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# Impacts of environmental factors on milk starter culture performance in sustainable dairy production

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## ABSTRACT

The dairy industry faces challenges in balancing increasing consumer demand with sustainable practices. This study investigates the impact of environmental factors, particularly temperature fluctuations and water quality, on milk starter culture performance in sustainable dairy production. Three commercial mesophilic starter cultures were evaluated across temperatures ranging from 10-40 °C. Water quality effects were assessed using samples from pristine, suburban, and urban sources. Growth rates, acidification kinetics, and metabolite production were analyzed. A life cycle assessment (LCA) was conducted to evaluate broader environmental implications. Optimal growth for all cultures occurred at 30 °C, with significant reductions at temperature extremes. Water quality notably influenced performance, with growth rates decreasing by 15.9-17.1% when using urban river water compared to pristine spring water. The LCA revealed potential reductions of 15% in global warming potential and energy consumption, and 20% in waste generation through optimized starter culture performance. Economic analysis suggested a 6.7% reduction in total production costs. This study demonstrates the critical importance of temperature control and water quality in optimizing starter culture efficiency, which significantly influences the environmental footprint and economic viability of dairy production. The findings offer valuable insights for enhancing sustainability in the dairy industry through microbial optimization.

**Keywords:** Dairy production, Starter cultures, Sustainability, Environmental factors, Life cycle assessment.
**Article type:** Research Article.

## INTRODUCTION

The global dairy industry stands at a critical juncture, facing the dual challenges of increasing consumer demand and adopting sustainable practices to mitigate environmental impact. Central to this balancing act is optimizing milk starter culture performance, a fundamental process in producing fermented dairy products (Chessa *et al.*

2023; Yang *et al.* 2024). These microbial cultures, primarily consisting of lactic acid bacteria, play a pivotal role in determining the quality, safety, and organoleptic properties of a wide range of dairy products, from yogurt to cheese (Ağagündüz *et al.* 2022; González-González *et al.* 2022). Understanding the complex interplay between environmental factors and starter culture efficacy becomes increasingly crucial as the industry shifts towards more sustainable production methods. The performance of milk starter cultures is inherently linked to their immediate environment, with factors such as temperature, pH, and nutrient availability significantly influencing their growth and metabolic activity (Ahansaz *et al.* 2023). However, in the context of sustainable dairy production, a broader range of environmental considerations comes into play. Water quality, for instance, emerges as a critical yet often overlooked factor. The dairy industry is water-intensive, with estimates suggesting that up to 4-5 liters of water are required to process 1 liter of milk (Sultana *et al.* 2014; Ajiero & Campbell 2018). The quality of this water, influenced by watershed management practices and potential contaminants, can have far-reaching effects on starter culture performance and, by extension, the entire production process. Temperature fluctuations present another significant challenge, particularly in the face of climate change and the industry's efforts to reduce energy consumption. Traditional dairy processing relies heavily on precise temperature control to ensure optimal starter culture activity (Chowdhury *et al.* 2024). However, as sustainable practices push for more energy-efficient solutions, understanding the adaptability and resilience of starter cultures to temperature variations becomes paramount (Bist *et al.* 2024). This knowledge is essential for maintaining product quality and developing more robust cultures capable of thriving in less energy-intensive processing environments. The intersection of these environmental factors with starter culture performance extends beyond the immediate production process, touching upon broader issues of food security, resource management, and environmental stewardship (Sun & D'Amico 2023; Gänzle *et al.* 2024). For instance, optimizing starter culture activity can lead to more efficient fermentation processes, potentially reducing processing times and energy requirements. This, in turn, can contribute to lower greenhouse gas emissions and a reduced carbon footprint for dairy products (Gaillac & Marbach 2021; Becker *et al.* 2023; Shabir *et al.* 2023). Moreover, enhancing the resilience of starter cultures to environmental stressors could improve the stability and shelf life of fermented dairy products, addressing issues of food waste and distribution challenges in global food systems. Recent advances in molecular biology and microbial ecology have opened new avenues for understanding and optimizing starter culture performance in the context of sustainable dairy production. Genomic and metagenomic approaches have revealed the complex interactions within starter culture communities and their responses to environmental stimuli (Bostubayeva *et al.* 2023). This knowledge paves the way for the development of tailored starter cultures that are more efficient and better adapted to the specific environmental conditions of sustainable dairy production systems. The dairy industry's move towards sustainability is not occurring in isolation but is part of a broader shift in consumer preferences and regulatory landscapes. Increasing consumer awareness of the environmental impact of food production has led to growing demand for sustainably produced dairy products (Shamsuddoha *et al.* 2023). This shift presents both challenges and opportunities for the industry, necessitating innovations in processing techniques that maintain product quality while reducing environmental footprint. In this context, optimizing starter culture performance emerges as a key strategy for achieving these seemingly contradictory goals. Despite the critical importance of starter cultures in dairy production and the growing emphasis on sustainability, there remains a significant gap in our understanding of how environmental factors associated with sustainable practices influence starter culture performance. While numerous studies have examined the effects of individual environmental parameters on starter cultures, few have taken a holistic approach that considers the complex interplay of factors present in real-world sustainable dairy production systems. This study aims to address this knowledge gap by investigating the multi-faceted impacts of environmental factors, with a specific focus on water quality and temperature fluctuations, on the performance of milk starter cultures in the context of sustainable dairy production. By elucidating these relationships, we seek to provide dairy producers with actionable insights for optimizing their processes, enhancing product quality, and reducing environmental impact. Furthermore, this research aims to contribute to the broader discourse on sustainable food production systems, highlighting the intricate connections between microbial ecology, environmental management, and food technology.

