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Optimization and Application of Lactic Acid Fermentation Process via Online Analysis Method

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ABSTRACT

Lactic acid, a vital organic acid with widespread applications in the food, pharmaceutical, cosmetic, and biomaterial industries, is the focus of our study. *Lactobacillus casei*, a key strain for lactic acid production, is influenced by environmental factors during fermentation, particularly pH, which significantly affects lactic acid accumulation and cell growth. Our study optimizes the fermentation process of *Lactobacillus casei* to investigate the impact of different pH conditions on lactic acid yield. We monitored the fermentation process in real-time using a fermenter combined with an automatic sampling system and ultra-high performance liquid chromatography (UPLC) to ensure precise control of lactic acid accumulation. The experiments reveal that pH 5.5 is optimal for lactic acid accumulation and cell growth. Furthermore, appropriate feeding strategies further increased the final lactic acid yield. These findings not only provide a reference for optimizing lactic acid production but also pave the way for refined control in industrial fermentation processes, potentially revolutionizing the field of lactic acid production and sparking a new wave of innovation and progress.

Keywords: Lactic Acid; *Lactobacillus casei*; Fermentation Optimization; pH Control, UPLC; Real-Time Monitoring

Abbreviations: UPLC: Ultra-High Performance Liquid Chromatography; LA: Lactic Acid; PLA: Polylactic Acid; TCP: Transmission Control Protocol; PLC: Programmable Logic Controller; CARE: Center for Application Research and Engineering; D2MS: Device and Data Management System

Introduction

Lactic acid (LA) is an important organic acid with a significant impact on various industries, including food, pharmaceuticals, cosmetics, and biomaterials. In recent years, lactic acid has attracted considerable attention as a high-value-added product, particularly in environmental, ecological, and medical fields [1-3]. It exists in two optical isomers: L-lactic acid and D-lactic acid. Due to its good compatibility with the human body, L-lactic acid is mainly used in the food and pharmaceutical industries as an additive, acidulant, flavoring agent, and emulsifier. It helps inhibit bacterial spore formation during food processing and is used in cosmetics, ointments, anti-acne solutions, moisturizers, and controlled-release drugs [4,5]. Furthermore, lactic acid is also employed in the chemical industry to produce organic solvents and polylactic acid (PLA) [6]. Being renewable and biodegradable, PLA is widely used in food packaging and plastic products as a substitute for petroleum-based materials, offering excellent biodegradability, biocompatibility, and flexibility. Therefore, efficiently producing lactic acid is a key issue in the fermentation industry. Traditional lactic acid fermentation adopts a batch fermentation process, but high substrate concentrations may inhibit strain growth, leading to extended lag phases and increased osmotic stress, affecting lactic acid yield and strain viability [7-9]. To address this substrate inhibition issue, this study controlled substrate concentration and optimized feeding strategies (e.g., feeding when the lactic acid concentration reaches a certain level) to reduce the inhibitory effects of high substrate concentrations on the strains [10-12].

Lactobacillus casei is highly sensitive to environmental changes during fermentation, particularly pH, significantly affecting its growth and lactic acid production [13,14]. Optimal pH conditions can maximize lactic acid accumulation, while excessively high or low pH may inhibit cell growth. Lactic acid accumulation can lead to a significant drop in pH, further hindering the metabolic activity of the strain [15,16]. Therefore, this study employed automated pH control and the addition of neutralizing agents (e.g., sodium hydroxide) to main-

tain an appropriate pH range, investigating the lactic acid accumulation patterns of *Lactobacillus casei* under different pH conditions. This approach is crucial for optimizing the fermentation process by alleviating the inhibitory effects of lactic acid accumulation. To further improve lactic acid production efficiency and product purity, this study employed UPLC to monitor the lactic acid fermentation process in real-time and applied feedback control technology to optimize fermentation parameters. Using UPLC for online lactic acid detection, fermentation conditions could be precisely adjusted, ensuring the entire process remained optimal. This method effectively improved the automation level of the fermentation process, ensured high-purity lactic acid production, and enhanced lactic acid yield and quality.

Materials and Methods

Strain

Lactobacillus casei (GDMCC 1.80) was obtained from the Guangdong Microbial Strain Collection Center.

Medium

All reagents in the following media were purchased from China National Pharmaceutical Group Chemical Reagents Co., Ltd. The composition of the medium is as follows.

