ (85 %) before being analysed.

SMP (lactic acid, formic acid, succinic acid, glycerol, ethanol) and xylose were determined through high-performance liquid chromatography (HPLC) according to the GLEFG1 method with an HP 1100 (IR HP1047A detector). $H_2SO_4$ (5 mM) as an isocratic eluent was used on the AMINEX HPX-87 H (300 ×7.8 mm) column, which was at 30 °C while the detector was at 35 °C. Unidentified SMP were estimated from soluble COD measurements.

### 2.6. Calculations

Acidification degree was used to describe the xylose conversion to VFA (on COD basis), based on the VFA concentrations measured in the reactor:

$$Acidification\ degree(\%) = \frac{\Sigma C_{VFA}}{C_{xyl.in}} \times 100 \quad (1)$$

Where $C_{VFA}$ stands for the total concentration of the measured VFAs (in g COD-VFA/L) in the reactor and $C_{xyl,in}$ stands for the xylose concentration (in g COD/L) in the reactor feeding (continuous reactors). For batch reactors, $C_{xyl,in}$ stands for the initial xylose concentration.

The carbon balance and the biomass and product yields were

calculated with the values from the table attached in Supp.Mat, which was based on Rombouts et al. (2018) and Regueira et al. (2018), and was expressed as below:

$$Carbon\ balance(\%) = \frac{\sum C_{sc} + C_x + C_{H_2}}{C_{xyl,in}} \cdot 100 \quad (2)$$

Where $C_{sc}$ stands for the total identified soluble compound concentrations in the reactor effluent including the non-consumed xylose, VFA and SMP (in Cmol/L), $C_x$ stands for the biomass concentration in the effluent (in Cmol/L), assuming $CH_{1.4}O_{0.4}N_{0.2}$ as the biomass formula (Rombouts et al., 2018), $C_{H2}$ stands for the hydrogen concentration, assuming all hydrogen came from formic acid (in Cmol/L) and calculated considering that all the COD loss (difference between the total COD in the feeding and the total COD in the effluent) is caused by hydrogen production.

$$Biomass\ yield(Y_{x/s}) \quad \left(\frac{Cmol\ biomass}{Cmol\ substrate}\right) = \frac{C_x}{C_{xyl,in} - C_{xyl,eff}} \quad (3)$$