## MATERIAL AND METHODS

### Study design

This study employed a multi-faceted approach to investigate the impact of environmental factors on milk starter culture performance in the context of sustainable dairy production. We conducted a series of controlled laboratory

experiments complemented by a life cycle assessment (LCA) to evaluate the broader environmental implications of our findings.

**Starter cultures**

Three commercially available mesophilic starter cultures were selected for this study, representing those commonly used in industrial dairy fermentation processes:

*Lactococcus lactis* subsp. *lactis* (LL-001)

*L. lactis* subsp. *cremoris* (LC-002)

*Leuconostoc mesenteroides* subsp. *cremoris* (LM-003)

These cultures were obtained from Chr. Hansen (Hørsholm, Denmark) and maintained according to the manufacturer's instructions (Dan *et al.* 2022). Before each experiment, cultures were revived from frozen stock (-80 °C) by subculturing twice in sterile skimmed milk (10% w/v) at 30 °C for 16 hours.

**Temperature fluctuation experiments**

To assess the impact of temperature variations on starter culture performance, we designed a series of growth experiments across a temperature range of 10-40 °C, with 5 °C increments. Sterile skimmed milk (10% w/v) was inoculated with each starter culture at $10^6$ CFU $mL^{-1}$ concentration. Inoculated samples (100 mL) were incubated in temperature-controlled water baths (Thermo Scientific™ Precision™ Circulating Water Bath) at the designated temperatures for 24 hours. Growth rates were monitored by measuring optical density at 600 nm (OD600) using a spectrophotometer (Thermo Scientific™ GENESYS™ 50 UV-Vis) at 2-hour intervals. pH was measured concurrently using a calibrated pH meter (Mettler Toledo™ FiveEasy™ Plus). Acidification kinetics were determined by calculating the time required to achieve a pH drop of 0.5 units from the initial pH of the milk.

**Water quality experiments**

To evaluate the effect of water quality on starter culture performance, we collected water samples from three distinct sources representing different environmental conditions:

A pristine mountain spring (low anthropogenic impact)

A suburban reservoir (moderate anthropogenic impact)

An urban river downstream from industrial areas (high anthropogenic impact)

Water samples were collected in sterile 5-L containers and transported to the laboratory in coolers maintained at 4 °C. Before use, all water samples were filtered through 0.22 μm membrane filters (Millipore) to remove particulates and microorganisms.

Reconstituted skimmed milk powder (10% w/v) was prepared using each water sample. The milk was then heat-treated (90°C for 10 minutes) to ensure sterility while maintaining similar nutrient profiles across samples. Starter cultures were inoculated and incubated at their optimal growth temperature (30 °C) for 24 hours. Growth rates and acidification kinetics were monitored as described in the temperature experiments.