Glucose: 20.0 g/L, Beef extract: 10.0 g/L, Peptone: 10.0 g/L, Yeast extract: 5.0 g/L, K_2HPO_4 (dipotassium phosphate): 2.0 g/L, $CH_3COONa{\cdot}3H_2O$ (sodium acetate trihydrate): 5.0 g/L, Ammonium citrate: 2.0 g/L, $MgSO_4{\cdot}7H_2O$ (magnesium sulfate heptahydrate): 0.1 g/L, $MnSO_4{\cdot}H_2O$ (manganese sulfate monohydrate): 0.05 g/L, Tween 80: 1.0 g/L

Dissolve all the ingredients in distilled water, adjust the pH to 6.2 ± 0.2 (using HCl or NaOH), and autoclave at 121 °C for 15 minutes.

Equipment and Consumables

CloudReady 1.5L×4 parallel bioreactor system with Device and Data Management System (D2MS), T&J Bio engineering (Shanghai) Co., Ltd. Quick Flow HT Sampler, T&J Bioengineering (Shanghai) Co., Ltd. Patrol UPLC, Waters Co., Ltd.

Chromatographic column: HSS T3 (Waters), 2.5 μm, 4.6 mm × 150 mm

Gradient Elution Procedure

The gradient elution procedure used a quaternary solvent manager, as shown in Table 1. The mobile phases included B (H_3PO_4) and D (Methanol). The flow rate was set at 0.8 mL/min, and the total gradient time was 15 minutes.

Table 1: Gradient elution program.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Mobile Phase C (%)	Mobile Phase D (%)
0	0	100	0	0
4	0	100	0	0
10	0	80	0	20
10.1	0	100	0	0
15	0	100	0	0

Chromatographic Analysis

Lactic acid was eluted at around 4 minutes and monitored in real-time using Patrol UPLC during fermentation (Figure 1).

Sampling Method and Data Collection

Figure 2 shows the system's specific technical route. A high-throughput parallel bioreactor optimized the microbial culture environment, providing optimal growth conditions. At the same time, the D2MS enabled real-time monitoring throughout the process. The Quick Flow HT automatic sampler achieved efficient sample filtration, quantification, and transfer, ensuring accurate sample processing. The samples were then analyzed using Patrol UPLC, and critical parameter data were uploaded to the host computer to achieve precise control and optimization of the culture process. The system used a 0.1μm ceramic membrane sampling rod for sample filtration (Figure 3), effectively replacing traditional processing steps of 12,000 rpm centrifugation for 5 minutes and 0.22 μm membrane filtration. The Patrol UPLC workstation, Empower, was configured with a timed injection sequence, exporting an upload template to the micro automatic sampler. Sampling times were set using the Modbus protocol, synchronizing reactor sampling, and chromatographic injection (Figure 4). The Empower and D2MS were networked using the Transmission Control Protocol (TCP), which created a shared folder under the same local area network. Relevant parameters were configured within the system, and the data acquisition program was run to write data to the high-throughput bioreactor Programmable Logic Controller (PLC) using the Snap7 protocol. Feedback control was achieved using the advanced automation features of the host computer (Figure 5).

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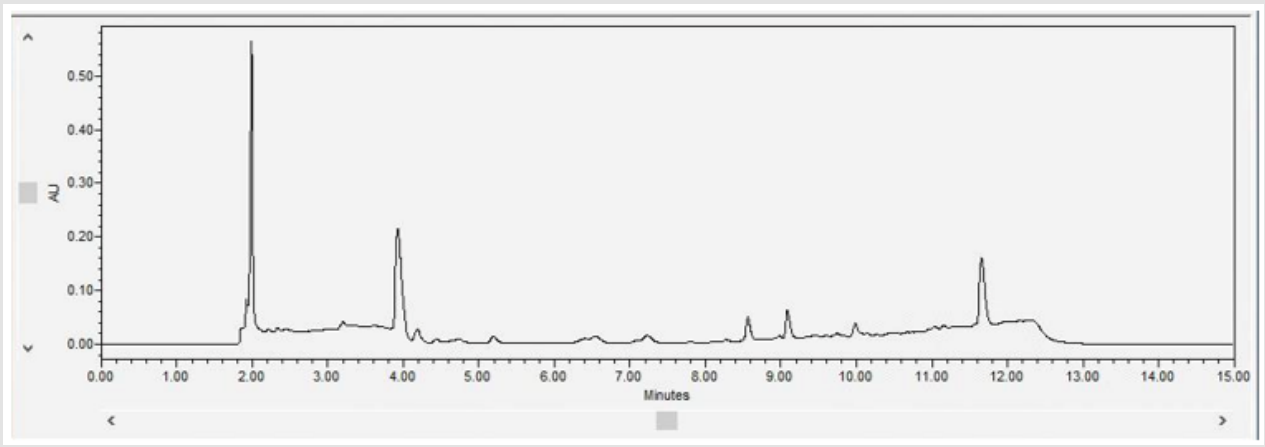