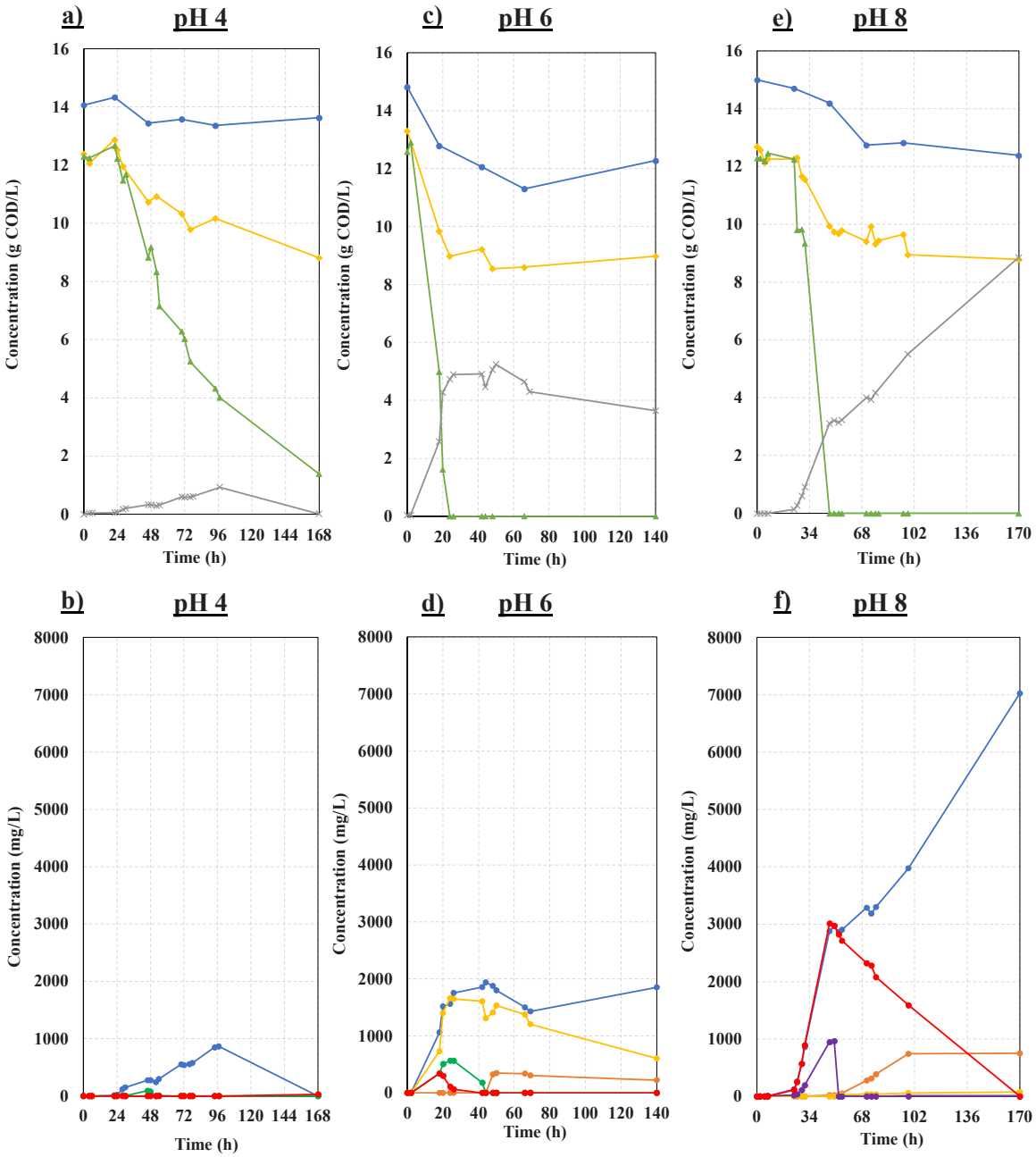



**Fig. 1.** COD measurements (a, c, e) (total COD, soluble COD, xylose, VFA) and product evolution (b, d, f) (acetic acid, propionic acid, butyric acid, formic acid, succinic acid, lactic acid) in the batch tests at pH 4, pH 6 and pH 8.

$$Product\ yield(Y_{p/s})\left(\frac{Cmol\ product}{Cmol\ substrate}\right) = \frac{C_p}{C_{xyl,in} - C_{xyl,eff}} \quad (4)$$

Where $C_{xyl,eff}$ stands for the xylose concentration in the reactor effluent (in Cmol/L) and $C_p$ stands for the targeted product concentration (in Cmol/L).

## 3. Results and discussion

### 3.1. pH screening

Different results were observed in the three batch experiments conducted with xylose at pH 4, 6 and 8 (Fig. 1). The test at pH 4 showed a behaviour consistent with product inhibition since there was xylose remanent (1.4 g COD/L) at the end of the experiment (Fig. 1a). Acetic acid was the only acid detected (Fig. 1b) reaching its maximum concentration (0.9 g/L) after 96 h, being afterwards completely consumed. In the test at pH 6, xylose was completely consumed after 24 h (Fig. 1c) resulting in higher VFA concentrations, with acetic (1.9 g/L), butyric (0.6 g/L) and propionic (0.2 g/L) acids as the main products at the end of the experiment (Fig. 1d). Xylose was also totally converted after 47 h in the test at pH 8 (Fig. e), with only acetic acid (7.0 g/L) as the main product at the end of the experiment (Fig. 1f).

These results demonstrated that pH clearly affected xylose conversion not only in terms of kinetics but also in terms of selectivity. Although the inoculum was adapted to slightly alkaline conditions, the maximum consumption rate of the three experiments (0.55 g xylose/L·h) and the lowest lag phase (< 18 h) was observed at pH 6. The tests at pH 4 and 8 had a similar lag phase (around 24 h), but the subsequent consumption rates were very different: 0.11 and 0.48 g xylose/L·h, respectively, indicating that the pH affects both microbial adaptation and xylose consumption.

The acidification degree also varied with pH. At the end of the tests, the acidification degree was 0 %, 29 % and 57 % for pH 4, 6 and 8, respectively, The gap between the soluble COD and VFA+ substrate corresponds to SMP, which usually appears during anaerobic fermentation process and they stand for alcohols, alkanes, alkenes or acids (Kunacheva et al., 2020). During the experiments, some SMP were identified, such as succinic acid at pH 4 (max. 0.1 g/L) and pH 6 (max. 0.6 g/L), and lactic acid at pH 8 (max. 1.0 g/L). These compounds were reported to be intermediates in the production of some VFA, such as propionic acid via acrylate pathway (Candry et al., 2020), which is consistent with the results obtained at pH 6 and pH 8 (Fig. 1b). The production of formic acid at pH 6 and pH 8 is consistent with the COD loss of around 2.5 g COD/L observed in these assays since hydrogen can be formed from this compound (González-Cabaleiro et al., 2015). The fraction of unidentified SMP also varied with pH, ranging from 0 % at pH 8, 60 % at pH 6 and 100 % at pH 4.