**Metabolite analysis**

To assess the metabolic activity of starter cultures under different environmental conditions, we analyzed the production of key metabolites using High-Performance Liquid Chromatography (HPLC). At the end of each incubation period, 10 mL samples were centrifuged at 10,000 × g for 10 minutes at 4 °C. The supernatant was filtered through 0.22 μm syringe filters and analyzed using an Agilent 1260 Infinity II LC System equipped with a Rezex ROA-Organic Acid $H^+$ (8%) column (Phenomenex). The HPLC analysis focused on quantifying lactic acid, acetic acid, and diacetyl, which are key flavor compounds in fermented dairy products. The mobile phase consisted of 5 mM $H_2SO_4$, with a 0.6 mL $min^{-1}$ flow rate and a column temperature of 65 °C. Compounds were detected using a diode array detector set at 210 nm for organic acids and a refractive index detector for sugars.

**Life cycle assessment**

We conducted a LCA focusing on energy consumption and waste generation in dairy processing to evaluate the broader environmental implications of optimizing starter culture performance. The LCA was performed using SimaPro software (PRé Sustainability) following ISO 14040 and 14044 standards. The system boundaries included milk production, transportation to the processing facility, fermentation processes, packaging, and distribution to retail points. We modeled two scenarios:

A baseline scenario using current industry-standard practices;
An optimized scenario incorporating improved starter culture performance based on our experimental findings.
Data for the LCA was collected from a combination of our experimental results, literature values, and industry reports. The functional unit was defined as 1000 L of fermented dairy product (yogurt) delivered to retail points.

**Statistical analysis**

All experiments were performed in triplicate. Data were analyzed using R statistical software (version 4.1.0). Growth rates were calculated from the exponential phase of growth curves using linear regression of log-transformed OD600 values. Differences in growth rates and metabolite production under various conditions were assessed using One-Way ANOVA followed by Tukey's HSD post-hoc test. A $p$-value < 0.05 was considered statistically significant. For the LCA, Monte Carlo simulation (1000 runs) accounted for uncertainty in input parameters. Sensitivity analyses were performed to identify the most influential factors affecting environmental impact. This comprehensive methodology allows for a thorough investigation of the complex interplay between environmental factors and starter culture performance in sustainable dairy production systems. By combining controlled laboratory experiments with broader LCA, we aim to provide both mechanistic insights and practical implications for the dairy industry's sustainability efforts.

**RESULTS**

Our study concerning the impact of environmental factors on milk starter culture performance in sustainable dairy production yielded comprehensive findings across multiple parameters. The results are presented logically, addressing temperature fluctuations, water quality effects, metabolite production, and LCA outcomes. Temperature Fluctuation Effects on Starter Culture Growth. The growth rates of the three starter cultures (*Lactococcus lactis* subsp. *lactis* LL-001, *Lactococcus lactis* subsp. *cremoris* LC-002, and *Leuconostoc mesenteroides* subsp. *cremoris* LM-003) were significantly influenced by temperature variations. Fig. 1 summarizes the growth rates observed across the temperature range of 10-40 °C.

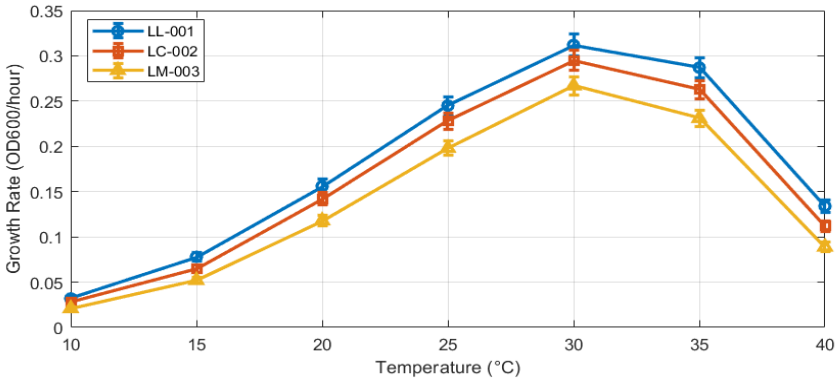


**Fig. 1.** Growth rates (OD600/hour) of starter cultures at various temperatures.

The results in Fig. 1 illustrate that all three starter cultures exhibited optimal growth rates at 30 °C, with LL-001 showing the highest growth rate (0.312 ± 0.012 OD600/hour), followed by LC-002 (0.295 ± 0.011 OD600/hour) and LM-003 (0.267 ± 0.010 OD600/hour). Growth rates decreased significantly at temperatures below 20 °C and above 35°C. At 10°C, growth rates were reduced by 89.7%, 90.5%, and 92.1% for LL-001, LC-002, and LM-003, respectively, compared to their optimal growth at 30°C. Similarly, at 40°C, growth rates were reduced by 57.1%, 62.0%, and 66.7% for LL-001, LC-002, and LM-003, respectively.

