Journal of Environmental Management 373 (2025) 123529

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Tailoring non-axenic lactic acid fermentation from cheese whey permeate targeting a flexible lactic acid platform

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ABSTRACT

Lactic acid (LA) is an important biobased platform chemical, with potential applications in synthetising a wide range of chemical products or serving as feedstock for various bioprocesses. Industrial LA production *via* pure culture fermentation is characterized by high operational costs and utilizes food-grade sugars, thereby reducing the feasibility of LA applications. In this context, our research focussed on valorising the largest dairy side stream, cheese whey permeate, through the use of mixed microbial communities. We evaluated the effect of different operational parameters (temperature, pH and hydraulic retention time) in non-axenic fermentations on productivity, yield, concentration, optical purity, and community. Our findings revealed that operating at mildly thermophilic conditions (45 °C) resulted in highly selective LA production, and significantly augmented the LA yield, and productivity, compared to higher temperatures (50–55 °C). In addition, operating at circumneutral pH conditions (6.0–6.5) led to significantly increased the LA fermentation performance compared to the conventional acid pH conditions ($\leq$5.5). This led to an unprecedented LA productivity of 27.4 g/L/h with a LA yield of 70.0% which is 2.5 times higher compared to previous reported maximum. Additionally, varying pH levels influenced the optical purity of LA: we achieved an optical L-LA purity of 98.3% at pH 6.0–6.5, and an optical D-LA purity of 91.3% at a pH of 5.5. A short hydraulic retention time of less than 12 h was crucial for selective LA production. This process also yielded a microbial biomass composed of 90.3–98.6% *Lactobacillus delbrueckii*, which could be potentially valorised as probiotic or protein ingredient in food or feed products. Our work shows that by careful selection of operational conditions, the overall performance can be significantly increased compared to the state-of-the-art. These results highlight the potential of non-sterile LA fermentation and show that careful selection of simple reactor operation parameters can maximize process performance. A preliminary assessment suggests that valorising EU cheese whey permeate could increase LA and poly-LA production by 40 and 125 times, respectively. This could also lead to the production of 4,000 kton protein-rich biomass, potentially reducing CO_2 emissions linked to EU food and feed production by 4.87% or 2.77% respectively.

1. Introduction

Lactic acid (LA) is a biobased platform chemical with the second largest market volume after ethanol. The estimated annual LA market volume is 1,350 kton, with an expected annual growth of 8–16% (Dusselier et al., 2013; Ma et al., 2023). LA is a versatile bulk chemical that can be converted into a wide range of products, including resins, antifreeze, and flavouring compounds (Dusselier et al., 2013). Furthermore, LA can be used as a substrate for other biological processes producing more valuable products such as microbial protein (€6,860 per tonne dry biomass) (Sakarika et al., 2022; Van Peteghem et al., 2022).

This promising platform chemical can be converted to the biopolymer poly-lactic acid (PLA). Depending on the optical purity (enantiomeric LA forms L(+) or D(−)), different biopolymers can be produced with various mechanical properties (Naser et al., 2021). L-LA is preferred in the pharmaceutical and food industries due to its enhanced assimilation in the human body compared to D-LA (Abdel-Rahman and Sonomoto, 2016; Rawoof et al., 2021). Lactic acid bacteria (LAB), the key biocatalysts in LA production, can utilize a broad range of substrates, including industrial side streams, food waste, and lignocellulosic materials (Abdel-Rahman and Sonomoto, 2016). Additionally, valuable by-products such as bacteriocins, exopolysaccharides, poly-β-hydroxybutyrate, and probiotic cultures are produced during LA fermentation (Mazzoli et al., 2014). The broad range of LA applications, along with the diverse products produced by LAB, make them ideal candidates for biorefinery concepts.

The economic feasibility of LA applications depends largely on the cost of the raw materials and the requirement for axenic conditions (Li

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https://doi.org/10.1016/j.jenvman.2024.123529
Received 12 July 2024; Received in revised form 20 November 2024; Accepted 27 November 2024

et al., 2014). On an industrial scale, LA production utilizes food-grade feedstocks such as sugar beet, corn, and wheat resulting in competition with food resources (Spekreijse et al., 2019). Those food-grade feedstocks can account for up to 40–70% of the LA production cost (Haris et al., 2024; Huang et al., 2023). In addition, industrial LA production relies on pure cultures requiring sterilisation, which increases operational expenditure (OPEX) (Peinemann et al., 2019) and capital expenditure (CAPEX) compared to non-axenic fermentation (Chen and Wan, 2017; López-Gómez et al., 2019). In addition to high substrate and sterilisation costs, the production is often performed in batch processes (Avidan and Greener, 2021; Morrissey et al., 2016), resulting in low productivities (0.93–4.32 g/L/h), compared to chemical production thereby increasing the overall production costs (Doran, 2013).

Latest research aims to reduce those costs by utilizing mixed communities and low-value feedstocks such as cheese whey permeate (CWP) (Huang et al., 2023; Schütterle et al., 2024). This industrial side stream has limited applications in the food and feed sector (e.g. bulk sweetener ingredient in bakery products) and, if not valorised, requires costly disposal treatments (Khezri et al., 2016; Zotta et al., 2020). Interestingly, CWP is one of the largest dairy streams, next to milk, giving it significant potential to be used as a feedstock for platform chemical production. In 2020, the European Union (EU) produced 47 million tons of CWP (Eurostat, 2020), which is rich in lactose (50–60 g/L) (Byland, 2003; Prazeres et al., 2012). Different studies conducted in continuous stirred tank reactors (CSTR) have shown that selective LA production can be achieved by adjusting specific operational parameters (Sakai and Ezaki, 2006; Wu et al., 2015; Yang et al., 2022). Hydraulic retention time (HRT) of less than 12 h has proven to be an effective selection tool for LAB with high specific growth rates, thereby enhancing overall LA selectivity (Choi et al., 2016; Sakarika et al., 2022). To further minimize the invasion of non-LAB, promote the growth of LAB, and enhance LA selectivity, other stringent conditions such as acidic pH (<5.0) and thermophilic temperatures (50–60 °C) can be applied (Akao et al., 2007; Itoh et al., 2012; D.-H. Kim et al., 2012). Consequently, these operational parameters (i.e. temperature, pH, and HRT) could lead to LAB dominance, enabling selective LA production while omitting sterilisation.

Previous research on non-axenic LA fermentation of side streams has often focused on optimizing a single parameter, usually LA selectivity, while other crucial factors like productivity, yield, concentration, and optical purity are often understudied. These parameters are essential for the economic feasibility and scalability of LA applications. Additionally, industrial processes tend to rely on food-grade feedstocks and are energy-intensive, posing sustainability challenges. To address these limitations, this study aims to optimize multiple operational reactor parameters (temperature: 45–55 °C, pH: 5.0–6.5, HRT: 10-1.5h) to significantly enhance key LA production metrics: productivity, yield, concentration, and optical purity. Such a comprehensive exploration of the combined effects of HRT, temperature, and pH on LA selectivity has not been previously conducted, making this study crucial for bridging the knowledge gap in selective LA production. By simultaneously utilizing one the largest industrial dairy side streams, CWP, and performing non-axenic fermentation, we aim not only to optimize LA production metrics but also contribute to a more sustainable and resource-efficient LA production process. In addition, we assessed the microbial community for LAB abundance, with a keen focus on their potential use as probiotic or protein ingredient in food or feed applications. By identifying operational conditions that ensure both high LAB abundance and microbial safety, we not only improve LA production but also create new opportunities for valorising CWP. This study offers a combined strategy for improving LA production while exploring the use of LAB produced under non-axenic conditions as food or feed ingredients.