Overall, pH 6 was the most promising pH value for the operation of the continuous reactor due to the high xylose consumption rate, the significant butyric acid concentration and the presence of unidentified SMP at the end of the experiment which ultimately could be converted to products of interest.

### 3.2. Boosting butyric and lactic acid during continuous xylose fermentation

Soon after the startup of the reactor, xylose was completely consumed (Fig. 2), achieving a consumption rate of 12 g COD /L·d, similar to the consumption rate reported by Rombouts et al. (2018) and higher than Qian et al. (2020), wherein a maximum consumption rate of 2.0 g COD/L·d was achieved. A COD loss of around 1.3 g COD/L was observed and attributed to hydrogen production since methanogenesis was discarded because of the low HRT applied (1 day). The estimated carbon balance is also consistent with the lack of methanisation, as it closes next to 100 % (Table 1). During the first 70 days of operation, unidentified SMP were produced, showed by the gap between the soluble COD and the VFA+xylose+SMP concentrations in the effluent (Fig. 2). However, from day 71 on, all the soluble organic compounds corresponded to VFA with an average concentration of 8 g COD/L, resulting in an acidification degree of 71 %, a shift from the conversion

**Table 1**
Carbon balance, biomass yield ($Y_{x/s}$), acetic acid yield ($Y_{a,s}$) and butyric acid yield ($Y_{b/s}$) during the different stages of the continuous reactor operation. Stage of pH 5 + HLa stands for the period in the continuous reactor at pH 5 with a 2 g COD/L lactic acid addition in the feeding.

| | pH 4.5 | pH 5 | pH 5 + HLa | pH 6 |
|---|---|---|---|---|
| Carbon balance (% Cmol) | 96 | 99 | 96 | 100 |
| $Y_{x/s}$ ($Cmol_x/Cmol_s$) | 0.11 | 0.09 | 0.11 | 0.12 |
| $Y_{a/s}$ ($Cmol/Cmol_s$) | 0.25 | 0.24 | 0.21 | 0.31 |
| $Y_{b/s}$ ($Cmol/Cmol_s$) | 0.36 | 0.38 | 0.42 | 0.28 |

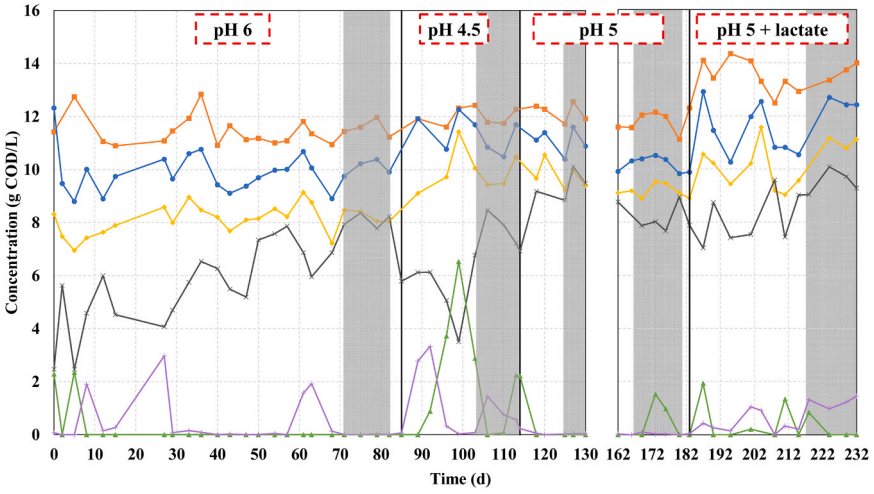



**Fig. 2.** COD measurements in the continuous reactor at different pH values (total influent, total effluent, soluble effluent, xylose, VFA, SMP). Shadowed areas correspond to steady state phases at each condition used for steady state analysis.

of xylose into SMP to the exclusive production of VFA. Butyric acid was the dominant product (4.1 g COD/L) followed by acetic acid (3.6 g COD/L), both accounting for more than 96 % (COD basis) of the soluble products (Fig. 3). These results are similar to those obtained by Rafay et al. (2022) and Temudo et al. (2007) when fermenting glucose at pH 5.4 and 5.5, respectively, with a similar organic loading rate (11.6 – 12 g COD/L·d). However, Qian et al. (2020) reported similar acetic acid (0.28 vs 0.31 Cmol/Cmol-s) but different butyric acid (0.04 vs 0.28 Cmol/Cmol-s) yields when fermenting xylose at the same pH value but under a much lower OLR (1.1 g COD/L·d vs 12 g COD/L·d), which indicates that the OLR (and/or HRT) also plays an important role in butyric acid yield. The latter is related to butyric acid production by chain elongation from acetic and lactic acids, whose production was reported to be boosted at high organic loading rates (Rombouts et al., 2020)