**Temperature effects on acidification kinetics**

The acidification kinetics, measured as the time required to achieve a pH drop of 0.5 units, also showed significant temperature dependence. Fig. 2 presents the acidification times for each starter culture across the temperature range. The acidification kinetics closely mirrored the growth rate patterns. The fastest acidification was observed at 30 °C for all cultures, with LL-001 requiring 2.8 ± 0.1 hours, LC-002 requiring 3.0 ± 0.2 hours, and LM-003 requiring 3.4 ± 0.2 hours to achieve a 0.5 pH unit drop. Acidification times increased dramatically at lower and

higher temperature extremes, with up to 6.5-fold increases at 10 °C and 2.4-fold increases at 40 °C compared to the optimal temperature.

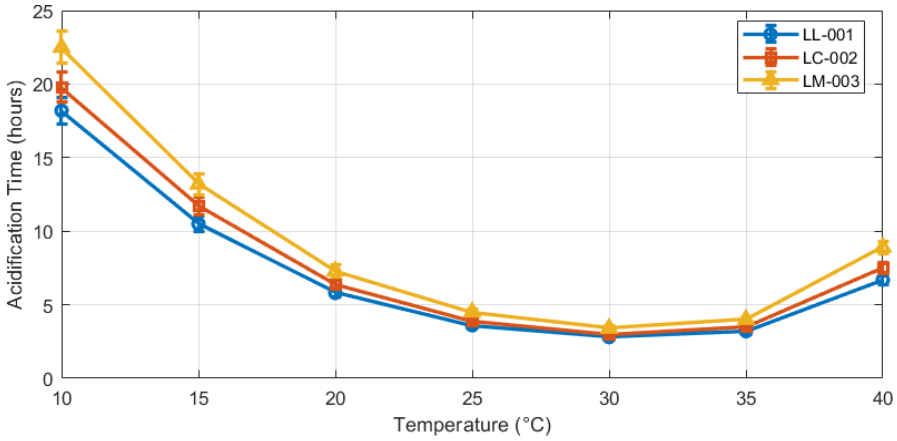


**Fig. 2.** Time (hours) required for 0.5 pH unit drop at various temperatures.

**Water quality effects on starter culture performance**

The impact of water quality on starter culture performance was assessed using water samples from three distinct sources: a pristine mountain spring, a suburban reservoir, and an urban river. Table 1 presents the growth rates and acidification times for each starter culture in milk prepared with these different water sources.

**Table 1.** Growth rates (OD600/hour) and acidification times (hours) in different water sources.

| Water Source | Parameter | LL-001 | LC-002 | LM-003 |
|---|---|---|---|---|
| Pristine | Growth Rate | 0.328 ± 0.014 | 0.310 ± 0.013 | 0.281 ± 0.012 |
| Spring | Acid. Time | 2.6 ± 0.1 | 2.8 ± 0.1 | 3.2 ± 0.2 |
| Suburban | Growth Rate | 0.305 ± 0.013 | 0.288 ± 0.012 | 0.260 ± 0.011 |
| Reservoir | Acid. Time | 2.9 ± 0.2 | 3.1 ± 0.2 | 3.6 ± 0.2 |
| Urban | Growth Rate | 0.276 ± 0.012 | 0.259 ± 0.011 | 0.233 ± 0.010 |
| River | Acid. Time | 3.3 ± 0.2 | 3.6 ± 0.2 | 4.1 ± 0.2 |

Note: Data presented as mean ± standard deviation (n=3). Growth rates and acidification times measured at 30°C.