2. Materials and methods

2.1. Cheese whey permeate characterization and nutrient supplementation

All experiments were conducted using CWP collected from Milcobel, a dairy product manufacturer in Langemark-Poelkapelle, Belgium. In this facility, cheese whey derived from mozzarella and cheddar cheese production was combined, and the whey proteins were separated via ultrafiltration. The remaining CWP, containing a limited amount of protein (0.30 ± 0.09%), was collected in batches, combined, and stored at −20 °C until use. Most LAB are auxotrophic for amino acids (Abdel-Rahman et al., 2013) and require organic nitrogen supplementation (e.g., protein) to support their growth. To determine the appropriate amount of supplemented organic nitrogen needed for LAB growth and high LA yields, a preliminary experiment was performed. The organic nitrogen used in this experiment was yeast extract (YE) due to its utilisation on an industrial scale (Proust et al., 2019). YE concentrations of 6 g/L, 9 g/L, and 12 g/L were evaluated in a CSTR, at relevant conditions for this study (45 °C, pH 5.5, and HRT 10h). Amongst the YE concentrations evaluated, 9 g/L was the lowest concentration that did not negatively impact the LA production (Fig. S1). Therefore, 9 g/L of YE was added to the CWP before the subsequent LA fermentations. Characterization of CWP (Table 1), with and without yeast extract supplementation, was performed by analysing concentrations of carbohydrates (lactose, galactose, glucose), organic acids (LA, acetic acid, formic acid, propionic acid, and butyric acid), anion and cation (Cl^-, NO_2^-,NO_3^-, PO_4^{3-}, SO_4^{2-}, Na^+, NH_4^+, K^+, Ca^{2+}, and Mg^{2+}), total nitrogen (TN), chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), pH, and electrical conductivity (EC). The

Table 1
Characterization of cheese whey permeate with and without yeast extract supplementation. The values presented are the average values of all the different cheese whey permeate batches used in this work (n ≥ 6).

Parameter	Cheese whey permeate	Cheese whey permeate supplemented with 9 g/L yeast extract	Unit
pH	6.08 ± 0.09	6.19 ± 0.09	/
EC	7.80 ± 0.38	8.45 ± 0.51	mS/cm
TN	0.29 ± 0.01	0.97 ± 0.08	g/L
Organic total nitrogen	0.18 ± 0.01	0.80 ± 0.02	g/L
COD	86.3 ± 2.4	83.0 ± 4.3	g/L
Carbohydrates			
Lactose	62.8 ± 6.1	65.3 ± 6.3	g/L
Glucose	0.18 ± 0.10	0.21 ± 0.11	g/L
Galactose	1.48 ± 0.40	1.79 ± 0.27	g/L
Organic acids			
Lactic acid	1.36 ± 0.03	1.41 ± 0.03	g/L
Acetic acid	0.21 ± 0.02	0.09 ± 0.03	g/L
Butyric acid	0.61 ± 0.05	0.28 ± 0.04	g/L
Propionic acid	ND	2.24 ± 1.14	mg/L
Citric acid	2.11 ± 0.08	9.07 ± 0.25	mg/L
Anions			
Cl^-	1,390 ± 57	1,545 ± 45	mg/L
NO_2^-	ND	ND	mg/L
NO_3^-	26.3 ± 2.7	29.3 ± 1.5	mg/L
PO_4^{3-}	1,473 ± 27	1,856 ± 15	mg/L
SO_4^{2-}	195 ± 12	264 ± 23	mg/L
Cations			
Na^+	535 ± 3	527 ± 2	mg/L
NH_4^+	138 ± 24	168 ± 10	mg/L
K^+	2,280 ± 57	2,667 ± 40	mg/L
Ca^{2+}	417 ± 5	387 ± 9	mg/L
Mg^{2+}	113 ± 5	109 ± 2	mg/L
TSS/VSS			
TSS	1.2 ± 0.3	1.6 ± 0.3	g/L
VSS	0.9 ± 0.3	1.1 ± 0.3	g/L

ND: not detected.

specific analytical techniques are described in detail in section 2.4.

2.2. Inoculum

Cheese whey, prior to reverse osmosis and ultrafiltration, was collected from Milcobel and stored at −20 °C. The cheese whey was used as inoculum for all the reactor runs. To ensure high microbial activity of the inoculum, it was incubated before reactor inoculation for 48 h at 45 °C, 50 °C, and 55 °C, depending on the respective LA fermentation in a CSTR.

2.3. Continuous fermentation of cheese whey permeate for lactic acid production

The non-axenic fermentation of CWP to LA was performed in a double jacketed glass CSTR with a total volume of 0.6 L and a working volume of 0.2 L, magnetically stirred at 400 rpm. A total of seven reactor runs, with varying temperature and pH were conducted under anaerobic conditions, which were ensured by purging the reactor with N_2 prior to inoculation and maintained using a rubber seal. Each reactor run evaluated either a specific temperature (45 °C, 50 °C, or 55 °C) or pH value (5.0, 5.5, 6.0, or 6.5). First, the optimal temperature (45 °C, 50 °C, or 55 °C) for LA productivity, selectivity, optical purity, and yield was determined at a fixed pH of 5.5. The pH was measured with a pH electrode (662–1774, VWR®) and was controlled at the targeted value through addition of 2 M NaOH using a Microdos MP pH controller (Verderflex VP). After establishing the optimal temperature for the LA fermentation parameters, different pH values (5.0, 5.5, 6.0, and 6.5) were evaluated at the optimal temperature. Each run began with a freshly incubated inoculum (10 v/v%), prepared by incubating unfiltered cheese whey at the specific reactor temperature (section 2.2). After inoculation, the reactor was operated in batch mode for 48 h, followed by continuous operation at an HRT of 10h. Once steady state was established, lower HRTs were gradually evaluated (10 h - 6 h - 3 h - 2.25 h - 1.5 h). Steady state operation was defined as a maximum difference of ±5% between LA concentration values over at least 10 HRTs. Reactor runs were immediately stopped if reactor failure occurred. The batch mode and subsequent continuous operation were performed with CWP, supplemented with 9 g/L YE (Table 1), with a concentration of 63 ± 6 g/L lactose, 0.2 ± 0.1 g/L glucose, and 1.5 ± 0.4 g/L galactose. A 15 mL sample was taken every 1–2 HRT and analysed for organic acids, pH, and electric conductivity. The remaining sample was stored at −20 °C. For steady state samples, additional operational parameters were measured including C2-C8 fatty acids, carbohydrates, and ethanol. For microbial community composition analysis, a 2 mL sample was centrifuged at 19,100 g for 5 min (Eppendorf™ Centrifuge 5430), the pellet was stored at −20 °C until analysis (section 2.5).

2.4. Analytical techniques

Organic acids such as LA, acetic acid, formic acid, propionic acid, and butyric acid were analysed with a 930 Compact Ion Chromatograph Flex (Metrohm®, Switzerland), using a Metrosep Organic acids 250/7.8 column with inline bicarbonate removal. The column was equipped with a Metrosep Organic acid Guard/4.6. Cations were analysed using a 761 Compact Ion Chromatograph (Metrohm®, Switzerland). The ion chromatograph was equipped with a Metrosep C6-250/4.0 column and a conductivity detector. Anions such as chloride, nitrate, sulphate were analysed using a Methrom 930 compact Ion Chromatograph, equipped with a conductivity detector and a Metrosep A supp 5–150/4.0 column (Metrohm®, Switzerland). C2-C8 fatty acids were analysed (including isoforms C4-C6) as described in Andersen et al. (2014). The optical D-LA and L-LA purity was measured using an enzymatic L-LA assay kit (Megazyme, United Kingdom). Lactose, galactose, glucose, and ethanol concentrations were determined by high-performance liquid chromatography (Shimadzu LC-2030C Plus Prominence-i-series) equipped with a refractive index detector (RID-20A), set at 40 °C, and an Aminex HPX-87H (Biorad) column protected with a micro guard cartridge. A mobile phase of 5 mM H_2SO_4 with 1% acetonitrile at a flow rate of 0.5 mL/min was used. The column temperature was kept at 30 °C. Determination of COD and TN were performed using commercial kits (NANOCOLOR®; MACHEREY-NAGEL GmbH & Co. KG). TSS and VSS were analysed according to Standard Methods (APHA, 2017). Finally, pH and electrical conductivity were measured with a pH electrode (SP10B, Consort) and EC electrode (SK21, Consort).