Trying to increase butyric acid and lactic acid yields, and following previous glucose (Temudo et al., 2007) and xylose (Qian et al., 2020; Wang et al., 2023) fermentation results, pH was decreased to 4.5 on day 85. The hypothesis is that, under more acidic conditions, $NADH/NAD^+$ balance shifts towards its acidic form, so the microorganisms would try to balance it by producing compounds in which this NADH excess is consumed, such as butyric acid. At pH 4.5, reactor performance was more unstable regarding VFA production and xylose conversion, which varied between 50 % and 100 % (Fig. 2). The soluble COD was only made up of non-converted xylose and VFA, with a maximum and minimum acidification degree of 72 % and 28 %, respectively. The product spectrum was still dominated by butyric (4.8 g COD/L) and acetic (2.6 g COD/L) acids, but a higher butyric acid yield (0.36 vs 0.28 Cmol/Cmol-s) and a lower acetic acid yield (0.25 vs 0.31 Cmol/Cmol-s) than the previous pH 6 stage were achieved. As expected, the pH decrease caused the detection of lactic acid (0.6 g COD/L), especially when butyric acid concentration or xylose conversion dropped. These results differ from previous studies at the same pH value but higher HRT (4.5–5 days) (Qian et al., 2020; Wang et al., 2023), where lactic acid was never detected and xylose conversion was around 90 %, suggesting the lactic acid production might be also influenced by HRT. Moreover, in these studies, butyric acid yields were significantly lower (0.09 and 0.17 Cmol/Cmol-s, respectively) than in this study. Comparing with studies fermenting glucose at acidic pH, Temudo et al. (2007) also noticed a decrease in glucose conversion at pH 5 or lower, needing a higher HRT to reach the total glucose conversion, which would be consistent with the abovementioned studies fermenting xylose at acidic pH.

Aiming at a more stable reactor performance and an improved xylose conversion maintaining the high butyric acid yield, pH was raised to 5 on day 114. This slight increase in pH favoured xylose consumption and VFA production (8.5 g COD/L) with negligible levels of unidentified SMP (Table 1; Fig. 2). The concentration of acetic acid was similar to pH 4.5 (Fig. 3), but butyric acid levels increased up to 5.6 g COD/L, resulting in a butyric acid yield of 0.36 Cmol/Cmol-s. This yield differs from those achieved by Tang et al. (2022) fermenting xylose at pH 5, where butyric acid (0.26 Cmol/Cmol-s), acetic acid (0.16 Cmol/Cmol-s) and caproic acid (0.19 Cmol/Cmol-s) were the main products. These differences are likely explained by the different operational parameters used, such as the organic loading rate (1.8 g COD/L·d vs 12 g COD/L·d) and the HRT (6 days vs 1 day) which could promote the chain elongation stage, since the overall yield of butyric and caproic acid precursors (acetic, butyric, lactic and caproic acid) reaches the same value (0.61 Cmol/Cmol-s) in both studies.

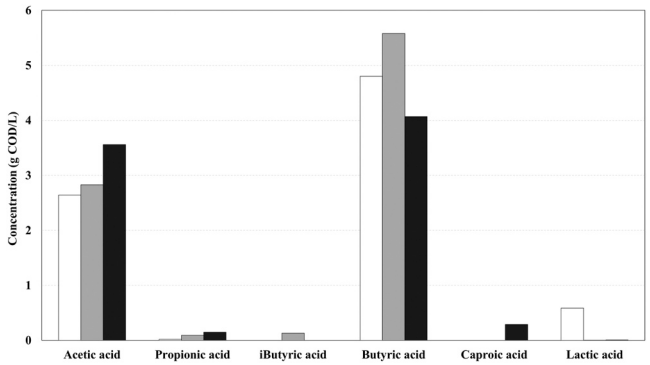



**Fig. 3.** COD concentrations of the main products obtained in the continuous reactor at different pH (□ pH 4.5, ■ pH 5 and ■ pH 6).

Predictably, the shift from pH 6 to more acidic conditions decreased the biomass concentration, since larger energy is needed for cell maintenance due to the higher cell membrane permeability at these acidic conditions (Infantes et al., 2012; Lv et al., 2022). The pH 5 stage showed the lowest biomass yield (0.08 Cmolx/Cmol-s) but the highest butyric acid yield (0.38 Cmol/Cmol-s), which suggests that the butyric acid production is not the most favourable pathway for biomass growth. This hypothesis is consistent with Tang et al. (2022) and Qian et al. (2020), where lower butyric acid yields (0.26 & 0.07 Cmol/Cmol-s) are associated with higher biomass yields (0.11 & 0.17 Cmolx/Cmol-s) are higher. However, no major implications were observed on reactor stability.