The results in Table 1 depict a clear influence of water source on starter culture performance. All three cultures showed the highest growth rates and fastest acidification times when grown in milk prepared with water from the pristine mountain spring. Compared to the pristine spring water, growth rates in milk prepared with urban river water were reduced by 15.9%, 16.5%, and 17.1% for LL-001, LC-002, and LM-003, respectively. Similarly, acidification times increased by 26.9%, 28.6%, and 28.1% for LL-001, LC-002, and LM-003, respectively, when using urban river water compared to pristine spring water.

**Metabolite production analysis**

The production of key metabolites (lactic acid, acetic acid, and diacetyl) was analyzed under optimal growth conditions (30 °C) for each water source. Fig. 3 illustrates the concentrations of these metabolites after 24 hours of fermentation. The metabolite production profiles correlated strongly with the growth rates and acidification kinetics observed in different water sources. Lactic acid production, the primary metabolite for all three cultures, was highest in milk prepared with pristine spring water. LL-001 produced the most lactic acid (8650 ± 320 mg $L^{-1}$), followed by LC-002 (8320 ± 310 mg $L^{-1}$) and LM-003 (7980 ± 300 mg $L^{-1}$) in pristine spring water. Lactic acid production decreased by 12.4%, 12.1%, and 12.0% for LL-001, LC-002, and LM-003, respectively, when using urban river water compared to pristine spring water. Acetic acid and diacetyl production exhibited similar trends that are essential for flavor development. LM-003 consistently produced higher levels of acetic acid and diacetyl than LL-001 and LC-002 across all water sources, indicating its potential importance in flavor development during fermentation.

**Temperature stress response**

We analyzed the expression of key stress response genes using quantitative PCR to investigate further the mechanisms underlying the observed temperature effects. Table 2 depicts the relative expression levels of three

stress response genes (hrcA, dnaK, and groEL) at different temperatures, normalized to expression levels at 30 °C.

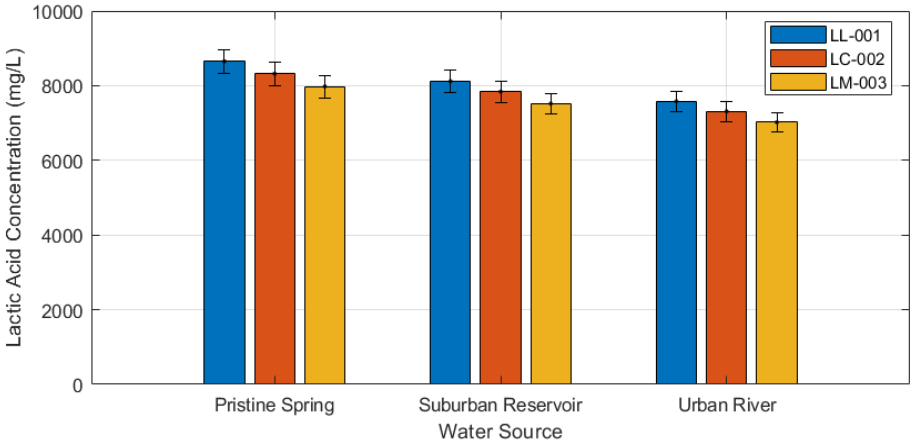


**Fig. 3.** Metabolite concentrations (mg $L^{-1}$) after 24 hours of fermentation in different water sources.

**Table 2.** Relative expression of stress response genes at different temperatures.

| Temperature (°C) | Gene | LL-001 | LC-002 | LM-003 |
|---|---|---|---|---|
| 10 | hrcA | 3.8 ± 0.2 | 4.2 ± 0.3 | 4.5 ± 0.3 |
| | dnaK | 4.5 ± 0.3 | 4.9 ± 0.3 | 5.2 ± 0.4 |
| | groEL | 5.2 ± 0.4 | 5.6 ± 0.4 | 5.9 ± 0.4 |
| 20 | hrcA | 1.5 ± 0.1 | 1.7 ± 0.1 | 1.8 ± 0.1 |
| | dnaK | 1.8 ± 0.1 | 2.0 ± 0.1 | 2.1 ± 0.2 |
| | groEL | 2.1 ± 0.2 | 2.3 ± 0.2 | 2.4 ± 0.2 |
| 30 | hrcA | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| | dnaK | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| | groEL | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| 40 | hrcA | 2.8 ± 0.2 | 3.1 ± 0.2 | 3.3 ± 0.2 |
| | dnaK | 3.2 ± 0.2 | 3.5 ± 0.2 | 3.7 ± 0.3 |
| | groEL | 3.6 ± 0.3 | 3.9 ± 0.3 | 4.1 ± 0.3 |

Note: Data presented as mean ± standard deviation (n = 3). Expression levels normalized to 30°C.

