



Caproic acid production is linked to biomass activity during xylose fermentation

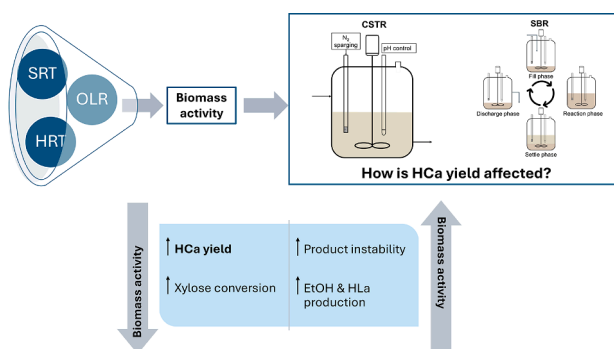
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HIGHLIGHTS

- Caproic acid production is favoured at low biomass activities.
- Electron donors are not fully consumed at high biomass activities.
- Sequential reactor operation leads to higher caproic acid yields.
- Short HRT leads to non-consumed xylose and no caproic acid production.
- High feed xylose concentration does not affect caproic but fosters butyric acid yield.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aims at addressing inconsistencies in literature regarding the organic loading rate (OLR) and hydraulic retention time (HRT) effect on caproic acid production, using biomass activity as an indicator. Xylose was fully consumed in the reference CSTR and SBR (HRT = 1-day; OLR = 12 g COD/(L·d)), but different caproic acid yields (0.02 vs 0.11 Cmole/Cmole-s) were observed, which was linked to differences in biomass activity (12 vs 3.5 g/(g VSS·d)). At HRT 0.5 days, xylose conversion was incomplete and lactic acid and ethanol appeared, reducing caproic acid production. However, increasing xylose concentration in the feeding to 24 g COD/L did not change the caproic acid yield (0.12 Cmole/Cmole-s), which was explained by similar biomass activity as in the reference SBR (4.8 g/(g VSS·d)). These findings indicate that the SBR is the optimal configuration, since it allows maintaining a low biomass activity and therefore a high caproic acid yield.

1. Introduction

Medium chain carboxylates (MCC) are promising products to recover organic carbon through anaerobic fermentation since they can be used as feed additives or as precursors for biofuel or biopolymer production

(O-Thong et al., 2020; Scarborough et al., 2018). One of the most common MCC is caproic acid, which can be obtained through reverse β-oxidation or fatty acid biosynthesis (FAB) (Han et al., 2018). Both pathways consist of a redox process in which a short chain carboxylate (SCC), such as butyric acid (C4), is elongated through an electron donor

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(ethanol or lactic acid), which provides two carbon atoms for the elongation to caproic acid (C6). The latter means that, to target caproic acid production in a single-stage mixed-culture fermentation process, the bioreactor operational conditions should provide the suitable window for the production of the electron donor and subsequent chain elongation.

Several studies reported that slightly acidic conditions (pH 5–6) were the optimal pH range for the chain elongation process as it is the best compromise between thermodynamics and microbial competition, as chain elongation is less thermodynamically favourable under alkaline conditions and at neutral pH the methanogens could outcompete with chain elongators (De Groof et al., 2019), but the proper organic loading rate (OLR) or HRT and solid retention time (SRT) range is still uncertain.

Since the electron donor availability is one of the limiting steps, its external supplementation emerges as a good alternative to enhance caproic acid yield. Adding punctually ethanol in a xylose fermentation system, the caproic acid yield rose from 0.20 Cmol/Cmol-s to around 0.27 Cmol/Cmol-s (Tang et al., 2022), while constant lactic acid supplementation using xylan as carbon source yielded around 0.15 Cmol/Cmol-s of caproic acid at different OLR values (Liu et al., 2022). However, this external addition entails higher operational costs, so other studies were focused on in-situ electron donor production.

Without an exogenous electron donor addition, caproic acid (0.05 Cmol/Cmol-s) was only detected in CSTR xylose fermentation when increasing the HRT from 4.5 to 9 days. These results corresponded to an OLR of 1.2 g COD/(L·d) (Wang et al., 2023), which is also consistent with Qian et al. (2020), where caproic acid was not detected with an HRT of 5 days (OLR 2.1 g COD/(L·d)) and Tang et al., (2022), where caproate (0.20 Cmol/Cmol-s) was detected with an OLR of 1.8 g COD/(L·d). Overall, it seems that a low OLR improve the caproic acid production.

However, other studies showed that chain elongation stage was boosted when increasing the OLR. Liu et al. (2022), using xylan and lactic acid as substrates, observed that rising the OLR from 2.9 to 11.6 g COD/L·d (by shortening the HRT from 8 to 2 days) led to the improvement of MCC yields (from 0.11 to 0.18 Cmol/Cmol-s), which is consistent with Rafay et al. (2022) fermenting glucose, in which chain elongation products rose when the OLR increased from 12 to 28.8 g COL/L·d (by increasing glucose concentration in the feeding from 10 to 24 g COD/L).