Figure 1: Lactic acid peaks at around 4 minutes.

Figure 2: Workflow: High-throughput reactor - Automatic sampler – UPLC.

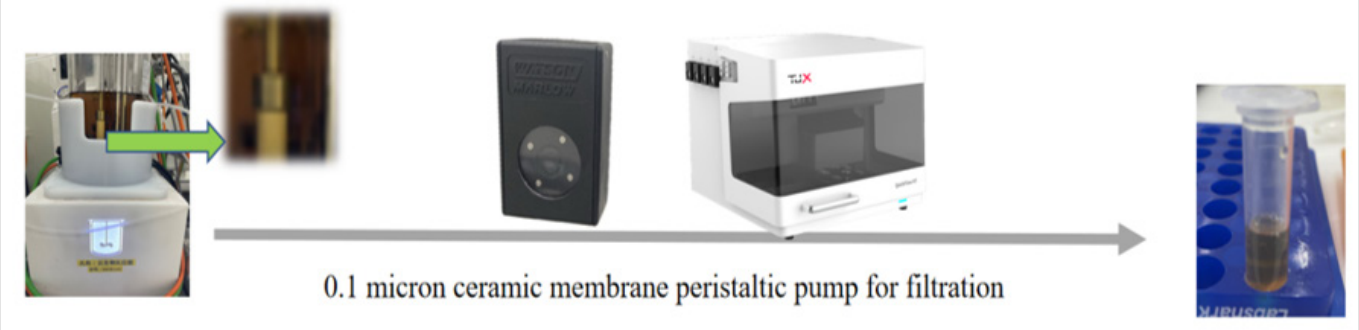

Figure 3: Sample filtration workflow.