Throughout reactor operation, lactic acid levels increased when butyric acid concentration dropped (days 5, 29, 63, 96, 111) (Supp. Mat), which was also reported by Rafay et al. (2022) fermenting glucose at higher organic loading rates. This pattern suggests that lactic acid plays a significant role in butyric acid production, as previously mentioned by Brodowski et al. (2022), or high butyric acid concentrations inhibit the process, so a more in-depth understanding of lactic and butyric acid interaction is needed.

### 3.3. Lactic acid-butyric acid batch tests

In order to understand the butyric and lactic acid alternation throughout the continuous reactor operation, batch tests were performed in duplicate. Two hypotheses were formulated to explain the observed behaviour: i) high butyric acid concentrations inhibit lactic acid conversion into butyric acid, or ii) butyric acid production is limited by lactic acid availability, so when lactic acid is fully consumed, butyric acid production drops. Tests with only xylose (X1, X2) were performed as controls; tests with xylose +lactic acid (XL1, XL2) were performed so as to understand if lactic acid availability is a process bottleneck; and finally, tests with xylose+ butyric acid (XB1, XB2) were designed aiming to understand if its concentration is inhibitory in the fermentation process.

Control duplicates (X1 and X2) behave similarly (Fig. 4a, b) and the results obtained were consistent with the previous experiment of pH screening (Fig. 1b, d), although the xylose consumption rate was slower (0.12 g xylose/L·h vs 0.55 g xylose/L·h) despite using a biomass adapted to xylose consumption. This behaviour might be explained by the difference between the substrate-to-inoculum ratio (16 vs 10) which ultimately could justify the lower consumption rate and therefore a product inhibition would occur.

Butyric acid duplicates (XB1 and XB2) showed that the presence of butyric acid affected the rate of xylose conversion since only 0.6 g COD/L of xylose was consumed after 24 h, suggesting that a microbial adaptation was needed for the new operational conditions. Despite this lag phase, xylose was completely consumed after 72 h. Interestingly, the addition of butyric acid modified the metabolic pathways of xylose fermentation, since more butyric acid (6.1 g COD/L; 0.21 Cmol/Cmol-s), in detriment of acetic acid (1.1 g COD/L; 0.14 Cmol/Cmol-s), was produced compared to the controls (0.08 and 0.21 Cmol/Cmol-s, respectively). These results do not discard the hypothesis related to butyric acid inhibition through lactic acid pathway, as lactic acid was not detected during the experiment.

On the contrary, lactic acid duplicates (XL1 and XL2) showed quite different trends during substrate conversion to the different products (Fig. 4e, f). In XL1, lactic acid and xylose are fully converted after 72 h