A high OLR could be applied either by lowering the HRT (hydraulic overload) or by increasing the substrate concentration in the reactor feeding (organic overload), and consequently, the implications are different. A hydraulic overload might cause a biomass growth limitation (reactor washout) and therefore a kinetic limitation (insufficient time for substrate or intermediates conversion). On the contrary, an organic overload might cause the incomplete substrate conversion due to product inhibition or high cellular density inhibition. Therefore, both overloads might drive to differences in microbial community composition and product selectivity.

The differences observed in literature suggest that the OLR might be incomplete, as it only takes into account the substrate fed and the HRT, thus ignoring other variables that may be crucial, such as the SRT or even the substrate feeding profile. Since the SRT and HRT are coupled in a CSTR reactor due to the permanent mixing, the solid phase is retained the same time as the liquid phase. In contrast, the Sequential Batch Reactor (SBR) emerges as a good operation alternative, as it gives the opportunity to decouple the HRT from the SRT by including a settle phase in the cycle, allowing to retain the biomass inside the reactor. Furthermore, this reactor configuration allows to alternate high and low substrate concentrations in the reactor (Rombouts et al., 2018), which might be interesting for MCC production, since lactic acid production was reported to be boosted when a high substrate concentration is available due to the fast growth rate of the lactic acid bacteria (Rombouts et al., 2020). Lago et al. (2023) reported that decoupling the SRT (30 days) from the HRT (10, 20 days) in a SBR reactor treating a

carbohydrate-rich residue at pH 6 caused the decrease of caproic acid production compared to the CSTR operation (4.1 % vs 25 % w/w) in favour of butyric and lactic acid, besides the same OLR (3 g COD/(L·d)) was applied in both reactors, thus reinforcing that the OLR is not the proper parameter to analyse the load impact on MCC production.

The biomass activity provides a better description of the reactor performance than HRT or OLR because it takes into account not only the load applied to the system but also the biomass yield and the SRT. and thus it helps to close the gap in understanding the effect of different loads on MCC production during mixed-culture fermentation. Therefore, the objective of this work was to evaluate the influence of biomass activity on xylose fermentation targeting caproic acid. For that purpose, two reactor configurations (CSTR and SBR) were assessed at similar HRT and OLR.

2. Materials and methods

2.1. Feedstock description

Same feedstock composition was applied in both the continuous and the sequential batch reactor. The carbon source was a standard hemi-cellulose monomer (synthetic xylose (Iris Biotech GmbH, Germany)), which was complemented with macronutrients and micronutrients (see Suppl. Mat.). The feedstock was kept at 4 °C to prevent xylose degradation.

2.2. Continuous reactor and sequential batch reactor

A continuous stirred tank reactor (CSTR) and a sequential batch reactor (SBR) were operated during 156 and 104 days, respectively, at varying operational conditions (Table 1). Both reactors were inoculated with the same biomass from a mesophilic anaerobic sludge digester (pH 7.4; HRT 54 d).

Two operational stages can be distinguished in the continuous reactor, according to the HRT applied, to assess the effect of a hydraulic overload: 1 day (CSTR-Ref, OLR of 12 g COD/L·d) and 0.5 days (CSTR-Hyd, OLR of 24 g COD/L·d).

The cycle of the SBR comprised the following four phases: charge phase (5 min), reaction phase (duration dependent on HRT), settling phase (3 min) and discharge phase (5 min). Three operational stages were applied to this reactor. During the first stage (SBR-Ref), the same HRT (1 day) and OLR (12 g COD/L·d) as in CSTR-Ref were applied in order to compare the two reactors. Once the pseudo-steady state was reached, the reactor was split in two to assess a hydraulic overload (by decreasing the HRT to 0.5 days, SBR-Hyd) and an organic overload (by increasing xylose concentration in the feedstock to 24 g COD/L, SBR-Org); increasing in both cases the OLR to 24 g COD/(L·d).

Reactors' performance was monitored via the measurement of the chemical oxygen demand (COD), carboxylates, soluble microbial compounds (SMP) (lactate, succinate, ethanol and formate) and xylose concentrations twice a week, while the total and suspended solids concentrations were measured once a week. For the SBR mode, a cycle characterisation was performed when pseudo-state was reached in each

Table 1
Experimental conditions of the reactors' operation.