2.5. Molecular techniques

The microbial community composition was analysed for every condition (temperature and pH) at HRT 6 h and 1.5 h, except for 55 °C due to reactor failure at HRT 10 h. The microbial biomass was disrupted by bead beating with a PowerLyzer (Qiagen, Venlo, the Netherlands). Subsequently, DNA was extracted with phenol/chloroform (Vilchez-Vargas et al., 2013). A 10 μL aliquot was sent out to LGC genomics GmbH (Berlin, Germany) for library preparation and sequencing on an Illumina Miseq platform with v3 chemistry. For amplification of the DNA extract, following primers were used 341F (5′-CCT ACG GGN GGC WGC AG −3′) and 785Rmod (5′-GAC TAC HVG GGT ATC TAA KCC-3′). Assembly and clean-up of the reads were based on the MiSeq SOP described by the Schloss lab. DADA2 (v. 1.16) was used to assemble the reads into contigs, perform alignment-based quality filtering and remove chimeras (Callahan et al., 2016). Taxonomy assignment was performed using a naïve Bayesian classifier and SILVA NR v132 clustering contigs into amplicon sequence variants (ASV).

2.6. Calculations

2.6.1. Lactic acid productivity

The LA productivity (P_{LA}) was calculated as the LA concentration (C_{LA}) divided by the HRT (Eq. 1.)

$$LA\ productivity\left(\frac{g}{L\cdot h}\right) = \frac{C_{LA}\left(\frac{g}{L}\right)}{HRT\ (h)} \tag{1}$$

2.6.2. Lactic acid selectivity

The LA selectivity (S_{LA}) was calculated as the C_{LA} divided by the concentration of all organic acids and ethanol produced (Eq. 2.)

$$LA\ selectivity\ (\%) = \frac{C_{LA}\left(\frac{g}{L}\right)}{\sum C_{Products}\left(\frac{g}{L}\right)} \times 100 \tag{2}$$

2.6.3. Lactic acid yield

The LA yield (Y_{LA}) was calculated as the C_{LA} divided by the concentration of carbohydrates (glucose, galactose, and lactose) present in the CWP before fermentation (Eq. 3.)

$$LA\ yield\ (\%) = \frac{C_{\ LA}\left(\frac{g}{L}\right)}{\sum C_{influent\ carbohydrates}\left(\frac{g}{L}\right)} \times 100 \tag{3}$$

2.6.4. L(+)- lactic acid purity/D(−)-lactic acid purity

The optical purity of L-LA ($OP_{L\text{-}LA}$) was defined as the concentration of L-LA concentration measured divided by the total concentration of LA (Eq. (4)). For the optical purity of D-LA ($OP_{D\text{-}LA}$), the total concentration of LA was subtracted from the L-LA concentration and divided by the total concentration of LA (Eq. 5.).

$$L-LA\ optical\ purity(\%) = \frac{C_{L-LA}\left(\frac{g}{L}\right)}{C_{LA}\left(\frac{g}{L}\right)} \times 100 \tag{4}$$

$$D - LA\ optical\ purity(\%) = \frac{C_{LA}\left(\frac{g}{L}\right) - C_{L-LA}\left(\frac{g}{L}\right)}{C_{LA}\left(\frac{g}{L}\right)} \times 100 \qquad (5)$$

3. Results & discussion

3.1. Steering a non-axenic selective lactic acid fermentation through process parameter manipulation

3.1.1. Lowering reactor temperature from 55 °C to 45 °C resulted in enhanced lactic acid production

To assess the effect of temperature (45–55 °C) on LA fermentation, the pH was controlled at 5.5 while the HRT was gradually decreased from 10 h to 1.5 h. At all evaluated temperatures (45 °C, 50 °C, and 55 °C), LA was the main product at concentrations of 9.0–47.4 g/L (Fig S2) followed by acetic acid at around 1.0 g/L, regardless of the condition. Low concentrations of other by-products such as butyric acid (up to 1.4 g/L), and formic acid (up to 0.5 g/L) were produced while ethanol or propionic acid were not detected. The highest LA selectivity in this study (97.8%) was found at 45 °C (Table 2). The current work achieved higher LA selectivity than previously reported in literature (5.0–89.0%) for LA fermentations at 45 °C (D.-H. Kim et al., 2012; Sakarika et al., 2022). We achieved similar LA selectivity (95.8%) at 50 °C (Table 2), which aligns with the findings of Kim et al. (2012), who achieved a LA selectivity of 97.0% at 50 °C. However, at 55 °C, we obtained a LA selectivity of only 80.7% (Table 2) which differs from results obtained in previous studies that reported a 100% LA selectivity at this temperature (D.-H. Kim et al., 2012). These studies focused mainly on temperatures ranging from 50 to 55 °C based on the hypothesis that higher temperatures enable selective LA production due to the stringent conditions (Akao et al., 2007; Choi et al., 2016; Yang et al., 2022). However, the LA selectivity we obtained at 55 °C was the lowest of all evaluated temperatures.

At 45 °C, the LA productivity ranged between 5.0 and 12.3 g/L/h depending on the HRT (Fig. 1A). When the temperature was 50 °C, an overall lower LA productivity of 3.7–9.6 g/L/h was observed. At 55 °C the LA productivity was considerably lower (0.9 g/L/h) at an HRT of 10 h, compared to the other temperatures (Fig. 1A). Hence, lower HRT values were not evaluated at this temperature. Previous non-axenic LA fermentation performed at 50 °C only achieved a LA productivity of 0.1–1.8 g/L/h at 50 °C (D.-H. Kim et al., 2012; Schütterle et al., 2024; Yang et al., 2022) which is considerably lower than the results obtained in our study at 50 °C (3.7–9.6 g/L/h). Non-axenic LA fermentations performed at 45 °C achieved a LA productivity of 2.0–9.9 g/L/h at a HRT of 1.5–12 h (Sakarika et al., 2022; Schütterle et al., 2024), which is in line with the results obtained here.

The LA yield and concentration were strongly impacted by the temperature. Depending on the HRT, experiments at 45 °C and 50 °C achieved LA yields of 24.5–100.9% and 23.9–72.9% (Fig. 1B) and concentrations of 15.3–47.4 g/L and 13.1–36.8 g/L (Fig. S2). The lowest LA yield of 15.6% was obtained at 55 °C, corresponding to a concentration of 9.0 g/L. Previous literature reports reached a comparable maximum LA yield (92.0%) at 45–50 °C (Choi et al., 2016; Sakarika et al., 2022). Nevertheless, other studies at 55 °C achieved higher LA yields (43–92%) (D.-H. Kim et al., 2012; Yang et al., 2022).

Table 2
Product selectivity obtained in all evaluated HRTs during continuous lactic acid fermentation at pH 5.5 and temperatures of 45 °C, 50 °C and 55 °C. Mean values and standard deviation are presented (n = 3). Both ethanol and propionic acid were not detected.

Temperature (°C)	Lactic acid (%)	Acetic acid (%)	Formic acid (%)	Butyric acid (%)
45	97.8 ± 0.4	2.1 ± 0.2	ND	ND
50	95.8 ± 1.7	1.9 ± 0.8	2.2 ± 0.9	ND
55	80.7 ± 2.0	10.9 ± 2.4	0.8 ± 0.7	7.6 ± 0.4

ND: not detected, hence not calculated.