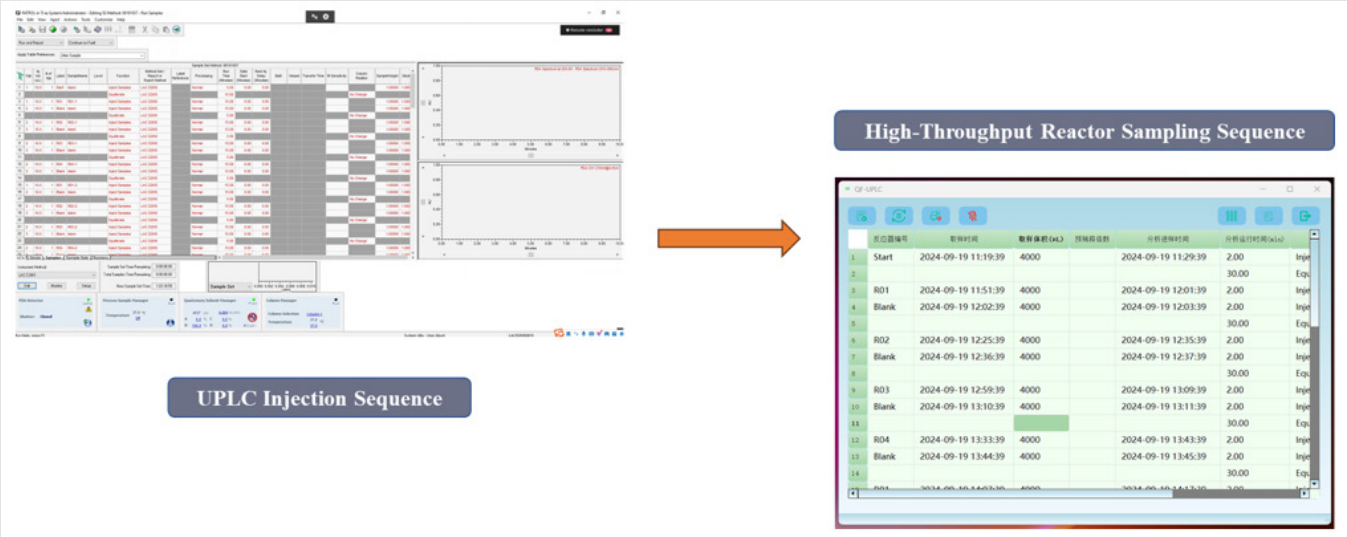

Figure 4: Reactor sampling and UPLC injection synchronization.

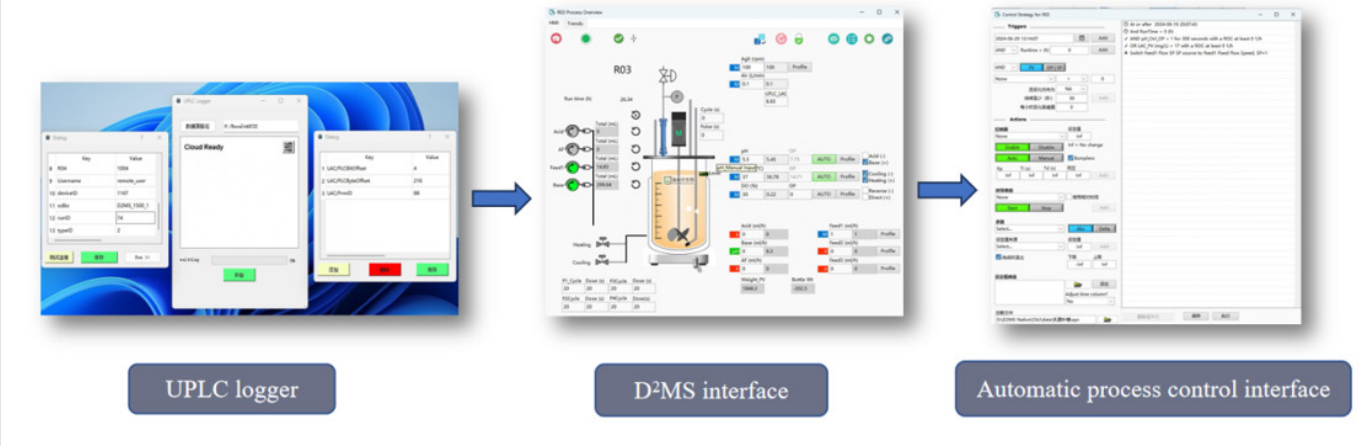

Figure 5: Steps to achieve feedback control using D2MS.

Results

Comparison of Batch Control Experimental Conditions

Table 2 shows the experimental conditions for each group. All groups maintained consistent temperature, stirring speed, inoculum size, and oxygen flow rate to explore the effect of pH on lactic acid yield and cell growth. The pH control gradient ranged from 6.5 to 3.5 to evaluate the metabolic performance of *Lactobacillus casei* under different pH environments.

Table 2: Batch control parameter.

	R1	R2	R3	R4
Inoculum amount	5%	5%	5%	5%
Temp.	37 °C	37 °C	37 °C	37 °C
pH	6.5	5.5	4.5	3.5
N_2	0.1vvm	0.1vvm	0.1vvm	0.1vvm
Agit.	50rpm	50rpm	50rpm	50rpm

Dynamic Analysis of Lactic Acid Accumulation and pH Changes

Figure 6 shows the dynamic changes in lactic acid accumulation and pH under different pH control conditions. In Group R2 (pH 5.5), the lactic acid accumulation rate was faster throughout the fermentation process, with a final lactic acid concentration of 32.13 g/L, the highest among all experimental groups. This indicates that pH 5.5 is the most suitable condition for lactic acid accumulation. In Group R4 (pH 3.5), lactic acid accumulation was rapid during the first 12 hours of fermentation, but the accumulation rate significantly decreased over time, resulting in the lowest final lactic acid yield. This may be due to the excessive acidification of the culture medium under low pH conditions, inhibiting the metabolic activity and growth of *Lactobacillus casei*. This result suggests that although low pH favors initial lactic acid accumulation, it hurts cell viability in the long term.