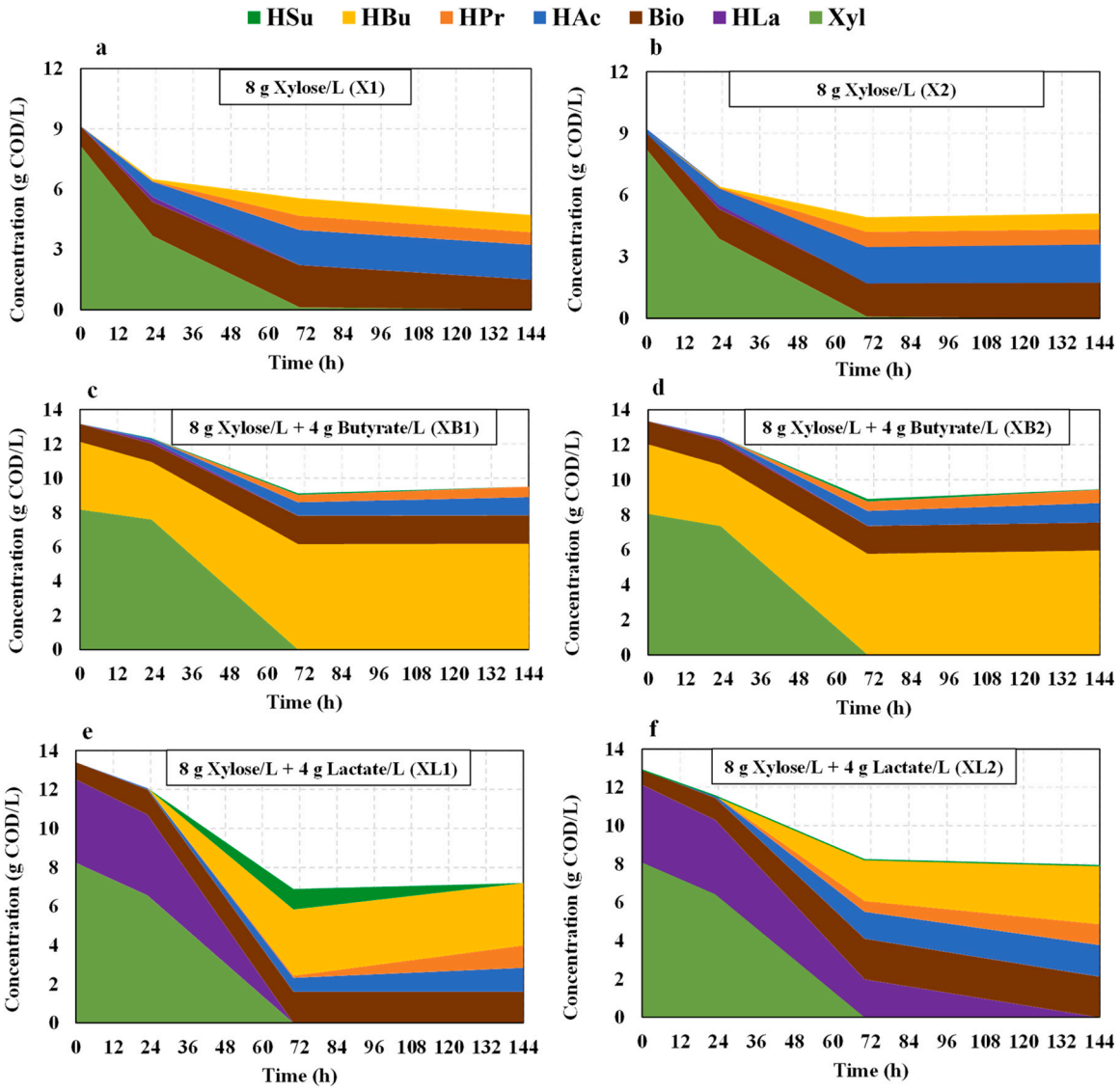



**Fig. 4.** COD measurements of different soluble organic fractions in batch tests with xylose (a, b), xylose + butyric acid (c, d) and xylose + lactic acid (e, f). Xylose, butyric acid and lactic acid concentrations are on COD basis.

into butyric acid (3.4 g COD/L), succinic acid (1.1 g COD/L), acetic acid (0.7 g COD/L), propionic acid (0.1 g COD/L) and a large concentration of unidentified SMP (4.3 g COD/L) (Fig. 4e). Xylose was also completely consumed after 72 h in XL2, but the product spectrum was quite different (Fig. 4f): succinic acid was not detected, instead a significant lactic acid concentration was achieved (2.0 g COD/L), together with butyric acid (2.1 g COD/L), acetic acid (1.4 g COD/L), propionic acid (0.6 g COD/L) and a lower unidentified SMP concentration (2.3 g COD/L). Despite the clear differences presented, both experiments converged into comparable results at the end of the experiment, with butyric acid as the main product (0.21 Cmol/Cmol-s), followed by acetic acid (0.10–0.14 Cmol/Cmol-s) and propionic acid (0.08 Cmol/Cmol-s). This final convergence suggests that both metabolic pathways are similar energetically, and very minor changes during fermentation process could drive to different intermediate products but to similar end products.

Both lactic and butyric acid tests yielded a significantly higher butyric acid production than the blank test (0.21 vs 0.08 Cmol/Cmol-s) so the presence of these compounds boosts the production of butyric acid, discarding the hypothesis of product inhibition. However, it still remains unclear if lactic acid acts as a precursor in butyric acid formation, so a lactic acid addition in the feeding of the continuous reactor was assessed so as to understand better the lactic acid role.

### 3.4. *Lactic acid-butyric acid interactions in continuous operation*

Lactic acid was added to the feeding of the continuous reactor (2 g COD/L) operated at pH 5 (Fig. 2). It can be observed that xylose consumption was not affected, but in contrast to the batch test, only half of the lactic acid added was converted to butyric acid (Fig. 5). The biomass concentration grew up from 0.7 to 1.0 g VSS/L. The 50 % lactic acid consumption might be explained by the XL1 and XL2 tests, in which it is observed that lactic acid has a lower consumption rate than xylose, which added to the low HRT applied (1 day), suggests that lactic acid is not fully converted due to a kinetic limitation.