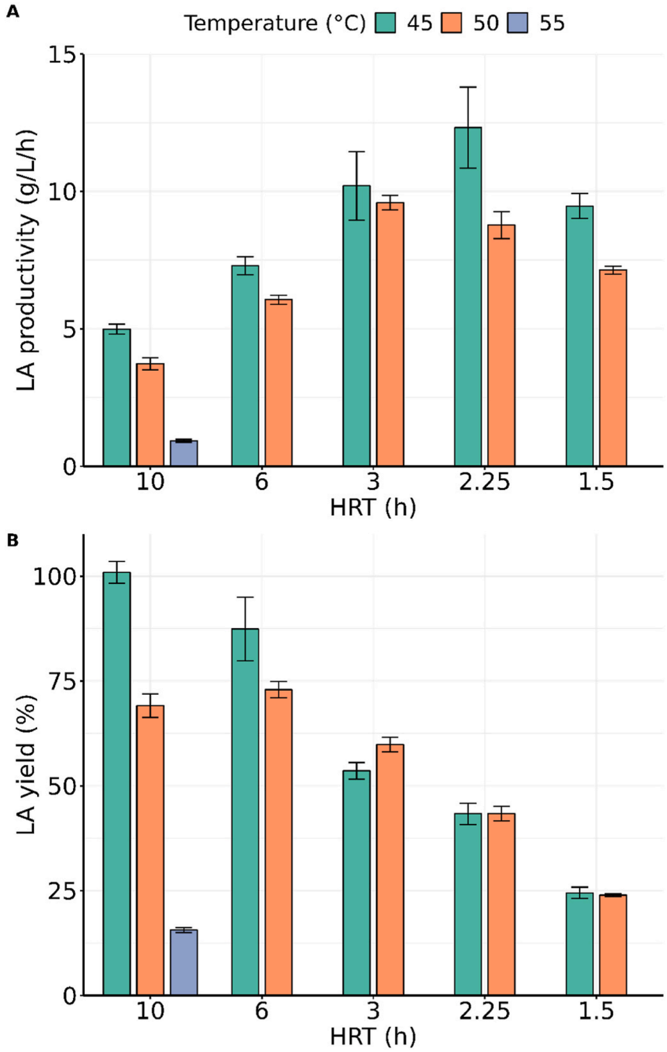

Fig. 1. The effect of temperature (45, 50, and 55 °C) at pH 5.5 on (A) lactic acid productivity and (B) lactic acid yield. Average values (n ≥ 3) ± standard deviation at steady state in each hydraulic retention time (HRT) are presented.

In summary, we observed improved LA fermentation performance as the temperature was decreased from 55 °C to 45 °C. This increase can be attributed to the growth kinetics and yields characteristic of thermophilic LAB species, which are typically isolated from dairy side streams (Marasco et al., 2022). These species, such as *Lactobacillus delbrueckii* and *Streptococcus thermophilus*, demonstrated a 147–170% increase in growth rate and a 170–194% increase in biomass yield with a temperature decrease from 50 °C to 45 °C (Adamberg et al., 2003). Moreover, no growth was observed for either species at 55 °C. However, other thermophilic LAB, such as *Bacillus coagulans* can be enriched at 55 °C (Choi et al., 2016). Nonetheless, those LAB species favouring temperatures of 55 °C, or higher, have lower growth rates and biomass yields compared to *Streptococci* and *Lactobacilli* (Borja et al., 1995; Glaser and Venus, 2017). It is highly likely that the microbial community established in our study at 55 °C had significantly lower biomass yield and growth rate compared to our microbial community at 45 °C. In combination with the shorter HRT (≤10h) applied in our study, compared to other studies (≥12h), this likely resulted in the washout of thermophilic LAB biomass and, consequently, reduced the overall LA fermentation performance. This could explain the discrepancy between the results we obtained at 55 °C and other studies.

Current state of the art often revolves around maximizing one or two

LA fermentation parameters. For example, industrial LA production in axenic batch reactors focuses on obtaining high LA yield and concentrations but often suffers from low productivity. The utilisation of mixed communities has demonstrated high LA selectivity but often lacks productivity, titres, or yields. Our results showed higher LA productivity, titres, and selectivity (P_{LA}: 12.3 g/L/h, C_{LA}: 28.1 g/L, S_{LA}: 97.6%) compared to previously reported maximum LA productivities of pure cultures (P_{LA}: 11.2 g/L/h, C_{LA}: 22.4 g/L, S_{LA}: 100%) and mixed communities (P_{LA}: 9.9 g/L/h, C_{LA}: 14.9 g/L, S_{LA}: 89%) achieved in a CSTR (Göksungur and Güvenç, 1997; Sakarika et al., 2022). However, as seen in our study and others, lower LA yields (43.0–45.4%) were obtained while maximizing LA productivity. This trade-off between yield and productivity is a common challenge in CSTR operations. Despite this, our study demonstrated significant improvements when maximizing LA yield. At an HRT of 10h (45 °C, pH 5.5) we obtained a LA yield of 100% (P_{LA}: 5.0 g/L/h, C_{LA}: 47 g/L, S_{LA}: 97.9%). In contrast, a pure culture LA fermentation (Göksungur and Güvenç, 1997) only achieved a 76.8% LA yield (P_{LA}: 4.0 g/L/h, C_{LA}: 40 g/L, S_{LA}: 100%) and for mixed community (Sakarika et al., 2022) a LA yield of 75.4% was achieved (P_{LA}: 3.9 g/L/h, C_{LA}: 23.4 g/L, S_{LA}: 74%). Notably, our non-axenic LA fermentation process can not only be maximized for LA productivity but also for LA yield while outperforming previous studies in both conditions.

3.1.2. Circumneutral pH can significantly improve lactic acid fermentation performance

Four continuous LA fermentations were controlled at pH values of 5.0, 5.5, 6.0, and 6.5 at 45 °C, which was the temperature that resulted in the best performance in this work (section 3.1.1). In each reactor run the HRT was gradually decreased from 10 h till 1.5 h. We achieved high LA selectivity (≥95.0%) in non-axenic LA fermentation independent of the operational pH. At all tested pH values LA was the main product (94.9–97.8%) followed by acetic acid and formic acid with a selectivity of 2.0–5.1% and 0.7–1.5%, respectively (Table 3). In contrast, previous studies evaluating higher pH values (6.0–6.5) reported a significant decrease in LA selectivity. Specifically, increasing the pH from 3.5 to 5.5 to 6.5, independent of the temperature, decreased the LA selectivity from 73-92% to 25–38% (Itoh et al., 2012; Yang et al., 2022).

Besides, increasing the pH from 5.0 to 6.5 resulted in a significant increase in LA productivity from 3.1 to 7.6 g/L/h to 5.5–27.4 g/L/h (Fig. 2A). To the best of our knowledge, the value of 27.4 g/L/h obtained at pH 6.5 and HRT 1.5 h is the highest LA productivity reported in axenic and non-axenic LA fermentation in a CSTR. In conjunction with an increased LA productivity, a positive effect on the LA yield and concentration was observed increasing the LA yield from 16.1 to 47.3% at pH 5.0 to 70.0–99.0% at pH 6.5 (Fig. 2B). A similar observation was made for the LA concentration which increased from 13.0 to 29.7 g/L at pH 5.0 to 44.2–53.1 g/L at pH 6.5 (Fig S3). Those findings are in line with previous studies as increasing the pH resulted in an LA productivity and yield increase. However, there an increase in pH resulted in overall lower LA selectivity which is not observed in our study (Itoh et al., 2012; Yang et al., 2022).

Overall, the lower LA productivity and LA yield obtained at lower pH values is most likely caused by the increased fraction of undissociated LA which can cause microbial growth inhibition, thereby resulting in lower biomass yields and, at some point, substrate utilisation (Itoh et al., 2012; Yang et al., 2022). However, in our study increasing the pH did not alter the LA selectivity which was observed in other studies (Yang et al., 2022). This difference is mostly likely due the HRT difference in our study (≤10h) and other studies (≥12h). A shorter HRT can apply a selection pressure towards fast growing organisms such as LAB outcompeting other organisms responsible for by-product formation (Rombouts et al., 2020).