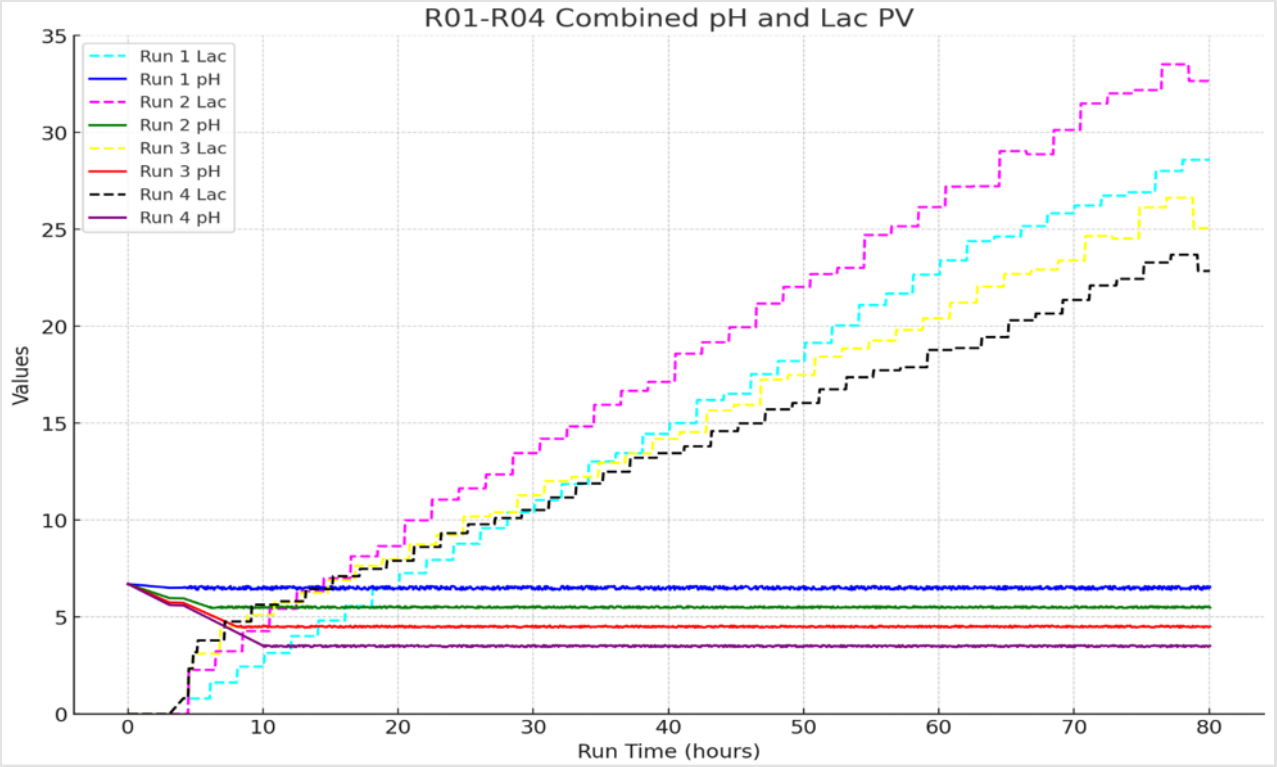

Figure 6: The pH and Lac process values for 4 reactors in the cultivation.

Lactobacillus Casei Wet Weight

The bar chart in Figure 7 shows each group's changes in cell wet weight over fermentation time. Group R2 (pH 5.5) exhibited significantly higher cell wet weight at various time points than other experimental groups, especially in the later stages of fermentation, where the wet weight reached the highest value. This further supports that pH 5.5 is the most favorable for *Lactobacillus casei* growth. In contrast, Group R4 (pH 3.5) showed significantly lower cell wet weight in the later stages of fermentation, consistent with the dynamic changes in lactic acid accumulation. This indicates low pH inhibited lactic acid accumulation and significantly suppressed cell proliferation.

Feeding Experiment

In Figure 8, the feeding experiment shows that when the lactic acid concentration exceeded 15 g/L, the basal medium was likely depleted, so the feeding program was initiated at around 30 hours. Lactic acid accumulation continued through feeding, with a final concentration of 33.77 g/L. This indicates timely feeding is vital in maintaining cell viability and promoting lactic acid accumulation.