	CSTR- Ref	CSTR- Hyd	SBR- Ref	SBR- Hyd	SBR- Org
Temperature (°C)	37	37	37	37	37
pH	6.0	6.0	6.0	6.0	6.0
Working volume (L)	1.0	1.0	1.2	1.2	1.2
Volume Exchange Ratio (%)	–	–	50	50	50
HRT (d)	1.0	0.5	1.0	0.5	1.0
Xylose feeding concentration (g COD/L)	12	12	12	12	24
OLR (g COD/(L·d))	12	24	12	24	24

operational stage (SBR-Ref: day 71, SBR-Hyd: day 99, SBR-Org: day 98).

2.3. Analytical methods

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

Carboxylates from C2 to C7 were measured by gas chromatography (Shimadzu UV-1800) with a DB-Wax column from Agilent Technologies (30 m × 0.250 mm × 0.25 μm). The detector temperature was set at 300 °C while the injector temperature was set at 200 °C. The carrier gas used was N₂. The samples were centrifugated and filtered (0.45 μm), and then acidified with 10 μM of concentrated H₃PO₄ (85 %) before being analysed.

SMP (lactic acid, formic acid, succinic acid, glycerol and ethanol) and xylose were determined through high-performance liquid chromatography (HPLC) according to the GLEFG1 method with an HP 1100 (IR HP1047A detector). H₂SO₄ (5 mM) as an isocratic eluent was used on the AMINEX HPX-87H (300 × 7.8 mm) column, which was at 30 °C while the detector was at 35 °C.

2.4. Calculations

The biomass exchange ratio (λ) represents the difference between biomass concentration in the reactor and biomass concentration in the effluent:

$$\lambda = \frac{X_{\text{reactor}}}{X_{\text{effluent}}} \quad (1)$$

Where X_{reactor} represents the biomass concentration within the reactor (in g VSS/L) and X_{effluent} the biomass concentration in the effluent (in g VSS/L). In a CSTR, λ is equal to one.

The product and biomass yields indicate the biomass growth and product formation per xylose consumed, respectively, and, considering that the concentration of biomass in the influent is negligible they are calculated as follows:

$$\text{Biomass yield} (Y_{x/s}) \left(\frac{\text{Cmol biomass}}{\text{Cmol substrate}} \right) = \frac{C_{x,\text{effluent}}}{C_{xyl,\text{influent}} - C_{xyl,\text{effluent}}} \quad (2)$$

$$\text{Product yield} (Y_{p/s}) \left(\frac{\text{Cmol product}}{\text{Cmol substrate}} \right) = \frac{C_{p,\text{effluent}}}{C_{xyl,\text{influent}} - C_{xyl,\text{effluent}}} \quad (3)$$

where $C_{x,\text{effluent}}$ stands for the biomass concentration in the effluent (in Cmol/L), $C_{p,\text{effluent}}$ represents the targeted product concentration in the effluent (in Cmol/L), $C_{xyl,\text{influent}}$ reflects total xylose concentration in the feeding of the reactor (in Cmol/L), $C_{xyl,\text{effluent}}$ constitutes the xylose concentration in the reactor effluent (in Cmol/L).

The average biomass activity indicates the xylose consumed per biomass and per day:

$$\text{Biomass activity} \left(\frac{\text{g xylose consumed}}{\text{g VSS} \cdot \text{d}} \right) = \frac{1}{Y_{x/s} \cdot f \cdot \text{HRT} \cdot \lambda} \quad (4)$$

where f is a conversion factor between the different units equal to 0.75 g xylose·Cmol-x/(g VSS · Cmol-s).

The maximum biomass activity in the SBR reactor represents the

theoretical maximum concentration of xylose the biomass can consume per day, and it is estimated as follows:

$$\text{Maximum biomass activity} \left(\frac{\text{g xylose consumed}}{\text{g VSS} \cdot \text{d}} \right) = \frac{\nu_{xyl}}{X_{\text{initial}}} \quad (5)$$

Where ν_{xyl} stands for the average xylose consumption rate (in g xylose/(L·d)), which is calculated from the substrate consumption profile obtained during cycle characterisations, and X_{initial} constitutes the biomass concentration in the reactor at the beginning of the cycle (in g VSS/L), which is estimated as the difference between the mass of biomass inside the reactor and the mass of biomass in the effluent divided by the reactor volume at the beginning of the reaction phase (1.2 L).

3. Results and discussion

3.1. Influence of reactor configuration in product selectivity

During the first stage (HRT 1 day; OLR 12 g COD/(L·d), xylose was completely consumed in both reactors (CSTR-Ref and SBR-Ref), and a similar concentration of carboxylates (7.5–8.5 g COD/L, Fig. 1 a,b) and biomass in the effluent (0.8–1.0 g VSS/L, Fig. c,d) was obtained. However, the biomass concentration inside the SBR was 3-fold (Fig. 1d) the biomass concentration inside the CSTR (Fig. 1c), increasing the biomass exchange ratio and decoupling the SRT (3.2 d vs 1.0 d) from the HRT. Consequently, the biomass activity in the SBR-Ref (3.5 g/(g VSS·d), Fig. 1f) was much lower than in CSTR-Ref (12 g/(g VSS·d), Fig. 1e).