Table 3
Average product selectivity of (n = 3) of continuous lactic acid fermentations at fixed temperature 45 °C and different pH values 5.0–6.5. Both ethanol and propionic acid were not detected.

pH	Lactic acid (%)	Acetic acid (%)	Formic acid (%)	Butyric acid (%)
5.0	94.9 ± 2.7	5.1 ± 2.8	ND	ND
5.5	97.8 ± 0.4	2.1 ± 0.2	ND	ND
6.0	97.3 ± 0.8	2.0 ± 0.4	0.7 ± 0.7	ND
6.5	96.2 ± 0.9	2.3 ± 0.7	1.5 ± 0.2	ND

ND: not detected, hence not calculated.

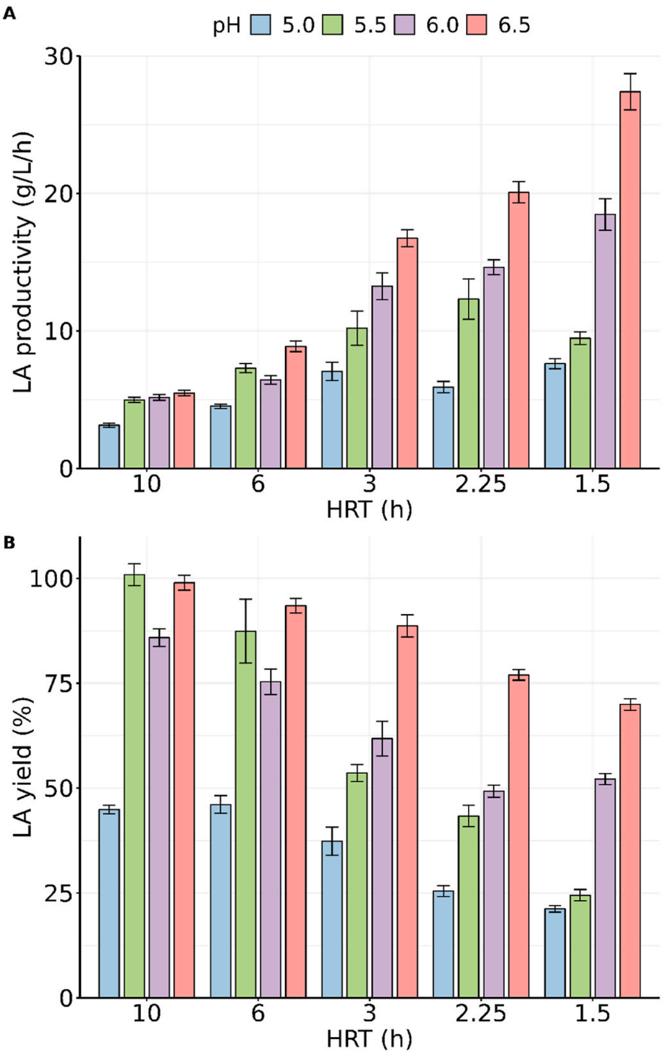

Fig. 2. The effect of pH values 5.0 till 6.5 at 45 °C on (A) lactic acid productivity (B) lactic acid yield. Average values (n ≥ 3) ± standard deviation at steady state in each HRT are presented.

Here, we achieved high LA selectivity (≥95.0%) under non-axenic LA fermentation independent from the operational pH. Additionally, increasing the pH from 5.0 to 6.5 showed an increase in LA yield (99.0%), concentration (53.1 g/L), and we reached the highest reported LA productivity of 27.4 g/L/h till now. It surpassed previous records of axenic (11.2 g/L/h) and non-axenic (9.9 g/L/h) LA fermentations, performed at 45 °C, with a factor of two or higher (Göksungur and Güvenç, 1997; Sakarika et al., 2022). Both previous productivity records only achieved a LA yield of around 50.0% while the LA fermentation we performed resulted in a LA yield of 70.0%. These results demonstrate that non-axenic operations can compete and even outperform axenic LA fermentations and simultaneously reducing the OPEX by 15% and CAPEX by 10%, compared to axenic operations. This can give rise to more LA applications becoming economically feasible (Chen and Wan, 2017; López-Gómez et al., 2019).

3.1.3. *A low HRT is crucial to achieve high lactic acid selectivity*

From the previous reactor experiments evaluating both pH (5.0–6.5) and temperature (45–55 °C), we can also infer the effect of the HRT on the fermentation performance. The evaluated HRTs did not significantly impact the LA selectivity which ranged between 92.3 and 98.3% (Table S2). This is most likely a consequence of LAB having a kinetic advantage at the short HRTs applied in our experiments over other microbial groups (Rombouts et al., 2020). Different continuous non-axenic fermentations with a HRT higher than 12h resulted in the co-generation of other products such as butyric acid and acetic acid at 45 °C (Sakarika et al., 2022) and 55 °C (Choi et al., 2016). In both cases, reducing the HRT from 48-120h to 6–12 h increased the LA selectivity from 6% up to 95% (Choi et al., 2016; Sakarika et al., 2022).

As commonly observed in CSTR operations, decreasing the HRT had a considerable positive effect on the LA productivity in our experiments. In all evaluated conditions decreasing the HRT from 10 h to 1.5 h (Figs. 1A and 2A) resulted in a LA productivity increase from 3.1 to 5.5 g/L/h to 7.6–27.4 g/L/h. However, decreasing the HRT negatively affected the LA yield, reducing it from 44.9-99.0% to 21.2–70.0% (Figs. 1B and 2B). Thus a clear trade-off exists between LA productivity and LA yield, which is also commonly observed in literature (Abdel-Rahman et al., 2016; Choi et al., 2016; D.-H. Kim et al., 2012). Both LA productivity and LA yield should be maximized as both can reduce costs through reducing reactor size and increasing overall product yields (Doran, 2013).

3.1.4. *Circumneutral pH of 6–6.5 favours the formation of L-lactic acid over D-lactic acid*

Highly optically pure LA is important for certain applications. For example, optically pure L-LA is preferred for pharmaceutical applications and poly-L-LA production while D-LA used for poly-D-LA production. Industrial production of either L-LA or D-LA production is accomplished using specific organisms that exclusively produce the respective enantiomers. To this end, we analysed optical purity during steady state operations (Fig. 3). Increasing the temperature from 45 °C to 55 °C, at pH 5.5, increased the L-LA from 12.6 ± 2.8% to 53.0% ± 6.5% (Fig. 3A). In contrast, other studies performed at 55 °C attained higher optical L-LA purity, reaching 96.7% (Akao et al., 2007; D.-H. Kim et al., 2012; Yang et al., 2022). Additionally, they found that in most cases *Bacillus coagulans*, a known L-LA producer, is the main contributor to the high L-LA optical purity (Akao et al., 2007; D.-H. Kim et al., 2012; Yang et al., 2022). However, as shown by Sakarika et al. (2022) non-axenic reactors operated at lower HRT (<6h) wash out *Bacillus coagulans.* Compared to other studies performing at 55 °C we operated at much lower HRT (≤10h), washing out *Bacillus coagulans*, which is most likely the cause of the low L-LA optical purity achieved in our study.

Additionally, at acidic pH (5.0–5.5) and 45 °C we achieved high D-LA optical purity with a maximum of 91.3% while more neutral pH values (6.0–6.5) yielded a maximum L-LA optical purity of 98.3% at 45 °C (Fig. 3B). Yang et al. (2022) attributed the shift in enantiomer production to a change in the community, which is also the cause in our study, as discussed in section 3.2. Nevertheless, we have shown that through selection of reactor operations high optical purities of L-LA or D-LA can obtained under non-axenic conditions. Provided the obtained purity is sufficient for purification, we can omit the need for sterilisation thereby enhancing the economic feasibility of LA applications.