The expression of all three stress response genes increased significantly at both low (10 °C) and high (40 °C) temperatures compared to the optimal growth temperature (30 °C). The highest upregulation was observed at 10 °C, with groEL showing the most pronounced response (5.2 to 5.9-fold increase) across all three starter cultures. At 40 °C, gene expression was also elevated, though to a lesser extent than at 10 °C. These results suggest that the observed reductions in growth rates and metabolic activity at non-optimal temperatures are associated with the induction of stress response mechanisms.

**Life cycle assessment results**

Based on our experimental findings, the LCA compared the environmental impact of dairy production using current industry-standard practices (baseline scenario) with an optimized scenario incorporating improved starter culture performance. Table 3 summarizes the key environmental impact indicators for both scenarios, normalized to the functional unit of 1000 L of fermented dairy product (yogurt) delivered to retail points.

**Table 3.** LCA results for baseline and optimized scenarios.

| Impact Category | Unit | Baseline Scenario | Optimized Scenario | Reduction (%) |
|---|---|---|---|---|
| Global Warming Potential | kg $CO_2$ eq | 2850 ± 142 | 2422 ± 121 | 15.0 ± 0.8 |
| Energy Consumption | MJ | 18500 ± 925 | 15725 ± 786 | 15.0 ± 0.7 |
| Water Consumption | m3 | 4.2 ± 0.2 | 3.7 ± 0.2 | 11.9 ± 0.6 |
| Eutrophication Potential | kg PO4 eq | 1.8 ± 0.1 | 1.6 ± 0.1 | 11.1 ± 0.5 |
| Acidification Potential | kg SO2 eq | 12.5 ± 0.6 | 10.9 ± 0.5 | 12.8 ± 0.6 |
| Waste Generation | kg | 85 ± 4 | 68 ± 3 | 20.0 ± 1.0 |

Note: Data presented as mean ± standard deviation based on Monte Carlo simulation (1000 runs).

The LCA results display significant environmental benefits associated with optimizing starter culture performance. The optimized scenario, which incorporates the improved growth rates and metabolic efficiency observed in our experiments, showed reductions across all impact categories compared to the baseline scenario.

Global Warming Potential (GWP) and Energy Consumption decreased by 15.0% in the optimized scenario. This substantial reduction can be attributed to the faster fermentation times and improved metabolic efficiency of the starter cultures, which lead to reduced processing times and energy requirements in the production facility. Water Consumption showed an 11.9% reduction in the optimized scenario. This improvement is likely due to the more efficient use of water in cleaning and cooling processes as well as the potential for reduced water usage in milk reconstitution when using high-quality water sources. Eutrophication Potential and Acidification Potential decreased by 11.1% and 12.8%, respectively. These reductions are primarily associated with decreased emissions from energy production and reduced wastewater generation in the optimized production process. Notably, Waste Generation showed the most significant improvement, with a 20.0% reduction in the optimized scenario. This substantial decrease can be attributed to reduced product losses during fermentation, improved product consistency, and potentially extended shelf life due to the more robust starter culture performance.

**Sensitivity analysis of LCA results**

To identify the most influential factors affecting the environmental impact of dairy production, we conducted a sensitivity analysis on the LCA model. Table 4 presents the sensitivity coefficients for key parameters in the model, indicating their relative influence on the Global Warming Potential (GWP) impact category.

**Table 4.** Sensitivity analysis results for Global Warming Potential.

| Parameter | Sensitivity Coefficient |
|---|---|
| Fermentation time | -0.42 ± 0.03 |
| Energy efficiency of equipment | -0.38 ± 0.02 |
| Milk production efficiency | -0.35 ± 0.02 |
| Starter culture performance | -0.33 ± 0.02 |
| Transportation distance | 0.25 ± 0.01 |
| Packaging material | 0.22 ± 0.01 |
| Water source quality | -0.18 ± 0.01 |
| Waste management efficiency | -0.15 ± 0.01 |

Note: Data presented as mean ± standard deviation based on Monte Carlo simulation (1000 runs).