This different biomass activity might explain the product spectra obtained in both reactors (Fig. 2). The SBR-Ref led to a higher caproic acid yield (0.11 vs 0.02 Cmol/Cmol-s) (Fig. 2) in detriment of a lower butyric acid yield (0.19 vs 0.28 Cmol/Cmol-s) compared to CSTR-Ref, suggesting that lower biomass activities foster the chain elongation stage. This fact would be consistent with the results of Wang et al., (2023), who observed that caproic acid was only produced when HRT was increased from 4.5 to 9 days, yielding 0.05 Cmol/Cmol-s with a biomass activity of 1.4 g/(g VSS·d). The similar caproic acid yield obtained in CSTR-Ref (0.02 Cmol/Cmol-s) with more restrictive conditions than studies at longer HRT and lower biomass activities (Qian et al., 2020; Wang et al., 2023) suggest an easier xylose uptake by the microorganism consortia present in this study.

A cycle characterisation was performed in SBR-Ref with the aim of finding out how substrate and products evolve during the cycle (Fig. 3a). It can be observed that xylose was rapidly consumed (2.9 g COD xylose/(L·h)) and converted mainly into acetic acid (3.4 g COD/L), butyric acid (3.0 g COD/L) and caproic acid (2.0 g COD/L). Lactic acid (max. 1.5 g COD/L) was produced during the first 2 h and subsequently consumed when xylose was no longer present in the reactor. At these conditions, the maximum biomass activity was 24 g/(g VSS·d) (Table 2), indicating that the system was far from being overloaded. Interestingly, two product profiles can be distinguished during the cycle: when xylose was still present in the medium (0–2 h interval) and when it was completely consumed (2–12 h interval). During first stage, 93 % of total acetic acid production was achieved, suggesting that it is mainly produced directly from xylose. Butyric acid is produced equally in both stages, which could indicate that is formed straightforwardly from xylose conversion, as reported in Rombouts et al., (2018), and also by the lactate-chain elongation, as demonstrated by Brodowski et al., 2022a. Caproic acid is mainly produced in the second stage (62 %), which leads to the