3.2. *Increasing temperature and pH can lead to a highly enriched Lactobacilli community*

The original composition of the inoculum was dominated by a *Streptococcus* species (ASV 4) (Fig. 4) and was substantially different from the microbial community observed during the different reactor operations. Our results show that the dominant genera are tightly connected with HRT, temperature, and pH, and, more importantly, that remarkably high enrichments of a single genus could be achieved, which is an important quality for the valorisation of the produced biomass in food or feed applications.

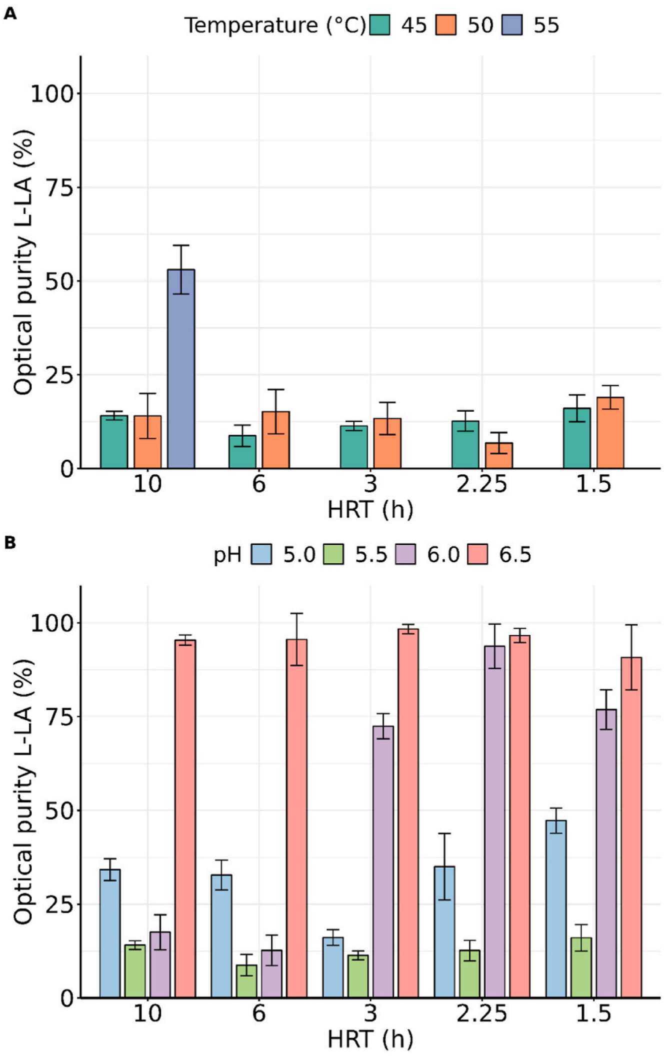

Fig. 3. The effect of (A) temperature (45, 50, and 55 °C) at pH 5.5 and (B) pH (5.0, 5.5, 6.0 and 6.5) at 45 °C on optical L-LA purity. Average values (n ≥ 3) ± standard deviation at steady state in each HRT are presented.

At 45 °C and pH 5.0, *Lactobacillus* was the dominant genus with a relative abundance of 74.3–90.3% composed of three different *Lactobacillus* ASVs (Fig. 4). Additionally, ASVs belonging to the *Rahnella*, *Serratia*, and *Lactococcus* genera were found at a relative abundance of 4.0–19.8% depending on the HRT. Both *Rahnella* and *Serratia* are facultative anaerobic, non-lactose fermenting opportunistic pathogens but can produce LA, acetic acid and ethanol from glucose (Barman et al., 2020; K. Y. Kim et al., 1997; Paradh, 2015) while *Lactobacillus* and *Lactoccocus* are both LAB (Onyeaka and Nwabor, 2022). Increasing the pH from 5.0 to 5.5 changed the relative abundance of *Lactobacillus* (ASV 1) from 30.5% to 93.5% while the other genera present at pH 5.0 noted a relative abundance below 2.0% at pH 5.5. At pH 6.0 and HRT 6 h, *Lactobacillus* (ASV 1) achieved a 99.2% relative abundance. Remarkably, ASV 1, belonging to the genus *Lactobacillus,* was not detected at pH 6.0, HRT 1.5 h. At that specific condition, the previously *Lactobacillus*-dominated community shifted towards a *Streptococci*-dominated community, with a relative abundance of 98.8%. Similarly, increasing the pH to 6.5 resulted in a relative abundance of 95.9–97.0% of the same three *Streptococcus* ASVs found at pH 6.0 and an HRT of 1.5 h. Additionally, raising the temperature from 45 °C to 50 °C at a fixed pH of 5.5 increased the relative abundance of *Lactobacillus* (ASV 1) from 90.3-93.5% to

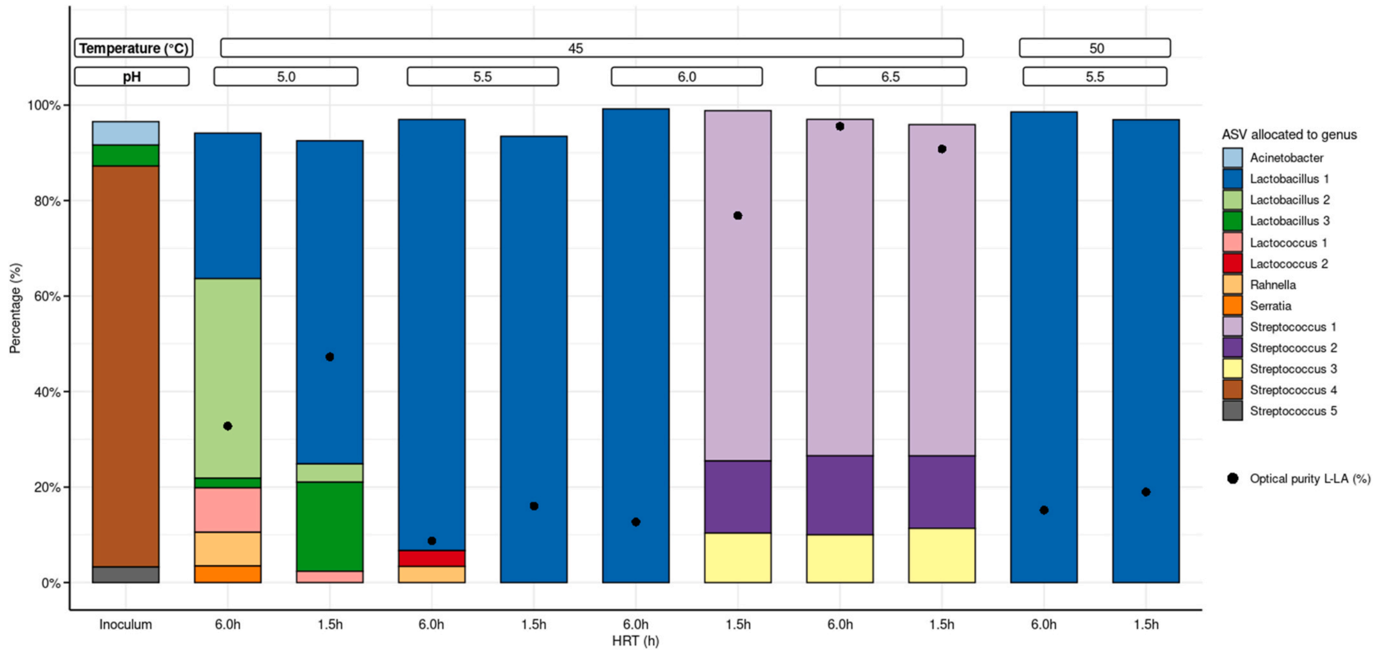

Fig. 4. Relative abundance of the microbial community composition of the top 13 abundant (≥2% relative abundance) ASVs assigned to the genus per condition and inoculum. Optical purity of L-lactic acid is represented by black dots.

97.0–98.6%.