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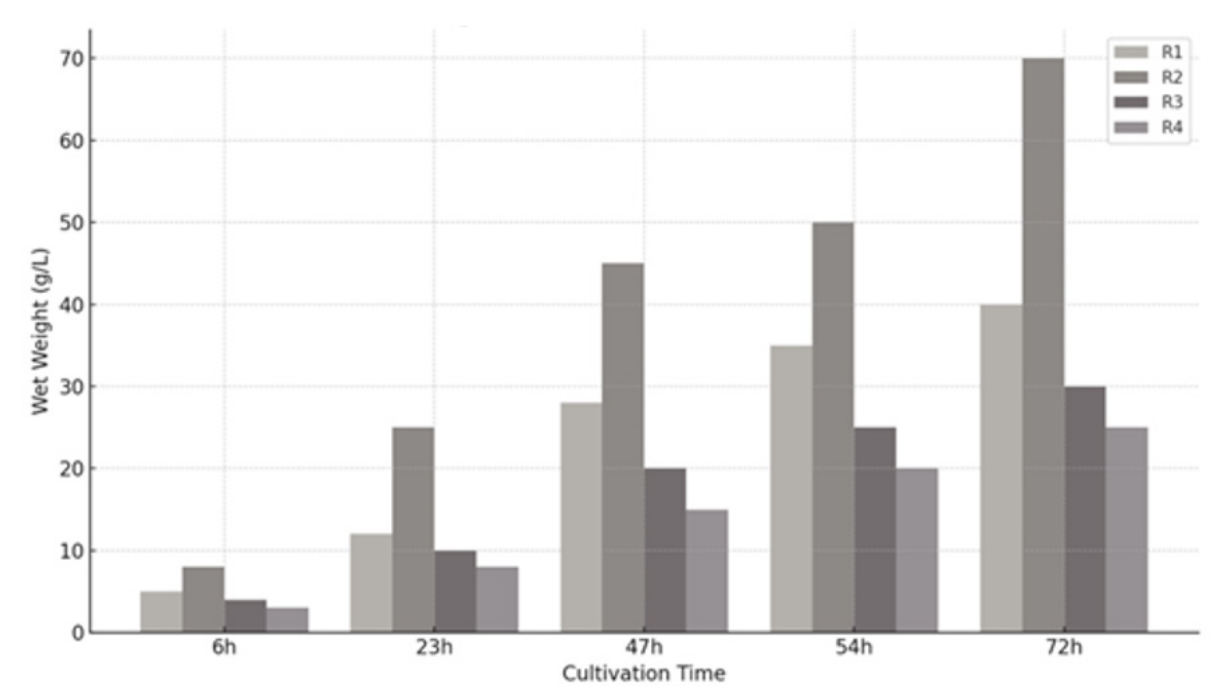

Figure 7: Wet weight of *Lactobacillus casei* for different reactors in 72h.