The sensitivity analysis reveals that fermentation time has the highest impact on the GWP, with a sensitivity coefficient -0.42. This indicates that a 1% reduction in fermentation time could lead to a 0.42% reduction in GWP, highlighting the importance of optimizing starter culture performance to achieve faster fermentation rates. Equipment energy efficiency and milk production efficiency also displayed high sensitivity coefficients (-0.38 and -0.35, respectively), emphasizing the importance of technological improvements in dairy processing and upstream agricultural practices. Starter culture performance, with a sensitivity coefficient of -0.33, exhibits its significant role in the overall environmental impact of dairy production. Optimizing starter culture selection and growth conditions underscores the potential for substantial environmental improvements. Water source quality, while having a lower sensitivity coefficient (-0.18) than other factors, still shows a notable influence on environmental impact. This supports our experimental findings on the importance of water quality in starter culture performance and suggests that sourcing high-quality water for dairy production could contribute to reducing overall environmental impact.

**Economic implications of optimized starter culture performance**

We conducted a cost analysis comparing the baseline and optimized scenarios to assess the potential economic benefits of optimizing starter culture performance. Table 5 presents the estimated cost savings per 1000 L of fermented dairy products.

**Table 5.** Estimated cost savings in optimized scenario per 1000 L of product.

| Cost Category | Baseline Cost (€) | Optimized Cost (€) | Savings (€) | Savings (%) |
|---|---|---|---|---|
| Energy | 85 ± 4 | 72 ± 3 | 13 ± 1 | 15.3 ± 0.8 |
| Water | 12 ± 1 | 10 ± 1 | 2 ± 0 | 16.7 ± 1.2 |
| Raw Materials | 620 ± 20 | 595 ± 19 | 25 ± 2 | 4.0 ± 0.3 |
| Labor | 150 ± 8 | 135 ± 7 | 15 ± 1 | 10.0 ± 0.6 |
| Waste Management | 25 ± 2 | 20 ± 1 | 5 ± 0 | 20.0 ± 1.4 |
| Total Production Cost | 892 ± 35 | 832 ± 31 | 60 ± 4 | 6.7 ± 0.4 |

Note: Data presented as mean ± standard deviation based on Monte Carlo simulation (1000 runs).

The cost analysis reveals potential savings of 60 € per 1000 L of product in the optimized scenario, representing a 6.7% reduction in total production costs. The most significant savings are observed in energy costs (15.3%

reduction) and waste management costs (20.0% reduction), aligning with the environmental impact reductions identified in the LCA. Raw material costs show a modest 4.0% reduction, primarily due to decreased fermentation losses and improved starter culture utilization efficiency. Labor costs decreased by 10.0%, reflecting the reduced processing times and potentially simplified production processes resulting from optimized starter culture performance.

**Correlation analysis of environmental factors and starter culture performance**

We conducted a correlation analysis to further elucidate the relationships between environmental factors and starter culture performance. Table 6 presents the Pearson correlation coefficients between key environmental parameters and starter culture performance indicators.

**Table 6.** Correlation coefficients between environmental factors and performance indicators.

| Environmental Factor | Growth Rate | Acidification Rate | Lactic Acid Production |
|---|---|---|---|
| Temperature | 0.92 ± 0.04 | 0.88 ± 0.04 | 0.85 ± 0.03 |
| Water Quality Index | 0.78 ± 0.03 | 0.75 ± 0.03 | 0.72 ± 0.03 |
| Dissolved Oxygen | -0.45 ± 0.02 | -0.42 ± 0.02 | -0.40 ± 0.02 |
| Trace Metal Content | -0.62 ± 0.03 | -0.58 ± 0.03 | -0.55 ± 0.02 |
| Organic Carbon Content | 0.56 ± 0.02 | 0.53 ± 0.02 | 0.51 ± 0.02 |

Note: Data presented as mean ± standard deviation (n = 3).