The pH was the most critical reactor parameter differentiating between *Lactobacillus* (pH 5.0–5.5) and *Streptococcus* (pH 6.0–6.5) community. These observations are in line with the findings of Schütterle et al. (2024). They observed similar community shifts, linked to the operational pH, in a non-axenic LA fermentation starting from acid cheese whey. At a pH of 5.5 (38–50 °C) they noted a 96.7–98.8% relative abundance of *Lactobacillus.* Increasing the pH to 6.5 at 44 °C decreased the relative abundance of *Lactobacillus* to 31.1% while *Streptococcus* became the dominant genus with a relative abundance of 65.5% (Schütterle et al., 2024). This shift in community, caused by the pH increase, can be linked to the optimal growth conditions and kinetics of these genera. The optimal growth temperature and pH for *Streptococci* are approximately 40–45 °C and within the pH range of 6.0–6.9 (Chen et al., 2016). In contrast, the optimal pH for *Lactobacilli* varies between 4.5 and 6.0 (Śliżewska and Chlebicz-Wójcik, 2020). Adamberg et al. (2003) evaluated the growth rate of different LAB at pH 6.5 (45 °C), noting that *Streptococcus thermophilus* had a growth rate of 2.25 h^{-1}, compared to 0.3–1.2 h^{-1} for various *Lactobacilli*. This discrepancy likely explains the dominance of *Streptococcus* at circumneutral pH in our study, outcompeting *Lactobacilli.*

Overall, the microbial community, with a high abundance of *Streptococcus*, showed an improved fermentation performance (P_{LA}: 27.4 g/L/h, Y_{LA}: 70.0%, C_{LA}: 44.2 g/L, $OP_{L\text{-}LA}$: 90.8%) compared to the *Lactobacillus*-dominated communities (P_{LA}: 12.3 g/L/h, Y_{LA}: 43.4%, C_{LA}: 28.1 g/L, $OP_{D\text{-}LA}$: 93.2%). The difference in optical purity between the different communities is linked to the presence of the representative dominant ASVs. A highly enriched *Lactobacillus* (ASV 1) community showed a high D-LA optical purity (84.0–93.2%). According to the basic local alignment search tool (BLAST), this ASV showed a 100% similarity with *Lactobacillus delbrueckii*, a known D-LA producer (Sahoo and Jayaraman, 2019). In contrast, the high L-LA optical purity (90.8–98.3%) was attributed to the exclusive L-LA production of all *Streptococcus* sp. (Toit et al., 2014). The increased fermentation performance at circumneutral pH is linked to the specific lactate production characteristic for the different genera. As shown by Adamberg et al. (2003), *Streptococcus* species had a 2.8 times higher specific lactate production rate (15.3 gLA. gCDW^{-1}. h^{-1}) at pH 6.5 compared to *Lactobacillus delbrueckii* (5.4 gLA. gCDW^{-1}.h^{-1}) at pH 5.5. This clearly indicates that the higher LA productivity obtained in our study are linked to the higher specific LA production rate attributed to *Streptococci*. Notably, neither *Streptococci* (ASV1-3) and *Lactobacillus* (ASV1) were detected in the initial inoculum, yet they emerged as the dominant ASVs due the selective influence of the reactor operational parameters. A study performed by Forrest et al. (2012) observed that independent of the inoculum source similar product spectra and fermentation performance can be obtained. Our study highlights, that non-axenic fermentations can be steered towards selective LA production (Choi et al., 2016; Schütterle et al., 2024; Yang et al., 2022) with high D-LA or L-LA optical purity (Akao et al., 2007; Sakai and Ezaki, 2006) due to selection of operational reactor operational parameters.

Besides LA production, the produced biomass can be of interest for the food and feed sector as a protein source or as probiotic. However, as we have an open fermentation the community composition is a determining factor to obtain a safe product. The safety of organisms can be assessed through several risk assessments. However, some organisms already received a Qualified Presumption of Safety (QPS) statute by the EFSA as they do not raise any safety concerns for humans, animals and the environment (EFSA et al., 2022). We achieved relative abundances up to 99.2% for *L. delbrueckii*, indicating that although the reactor was operated in non-axenic conditions, we could obtain a highly enriched community of one species without losing performance or reactor stability. Even though high relative abundance does not ensure the safety of the product our findings imply the potential utilisation of well-monitored non-axenic processes to produce biomass composed of mainly one microbial species for food applications. *L. delbrueckii* is considered as a safe organism, with probiotic properties, for food and feed applications due to its longstanding use in the food industry as dairy starter (Aghababaie et al., 2011; Beitel et al., 2020) but additionally it received a QPS statute by EFSA and Generally Regarded As Safe (GRAS) status by FDA (EFSA et al., 2022; FDA, 2012). In the case of food ingredients, supplementation of this biomass could enhance flavour and bring immunostimulant properties (Fang et al., 2020; Guglielmotti et al., 2007). For livestock feed, the biomass can enhance the feed conversion rates and act as an antibiotic replacement (Mo et al., 2022; Suda et al., 2021).

3.3. *Alternative lactic acid production scenarios can be achieved through selection of operational parameters*

By carefully selecting key operational parameters, we can maximize all LA production metrics. Specifically, lowering the temperature from 55 °C to 45 °C enhanced LA productivity, concentration, selectivity, and yield although it led to a decrease in optical L-LA purity. Operating at circumneutral pH range of 6.0–6.5, at 45 °C, significantly improved the overall LA performance. Additionally, running the reactor at low HRT was the decisive factor to obtain high LA selectivity.

Inevitably trade-offs exist between the LA production parameters which should be considered when designing operations for certain LA applications. In the following section, we evaluate a number of operational parameters associated with different production scenarios within the LA biorefinery (e.g. bulk platform chemical, biopolymer production or intermediate for microbial protein (MP)). The key trade-off between productivity and yield is visualized in Fig. 5, which contains our results as well as data found in literature (Table S1). All data obtained from non-axenic and axenic LA fermentations have been organized by operational temperature (37–55 °C) and pH (5.0–6.5).

If the aim is to produce LA as a platform chemical, operation should target a combination of maximum LA yield and LA productivity (top right corner of Fig. 5) since this minimizes overall production costs (Doran, 2013; Pothakos et al., 2018). However, a trade-off between both LA metrics can be observed (Fig. 5). To ensure high yields while maintaining productivity at the highest possible levels, the process should be operated at 45 °C, HRT 10h and a pH between 5.5 and 6.5 (grey area in Fig. 5). In our tests, this led to a combination of high LA selectivity (96.2–97.8%), yield (85.9–100.9%), and concentration (46.6–51.1 g/L), while achieving relatively high LA productivity (5.0–5.5 g/L/h) compared to other studies.

For LA applications requiring high optical purity (e.g. PLA and pharmaceutical applications), pure cultures are currently employed to produce either L-LA or D-LA. However, our study demonstrated that high optical purity and LA yield can also be achieved under non-axenic conditions. Specifically, operating at temperatures of 45–50 °C and pH 5.5 (blue square in Fig. 5) is optimal for D-LA production with an optical purity of 85.9–91.3% and a LA yield of 87.4–100.9%. In contrast, operating at 45 °C and pH 6.5 results in L-LA production at an optical purity of 95.6% and a LA yield of 93.5–99.0% (blue square in Fig. 5).

In case the produced LA is intended to be used as a substrate in biological processes (e.g. MP) maximizing relative abundance of LAB should be prioritized over yield and optical purity since the unconverted substrate will be fully consumed in the subsequent process. High abundance of LAB can provide an added advantage by enhancing the nutritional value of MP through its probiotic properties. To remove any concerns on the safety of the microbial biomass produced under non-axenic conditions, the operational conditions should be carefully selected. In this respect, 45 °C at pH 6.0 and HRT 6h and 50 °C at pH 5.5 is recommended for subsequent MP production as it showed 90.3–98.6% (brown square in Fig. 5) relative abundance of *L. delbrueckii*. This species has a longstanding use in the dairy industry and as a probiotic which could increase the functional properties of the MP product, if used as live cultures.