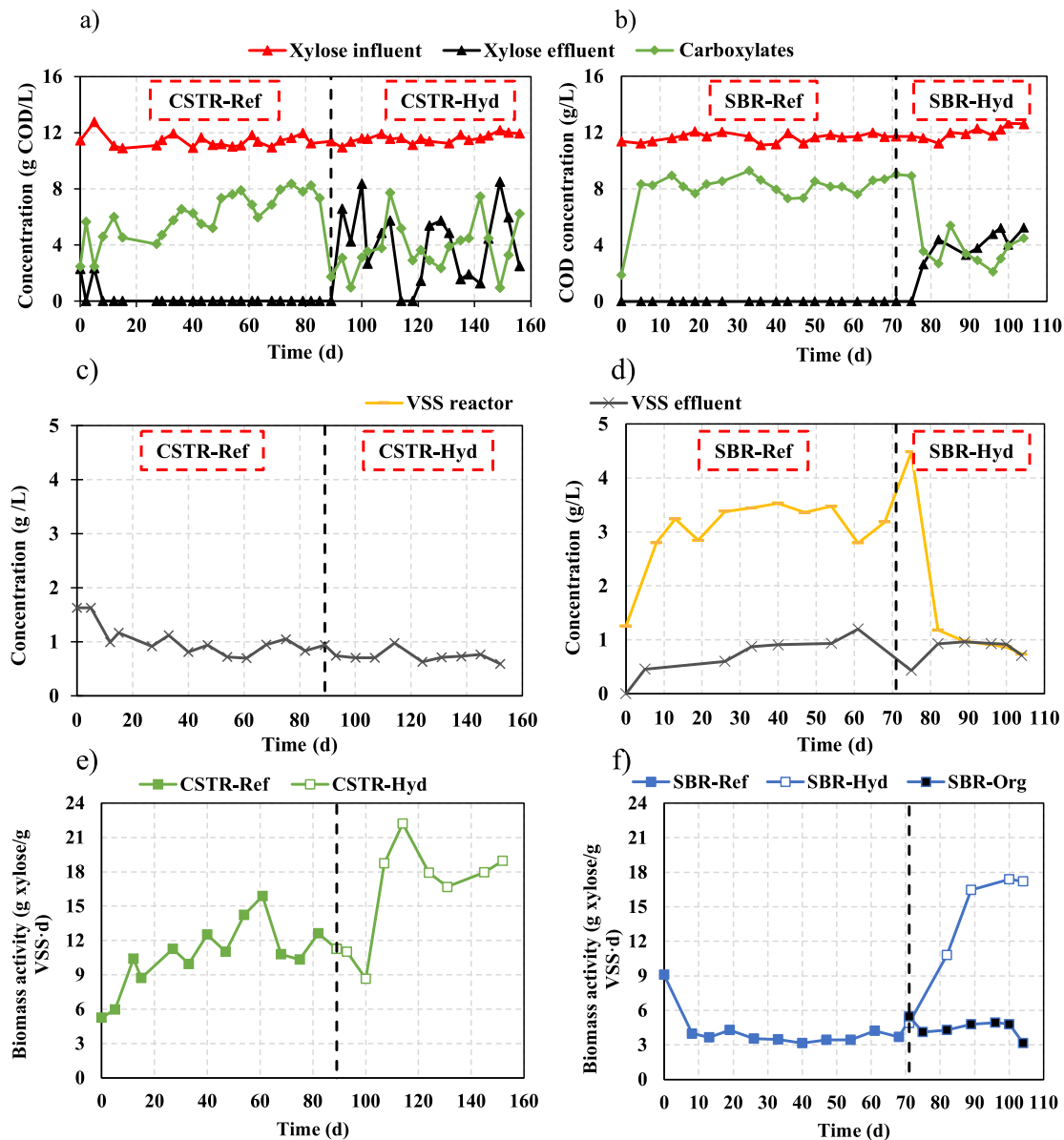


Fig. 1. Xylose and carboxylates concentration, biomass concentration and biomass activity during CSTR (a, c, e) and SBR (b, d, f) performance.

hypothesis that its production is dependent on the prior formation of other products (i.e lactic and butyric acid).

3.2. Hydraulic overload: does reactor configuration matter?

The hydraulic overload led to high instability in the CSTR operation (CSTR-Hyd), since xylose concentration in the effluent fluctuated between 0 and 8 g COD/L, and therefore, the carboxylates concentration varied accordingly from 1 to 8 g COD/L (Fig. 1a). The operation of the SBR was more stable (SBR-Hyd), but xylose conversion and carboxylate production dropped as well (Fig. 1b). The latter indicates that the low HRT applied in both reactors hampered substrate conversion into carboxylates. Interestingly, the biomass concentration inside the SBR reactor decreased significantly (Fig. 1d), equalling the biomass concentration in the effluent, and thus leading to the convergence of SRT

and HRT. As a consequence, the biomass activity in CSTR-Hyd and in the SBR-Hyd were similar (≈ 18 g/(g VSS-d)).

Despite the same biomass activity in both reactors, the product spectra were quite different. Similar acetic acid (0.27 vs 0.30 Cmol/Cmol-s), caproic acid (not detected) and lactic acid yields (0.19 vs 0.20 Cmol/Cmol-s) were achieved (Fig. 2), but differences were noticed in butyric acid (0.21 vs 0.07 Cmol/Cmol-s) and ethanol yields (0 vs 0.06 Cmol/Cmol-s). As butyric acid was reported to be produced either directly from xylose (Rombouts et al., 2018) or by chain elongation of acetate (Spirito et al., 2014), the increase of ethanol and lactic acid yields are consistent with the drop of butyric acid yield and that caproic was no longer produced in both operations.

The hydraulic overload (SBR-Hyd) led to lower (50 %) and slower (0.6 g COD xylose/(L-h)) xylose conversion (Fig. 3b). However, the maximum biomass activity increased until 36 g/(g VSS-d) (Table 2),