## 4. Conclusions

In this study, xylose fermentation was steered towards butyric and lactic acid production by changing pH. The butyric acid yield was optimised at pH 5 (0.36 Cmol/Cmol-s) but a constant lactic acid production could not be reached. The alternation of butyric and lactic acid

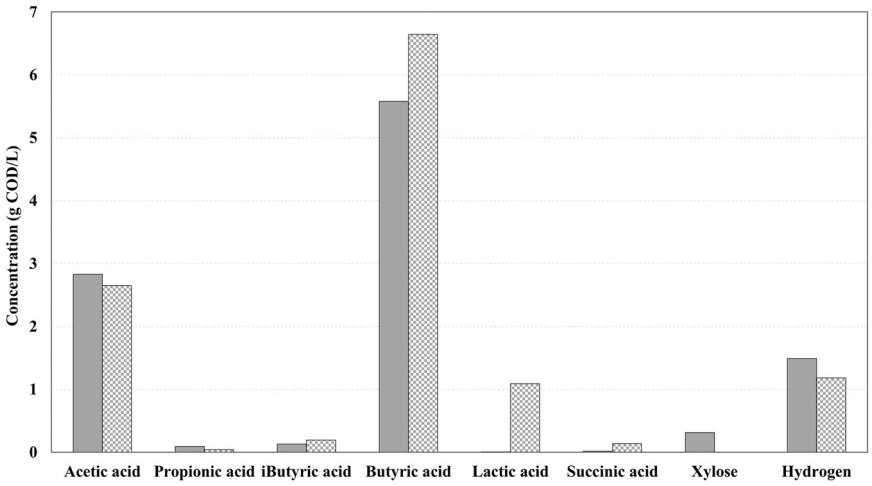



**Fig. 5.** COD concentrations in the continuous reactor at pH 5 without (■) and with (▩) lactic acid addition in the feeding.

in the continuous reactor suggests that lactic acid acts as an intermediate in butyric acid production, which was further confirmed. Overall, modifying pH is not sufficient to achieve constant lactic acid production, and thus, other strategies, such as modifying the organic loading rate or the medium used, must be investigated.

E-supplementary data of this work can be found in the online version of the paper.

## CRediT authorship contribution statement

**Juan Iglesias-Riobó**: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization; **Miguel Mauricio-Iglesias**: Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization, **Marta Carballa**: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marta Carballa reports financial support was provided by European Regional Development Fund. Juan Iglesias-Riobó, Miguel Mauricio Iglesias and Marta Carballa report financial support was provided by Xunta de Galicia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

This research was funded by the Spanish Government through the CELL4CHEM project (PCI2021-121989, ERACoBioTech 3rd call). The authors belong to a Galician Competitive Research Group (ED431C-2021/37).

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.119206.

## References

Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T., 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2010.11.006.

APHA, 2017. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. https://doi.org/10.1016/j.pecs.2008.06.002.

Brodowski, F., Łężyk, M., Gutowska, N., Kabasakal, T., Oleskowicz-Popiel, P., 2022. Influence of lactate to acetate ratio on biological production of medium chain carboxylates via open culture fermentation. Sci. Total Environ. 851. https://doi.org/10.1016/j.scitotenv.2022.158171.

Candry, P., Radić, L., Favere, J., Carvajal-Arroyo, J.M., Rabaey, K., Ganigué, R., 2020. Mildly acidic pH selects for chain elongation to caproic acid over alternative pathways during lactic acid fermentation. Water Res. 186 https://doi.org/10.1016/j.watres.2020.116396.

Cavalcante, W. de A., Leitão, R.C., Gehring, T.A., Angenent, L.T., Santaella, S.T., 2017. Anaerobic fermentation for n-caproic acid production: A review. Process Biochemistry. https://doi.org/10.1016/j.procbio.2016.12.024.

González-Cabaleiro, R., Lema, J.M., Rodríguez, J., 2015. Metabolic energy-based modelling explains product yielding in anaerobic mixed culture fermentations. PLoS One 10. https://doi.org/10.1371/journal.pone.0126739.

Han, W., He, P., Shao, L., Lü, F., 2018. Metabolic interactions of a chain elongation microbiome. Appl. Environ. Microbiol 84. https://doi.org/10.1128/AEM.01614-18.

Infantes, D., González del Campo, A., Villaseñor, J., Fernández, F.J., 2012. Kinetic model and study of the influence of pH, temperature and undissociated acids on acidogenic fermentation. Biochem Eng. J. 66, 66–72. https://doi.org/10.1016/j.bej.2012.04.017.

Kohn, R.A., Boston, R.C., Mcnamara, J.P., France, J., Beever, D.E., 2000. The role of thermodynamics in controlling rumen metabolism. Model. Nutr. Util. Farm Anim.

Kunacheva, C., Soh, Y.N.A., Stuckey, D.C., 2020. Identification of soluble microbial products (SMPs) from the fermentation and methanogenic phases of anaerobic digestion. Sci. Total Environ. 698. https://doi.org/10.1016/j.scitotenv.2019.134177.