3.4. *A preliminary assessment of the potential to valorise cheese whey permeate in different lactic acid-based applications*

CWP has a significant potential as a feedstock to produce biobased products. A preliminary assessment was conducted to explore the potential of this stream for producing LA or LA-derived products (e.g., bulk platform chemicals, PLA, or MP), focusing on current production capabilities and potential CO_2 reductions within the EU (see SI section S4).

Based on a scenario using LA as a platform chemical and the results

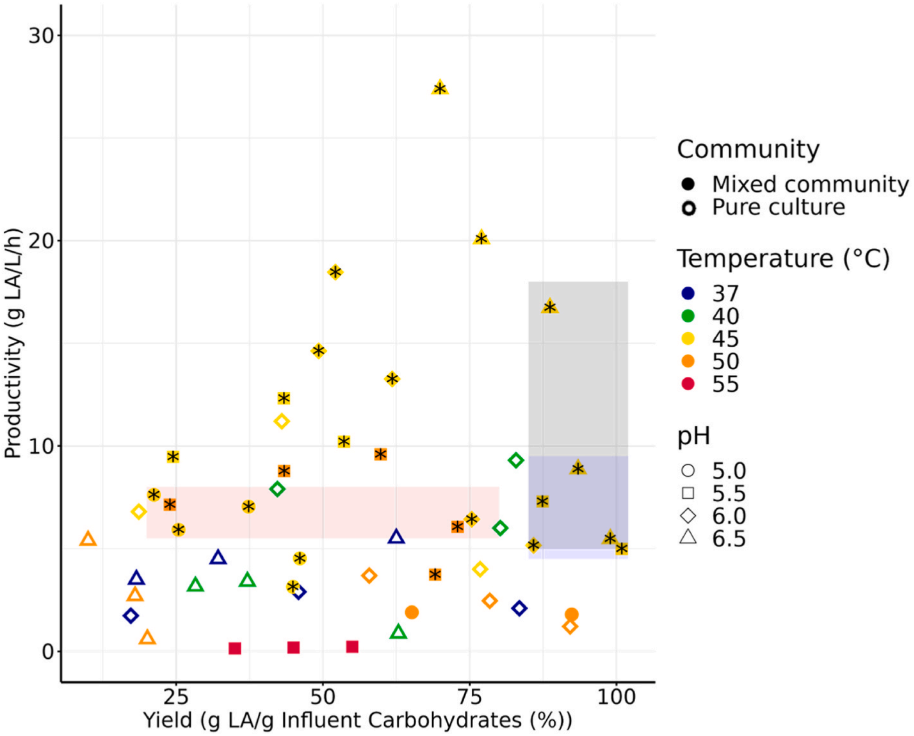

Fig. 5. Productivity and yield of lactic acid (LA) production obtained from different studies. The mixed communities shown achieved a LA selectivity of 95% or above. Results are organized based on reactor pH and temperature. Asterisks indicate results obtained in this study. The coloured areas show clusters of data points that achieved similar values of key performance indicators. Each different coloured area is a visualisation of different production scenarios: the grey area shows data where LA could serve as platform chemical, the blue area shows data where optical pure L-LA or D-LA can be achieved, and the brown area arrange data for LA production with a sequential production step such as microbial protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

obtained in our study, the current amount of CWP being produced can yield up to 2,600 kton of LA (see S4. Calculations). In the case LA is used for PLA production both LA enantiomers are important as they can yield polymers with different mechanical properties. Taking in account the yields and optical purity obtained in our study, 1,400 kton poly-L-LA or 1,200 kton of poly-D-LA can be obtained through the valorisation of Europe's CWP. To put this in perspective, annually the EU produces 65 kton of LA and 7 kton of PLA (Balla et al., 2021; Spekreijse et al., 2019). This production is based upon the utilisation of food-grade sugars derived from crops which is in direct competition with food production while causing environmental damage. The utilisation of CWP could completely circumvent the need for using food-grade feedstocks, while reducing costs, to produce plastics or chemicals while increasing the EU annual production 40 times for LA as platform chemical and 125 times for PLA production (see S4. Calculations). If production of PLA on CWP replaced fossil fuel-based PET, this could reduce CO_2 emissions by 1.9 million ton CO_2 equivalents each year (Moretti et al., 2021). In addition, the use of CWP as feedstock can reduce up to 16.5% of the acidification, 33.0% of the eutrophication, and up to 51.5% of the total water use related to feedstock production for industrial LA fermentation compared to LA fermentations relying on food-grade feedstocks (Moretti et al., 2021).

In the case CWP is used to produce MP, safety is of prime importance and only reactor conditions were considered where the community consisted out of 98.0% *L. delbrueckii.* Under these conditions 70% of the lactose was converted to LA. This scenario could lead to the production of 0.4 million ton of proteins containing all essential amino acids, assuming a theoretical biomass yield of 0.63 gDM/g-C electron donor consumed (Erdman et al., 1977; Páez et al., 2008). In case the MP is used as feed, this could result in the reduction of protein import in the EU by 2.23% (see S4. Calculations). This could reduce up to 2.5 million ton CO_2 equivalents annually, depending on the livestock (Mancuso et al., 2019; Sharma et al., 2018; Tallentire et al., 2018). Using the MP directly for protein replacement in food products could result in a 2.77% reduction of the annual EU protein consumption. As a result, this could have an annual CO_2 offset of 18 million ton CO_2 equivalents (or 4.87% of total CO_2 equivalents related to animal protein production) (see S4. Calculations). In summary, CWP can be used as a feedstock for a flexible LA platform and result in high quantities of LA, PLA, and MP that could reduce environment impact caused through current production of food, feed, chemicals, or plastics from primary resources.

4. Conclusions

In this work, we showed that non-axenic LA fermentation can be steered through careful selection of reactor parameters (i.e. temperature, pH, and HRT). Decreasing the temperature from 55 °C to 45 °C, improved LA productivity, yield, and selectivity. A pH of 6.5 (at 45 °C) yielded the highest LA productivity (27.4 g/L/h) recorded to date (Y_{LA}: 70.0%, C_{LA}: 44.2 g/L, $OP_{L\text{-}LA}$: 90.8%). Circumneutral pH (6.5) favoured the formation of L-LA, with a maximal optical purity of 98.3% (P_{LA}: 16.7 g/L/h, Y_{LA}: 88.7%, C_{LA}: 51.5 g/L), while a mildly acidic pH resulted in a maximal D-LA optical purity of 93.2% (P_{LA}: 7.3 g/L/h, Y_{LA}: 87.4%, C_{LA}: 46.0 g/L). The high D-LA optical purity coincided with a 99.2% relative abundance of a single *Lactobacillus* (ASV 1) species, even in non-axenic conditions. Furthermore, high L-LA optical purity was associated with an increased relative abundance of three different *Streptococcus* ASVs. In addition to the results obtained, we elaborated how operational parameters can be tuned to meet different production targets or applications. A preliminary impact assessment was provided for three LA applications, as platform chemical, for PLA and MP production).

CRediT authorship contribution statement

Brecht Delmoitié: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Myrsini Sakarika:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Korneel Rabaey:** Writing – review & editing, Conceptualization. **Heleen De Wever:** Writing – review & editing, Supervision, Conceptualization. **Alberte Regueira:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

B.D. was supported by the Research Foundation Flanders (FWO-Vlaanderen; Grant number: 1S20323N). M.S. was supported by Ghent University (BOF.PDO.2021.0036.01). A.R. acknowledges the support of the Xunta de Galicia through a postdoctoral fellowship (ED481B-2021-012). A.R. belongs to a Galician Competitive Research Group (GRC ED431C 2021/37), cofunded by ERDF (EU).

The authors gratefully acknowledge Milcobel for providing the substrates used in this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.123529.

Data availability

The raw fastq files that served as a basis for the bacterial community analysis were deposited in the National Center for Biotechnology Information (NCBI) database (accession number: PRJNA949127). All other data can be made available upon request.

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