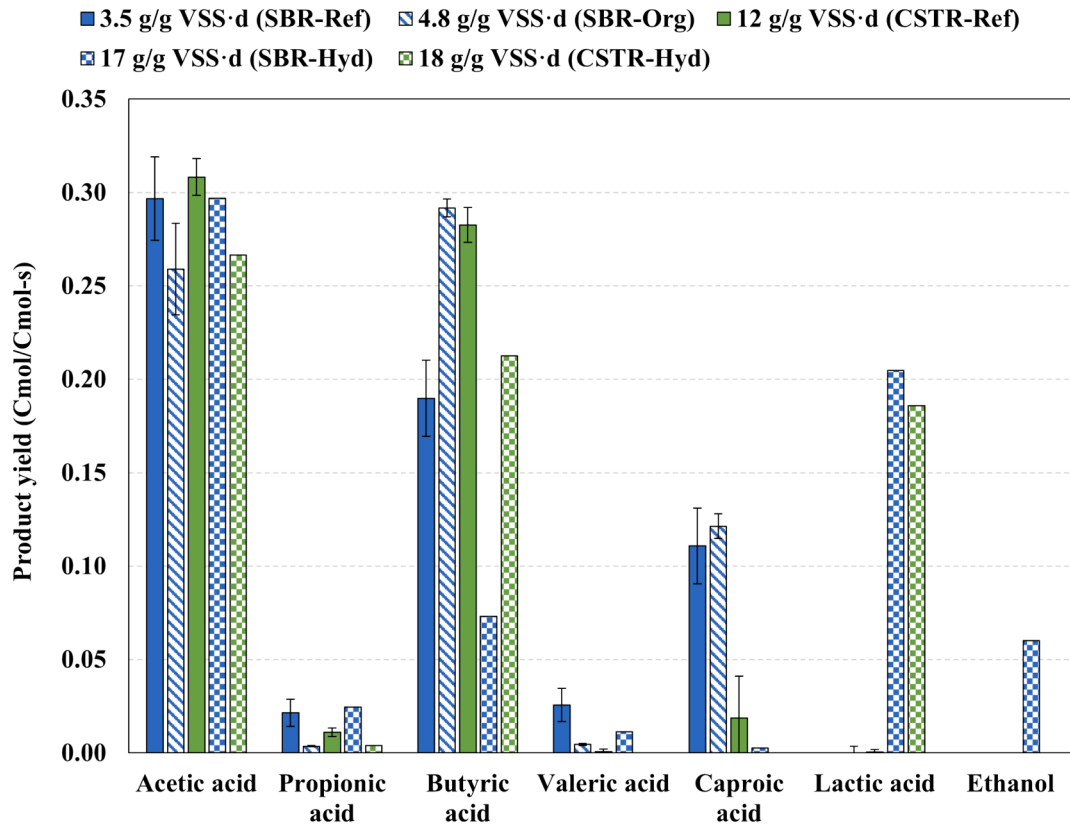


Fig. 2. Product spectra of different operations organised from the lowest to the highest biomass activity. Blue colour corresponds to SBR and green colour to CSTR.

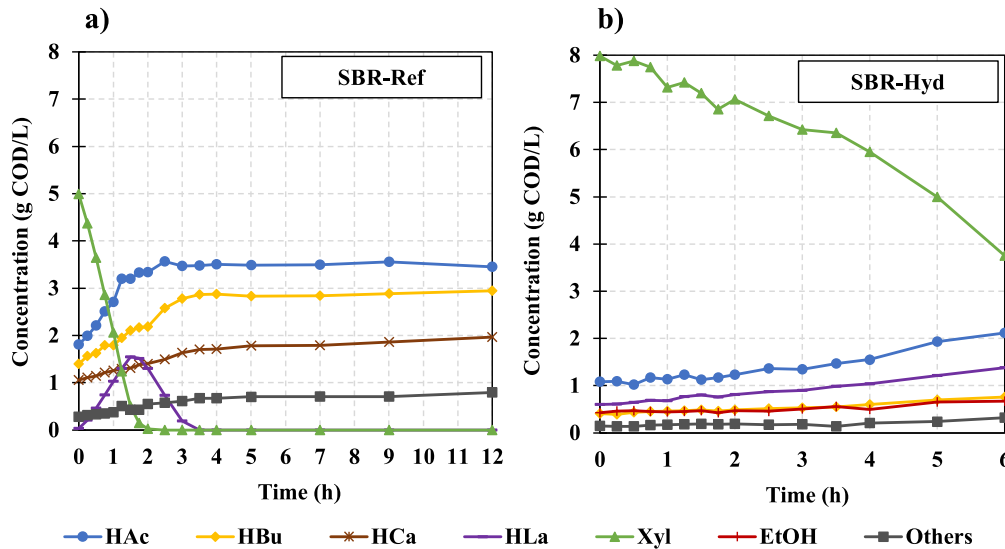


Fig. 3. Substrate (Xyl) and product (acetic acid (HAc), butyric acid (HBU), caproic acid (HCa), lactic acid (HLa) and ethanol (EtOH)) profile during one cycle in SBR-Ref (a) and SBR-Hyd (b).

Table 2

Biomass activity, maximum biomass activity and lactic acid production rate per unit biomass during one SBR cycle. Columns are organised from the lowest to highest biomass activity.

Parameter	SBR-Ref	SBR-Org	SBR-Hyd
Biomass activity (g/g VSS·d)	3.5	4.8	17
Maximum biomass activity (g/g VSS·d)	24	22	36
Lactic acid production rate (g/g VSS·d)	8.7	10	6.9

which means the microbial community can cope with higher organic loads but there was not enough biomass concentration inside the reactor at this HRT value. Moreover, although lactic acid was present as an end product, its specific production rate, calculated from lactic acid profile in cycle characterisation, decreased compared with SBR-Ref from 8.7 to 6.9 g/(g VSS·d), suggesting that these operational conditions did not improve the selectivity towards lactic acid.