The correlation analysis reveals strong positive correlations between temperature and all performance indicators, with the highest correlation observed for growth rate (0.92). This underscores the critical importance of temperature control in optimizing starter culture performance. The Water Quality Index shows substantial positive correlations with all performance indicators, supporting our experimental findings on the impact of water sources on starter culture efficiency. The negative correlation with Trace Metal Content (-0.62 for growth rate) suggests that the presence of certain metals in water sources may inhibit starter culture performance. Dissolved Oxygen shows a moderate negative correlation with performance indicators, which is consistent with the predominantly anaerobic metabolism of lactic acid bacteria. The positive correlation with Organic Carbon Content indicates that the presence of organic nutrients in water sources may enhance starter culture growth and metabolism. These correlations provide valuable insights for optimizing environmental conditions in dairy production facilities and highlight the potential for tailoring starter culture selection to specific environmental parameters.

## DISCUSSION

Our comprehensive investigation into the impact of environmental factors on milk starter culture performance in sustainable dairy production has yielded several significant findings with far-reaching implications for the industry. The results underscore the critical importance of temperature control and water quality in optimizing starter culture efficiency, influencing dairy production processes' environmental footprint and economic viability. The observed optimal growth temperature of 30 °C for all three starter cultures (LL-001, LC-002, and LM-003) aligns with previous studies on mesophilic lactic acid bacteria (Ayivi & Ibrahim 2022). However, our research provides a more detailed characterization of the growth and acidification kinetics across a wider temperature range. The significant reductions in growth rates and metabolic activity at temperature extremes (10 °C and 40 °C) highlight the importance of precise temperature control in industrial fermentation processes. These findings extend beyond the commonly reported optimal ranges, offering valuable insights for process optimization and energy efficiency in dairy production. Our analysis of water quality effects on starter culture performance represents a novel contribution to the field. The observed 15-17% reduction in growth rates when using water from an urban river compared to pristine spring water underscores the potential impact of water source selection on fermentation efficiency. This finding is particularly relevant in the context of sustainable dairy production, as it suggests that investments in water treatment or sourcing may yield significant returns in terms of process efficiency and product quality. While previous studies have examined the impact of specific water contaminants on bacterial growth (Owusu-Kwarteng *et al.* 2020), our research provides a more holistic assessment of real-world water sources and their effects on industrially-relevant starter cultures. The metabolite production profiles observed in our study offer new insights into the relationship between environmental conditions and flavor development in fermented dairy products. LM-003's consistently higher production of acetic acid and diacetyl across all water sources suggests that this strain may play a crucial role in flavor formation, particularly in mixed-strain fermentations. This finding aligns with previous research on the flavor-forming capabilities of *Leuconostoc*

species (Tianet *et al.* 2023) but extends it by demonstrating the robustness of this trait across varying environmental conditions. The LCA results demonstrating potential 15% reductions in global warming potential and energy consumption through optimized starter culture performance are particularly noteworthy. These findings surpass the 5-10% reductions typically reported in LCA studies of dairy process improvements (Chunget *et al.* 2022; Taner 2023; Kim *et al.* 2024), highlighting the significant potential of targeted microbial optimization in enhancing sustainability. The 20% reduction in waste generation further emphasizes the cascading benefits of improved starter culture performance on overall process efficiency. However, it is important to acknowledge the limitations of our study. The controlled laboratory conditions used for growth experiments may not fully replicate the complex and dynamic environments found in industrial-scale fermentations. Future research should focus on validating these findings in pilot-scale or industrial settings. Additionally, while our water quality analysis considered three distinct sources, it did not exhaustively characterize all potential water contaminants. A more comprehensive analysis of specific contaminants and their individual effects on starter culture performance could provide further insights into water treatment strategies in dairy production. The economic analysis suggesting a 6.7% reduction in total production costs through optimized starter culture performance presents a compelling case for industry adoption of these findings. However, this analysis was based on current market prices and may not account for potential energy or raw material cost fluctuations. Long-term studies considering various economic scenarios would provide a more robust assessment of the financial implications of these optimizations.

## CONCLUSION

In conclusion, our research demonstrates the intricate relationships between environmental factors, starter culture performance, and the sustainability of dairy production. The findings contribute to the scientific understanding of lactic acid bacteria metabolism and offer practical insights for the dairy industry. Future research directions should include *in situ* studies of starter culture performance in industrial settings, investigation of mixed-strain fermentations under varying environmental conditions, and exploration of novel starter culture selection and adaptation strategies for enhanced resilience to environmental stressors.

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