Lv, N., Cai, G., Pan, X., Li, Y., Wang, R., Li, J., Li, C., Zhu, G., 2022. pH and hydraulic retention time regulation for anaerobic fermentation: focus on volatile fatty acids production/distribution, microbial community succession and interactive correlation. Bioresour. Technol. 347 https://doi.org/10.1016/j.biortech.2021.126310.

Okolie, J.A., Epelle, E.I., Tabat, M.E., Orivri, U., Amenaghawon, A.N., Okoye, P.U., Gunes, B., 2022. Waste biomass valorization for the production of biofuels and value-added products: a comprehensive review of thermochemical, biological and integrated processes. Process Saf. Environ. Prot. https://doi.org/10.1016/j.psep.2021.12.049.

Qian, D.K., Geng, Z.Q., Sun, T., Dai, K., Zhang, W., Jianxiong Zeng, R., Zhang, F., 2020. Caproate production from xylose by mesophilic mixed culture fermentation. Bioresour. Technol. 308 https://doi.org/10.1016/j.biortech.2020.123318.

Rafay, R., Allegue, T., Fowler, S.J., Rodríguez, J., 2022. Exploring the limits of carbohydrate conversion and product formation in open mixed culture fermentation. J. Environ. Chem. Eng. 10 https://doi.org/10.1016/j.jece.2022.107513.

Regueira, A., Bevilacqua, R., Lema, J.M., Carballa, M., Mauricio-Iglesias, M., 2020. A metabolic model for targeted volatile fatty acids production by cofermentation of carbohydrates and proteins. Bioresour. Technol. 298 https://doi.org/10.1016/j.biortech.2019.122535.

Regueira, A., González-Cabaleiro, R., Ofiţeru, I.D., Rodríguez, J., Lema, J.M., 2018. Electron bifurcation mechanism and homoacetogenesis explain products yields in

mixed culture anaerobic fermentations. Water Res. 141, 349–356. https://doi.org/10.1016/j.watres.2018.05.013.

Rombouts, J.L., Kranendonk, E.M.M., Regueira, A., Weissbrodt, D.G., Kleerebezem, R., van Loosdrecht, M.C.M., 2020. Selecting for lactic acid producing and utilising bacteria in anaerobic enrichment cultures. Biotechnol. Bioeng. 117, 1281–1293. https://doi.org/10.1002/bit.27301.

Rombouts, J.L., Mos, G., Weissbrodt, D.G., Kleerebezem, R., Van Loosdrecht, M.C.M., 2018. Diversity and metabolism of xylose and glucose fermenting microbial communities in sequencing batch or continuous culturing. FEMS Microbiol Ecol. 95 https://doi.org/10.1093/femsec/fiy233.

Saavedra del Oso, M., Mauricio-Iglesias, M., Hospido, A., Steubing, B., 2023. Prospective LCA to provide environmental guidance for developing waste-to-PHA biorefineries. J. Clean. Prod. 383, 135331 https://doi.org/10.1016/j.jclepro.2022.135331.

Scarborough, M.J., Lynch, G., Dickson, M., McGee, M., Donohue, T.J., Noguera, D.R., 2018. Increasing the economic value of lignocellulosic stillage through medium-chain fatty acid production. Biotechnol Biofuels 11. https://doi.org/10.1186/s13068-018-1193-x.

Spirito, C.M., Richter, H., Rabaey, K., Stams, A.J.M., Angenent, L.T., 2014. Chain elongation in anaerobic reactor microbiomes to recover resources from waste. Curr. Opin. Biotechnol. https://doi.org/10.1016/j.copbio.2014.01.003.

Tang, J., Dai, K., Wang, Q.-T., Zheng, S.-J., Hong, S.-D., Jianxiong Zeng, R., Zhang, F., 2022. Caproate production from xylose via the fatty acid biosynthesis pathway by genus Caproiciproducens dominated mixed culture fermentation. Bioresour. Technol. 351, 126978 https://doi.org/10.1016/j.biortech.2022.126978.

Temudo, M.F., Kleerebezem, R., Van Loosdrecht, M., 2007. Influence of the pH on (Open) mixed culture fermentation of glucose: a chemostat study. Biotechnol. Bioeng. 98, 69–79. https://doi.org/10.1002/bit.21412.

Temudo, M.F., Mato, T., Kleerebezem, R., Van Loosdrecht, M.C.M., 2009. Xylose anaerobic conversion by open-mixed cultures. Appl. Microbiol Biotechnol. 82, 231–239. https://doi.org/10.1007/s00253-008-1749-y.

Wang, Q., Dai, K., Tang, J., Hong, S., Zheng, S., Sun, T., Zeng, R.J., Zhang, F., 2023. Low-temperature caproate production, microbial diversity, and metabolic pathway in xylose anaerobic fermentation. Front Environ. Sci. Eng. 17 https://doi.org/10.1007/s11783-023-1637-9.