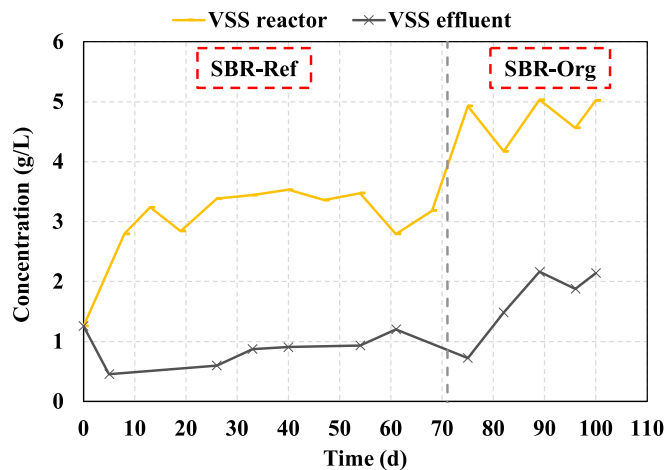


Fig. 4. Biomass concentration in the reactor and in the effluent of SBR-Ref and SBR-Org.

3.3. Organic overload in SBR operation (SBR-Org)

The cycle characterisations demonstrated that the SBR mode can maintain high caproic acid yields with a considerable OLR applied, but the system is too sensitive to SRT decrease due to the decrease in biomass concentration inside the reactor. However, it still remains unclear how the process selectivity would be affected at higher organic loads with enough biomass concentration in the reactor. Therefore, in parallel to SBR-Hyd, an organic overload (SBR-Org) was assessed by increasing the xylose concentration in the reactor feeding from 12 to 24 g COD/L at HRT 1 day, thus equalling the OLR to the SBR-Hyd (24 g COD/(L·d)).

Conversely to SBR-Hyd, the organic overload led to a rise of biomass concentration (Fig. 4) in the reactor (4.5 g VSS/L) and in the effluent (1.5–2 g VSS/L), corresponding to a SRT of 2.5 days. This SRT change did not affect biomass yield (0.12 Cmol x/Cmol·s) or substrate conversion, as xylose was fully consumed, leading to a biomass activity of 4.8 g/(g VSS·d) (Fig. 1f). Similar product spectra than SBR-Ref (Fig. 2) was reported, except for butyric acid yield, which was higher (0.29 vs. 0.19 Cmol/Cmol·s).

Cycle characterisation revealed that the organic overload in SBR-Org resulted in a higher xylose consumption rate (3.8 vs 2.9 g COD xylose/(L·h)), but similar maximum biomass activity (22 g/(g VSS·d), Table 2), thus confirming that the system can assimilate elevated substrate

Table 3

Summary of main parameters and results of CSTR and SBR operations organised from the lowest to the highest biomass activity.

Parameter	SBR-Ref	SBR-Org	CSTR-Ref	SBR-Hyd	CSTR-Hyd
SRT (d)	3.2	2.3	1.0	0.5	0.5
Biomass exchange ratio (λ)	3.2	2.3	1.0	1.0	1.0
Biomass activity (g/g VSS·d)	3.5	4.8	12	17	18
Carbon balance (% Cmol)	98	101	100	103	106
Xylose conversion (%)	100	100	100	61	66
$Y_{x/s}$ (Cmol/Cmol)	0.12	0.12	0.12	0.16	0.15
$Y_{Ca/s}$ (Cmol/Cmol)	0.11	0.12	0.02	0.00	0.00
$Y_{Bu/s}$ (Cmol/Cmol)	0.19	0.29	0.28	0.07	0.21

concentrations without a product inhibition. Lactic acid reached a higher maximum concentration (4.4 g COD/L, Fig. 5) than in SBR-Ref (1.5 g COD/L, Fig. 3), due to the specific lactic acid production rate was similar (10 vs 8.7 g/g VSS·d, Table 2). Literature reported that lactate could be oxidised to acetic acid, converted to propionic acid via acrylate pathway or used as electron donor in reverse β -oxidation

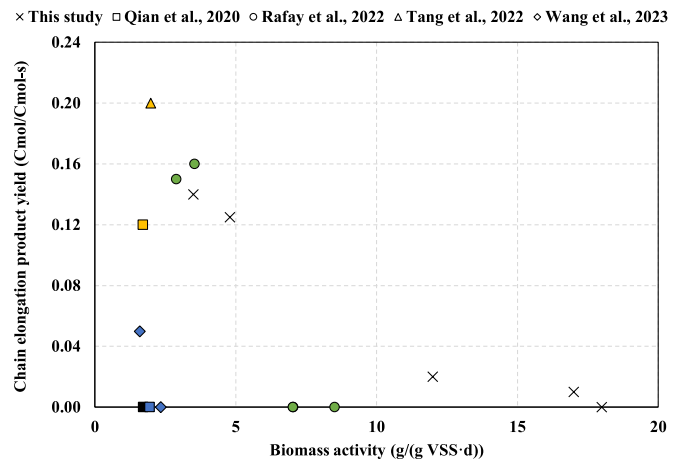


Fig. 6. Comparison of biomass activities and chain elongation product yield. Black markers (■) stands for pH 6, blue markers (■) for pH 5.5, green markers (●) for pH 5.4 and yellow markers (■) for pH 5.