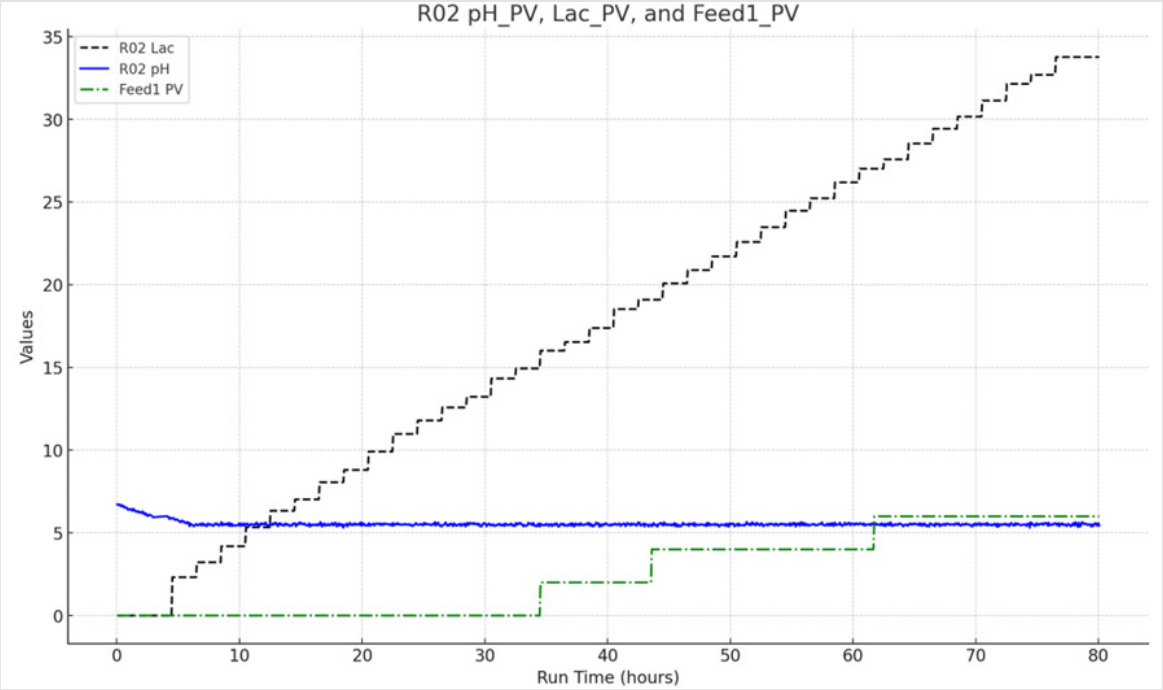

Figure 8: Timed feeding sequence based on lactic acid yield.

Correlation Between pH and Lactic Acid Production

From the lactic acid accumulation curves in Figures 1 & 2, different pH control strategies significantly impact the dynamics of lactic acid accumulation. Higher pH (e.g., 6.5) led to slower initial lactic acid accumulation but higher final yields, while lower pH (e.g., 3.5) resulted in rapid initial accumulation but significantly slowed later stages. This may be due to excessive acidic conditions inhibiting the metabolic activity of lactic acid bacteria, affecting sustained accumulation and average cell growth. Through the above analysis, pH 5.5 exhibited optimal lactic acid yield and cell growth in all aspects. Future research can further explore the physiological adaptation of lactic acid bacteria to different pH environments and how to achieve optimal lactic acid production through precise pH control.

Discussion

Standard production techniques in the microbial fermentation process of lactic acid include batch feeding and continuous fermentation [17]. Batch feeding often requires strains with high tolerance, and to maintain nutrient supply during cultivation, high concentrations of feed are used, which can lead to substrate inhibition [18,19]. In the production phase, product inhibition can also occur due to the gradual accumulation of the product [20,21]. Continuous fermentation, however, can achieve high-density fermentation with lower substrate concentrations, reducing substrate inhibition. Moreover, products are continuously removed during production, minimizing product inhibition [22,23]. One of the main challenges with continuous fermentation is the proper supply of the medium, often leading to medium waste in traditional production processes [24]. In this study, precise integration of microbial fermentation and product detection processes was achieved using an automated sampling device, enabling comprehensive monitoring of bioprocesses. Real-time feedback control based on detection results facilitated rapid optimization of production processes.

The study results allow real-time tracking of various biochemical parameters throughout the entire bioreactor fermentation process, beyond pH and dissolved oxygen, aiding researchers in understanding process variations clearly. This approach helps researchers better time and adjust the feeding amount for batch feeding processes, reducing substrate and product inhibition risks. For continuous fermentation, precise medium supply can be ensured, enhancing medium utilization efficiency, lowering production costs, and improving product quality. Furthermore, these results can supplement substrates singly or in combination, reducing strain stress during fermentation, lowering osmotic pressure risks, and enhancing production efficiency, thereby achieving efficient, economical precision fermentation.

Conclusion

This study analyzed lactic acid accumulation by *Lactobacillus casei* under different pH conditions, finding that pH 5.5 is the most favorable condition for lactic acid production and cell growth. Moreover, timely feeding was essential in maintaining sustained lactic acid accumulation. These findings provide valuable insights for optimizing lactic acid production and lay a foundation for intelligent fermentation process control.

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Credit Authorship Contribution Statement

Xingli Shi: Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. Jinyan Chai: Writing – review & editing, Project administration, Methodology, Conceptualization. Zhiguo Xu: Formal Analysis, Writing – original draft. Zhixin Guan: Investigation, Software. Junxuan Zhang: Formal analysis, Data curation. Huanghe Cheng: Formal analysis, Data curation. Xing Wang: Formal analysis, Data curation.

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.

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