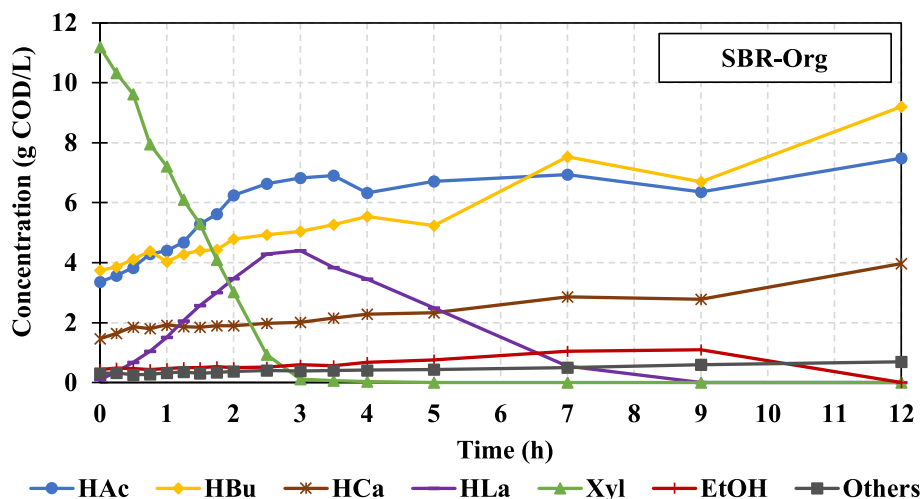


Fig. 5. Substrate (Xyl) and product (acetic acid (HAc), butyric acid (HBu), caproic acid (HCa), lactic acid (HLA) and ethanol (EtOH)) profile during one cycle of SBR-Org.

process for the production of butyric and caproic acid (Brodowski et al., 2022b; Candry et al., 2020; Spirito et al., 2014). It is hypothesised that the higher xylose concentration fed into the system led to a higher lactic acid concentration which inhibited acrylate pathway and lactate oxidation in favour of butyric acid production, specially using monosaccharides as substrate (De Groof et al., 2020; Jankowska et al., 2018), as the decrease in odd carboxylates (propionic and valeric acid) and acetic acid yields (-0.1 Cmol/Cmol-s) is equal to the increase in butyric acid yield ($+0.1$ Cmol/Cmol-s).

3.4. Influence of biomass activity on chain elongation stage

Regardless of reactor configuration and operational conditions, a close relation between biomass activity and caproic acid yield is observed, as lower biomass activities are associated with higher caproic acid yields (Table 3).

To compare these findings with literature, biomass activity was calculated for some studies fermenting xylose or glucose (Qian et al., 2020; Rafay et al., 2022; Tang et al., 2022; Wang et al., 2023), and related to chain elongation product yield (Fig. 6). Caproic acid and valeric acid were considered here as chain elongation products as valeric acid cannot be produced directly by the acidification of either xylose or glucose. Overall, it is confirmed that there exist a biomass activity window in which chain elongation process is favoured, regardless of pH (between 5 and 6) and substrate (glucose or xylose). Yet, the highest caproic acid yields were obtained at various pH, HRT and reactor mode conditions, which suggest that the optimal operational conditions for chain elongation may vary depending on substrate characteristics.

4. Conclusions

The interplay between biomass activity and caproic acid yield in xylose mixed-culture fermentation was assessed in this study. Higher caproic acid yields were obtained at lower biomass activities, regardless the OLR applied or the reactor configuration. The hydraulic overload induces reactor operation instability, leading to a higher biomass activity and to the appearance of electron donors as end products. In contrast, SBR demonstrated resilience to organic overload without altering caproic acid yield. Overall, this study brings more light to the interactions among HRT, SRT and OLR to optimise the medium chain carboxylates production.

CRedit authorship contribution statement

Juan Iglesias-Riobó: conceptualization, methodology, investigation, writing – Original draft, Writing – Review & editing. **Miguel Mauricio-Iglesias:** conceptualization, methodology, writing – Original draft, Writing – Review & editing, supervision. **Marta Carballa:** conceptualization, methodology, resources, writing – Original draft, Writing – Review & editing, supervision, project administration, funding acquisition.

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].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2024.131952>.

Data availability

Data will be made available on request.

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