RESEARCH ARTICLE | JUNE 05 2023

Effect of lanthanide ions stress on lactic acid fermentation performance of *Streptococcus thermophilus* during milk processing FREE

M. A. Vargas-Vargas ✉; R. A. Hernández-Chaverri; A. Jiménez-Silva; R. Malaka

AIP Conference Proceedings 2628, 050034 (2023)
https://doi.org/10.1063/5.0144195

Effect of Lanthanide Ions Stress on Lactic Acid Fermentation Performance of *Streptococcus thermophilus* during Milk Processing

M. A. Vargas-Vargas[1,a)], R. A. Hernández-Chaverri[2,b)], A. Jiménez-Silva[3,c)], and R. Malaka[4,d)]

[1]*Research Laboratory of Chemistry and Applied Biosciences, National Technical University (UTN), Costa Rica*
[2]*Bioenergetic and Environmental Research Unit, State Distance University (UNED), San José, Costa Rica*
[3]*Chemical Quality Assurance Laboratory, Quality and Productivity Center (CECAPRO), National Technical University (UTN); Alajuela, Costa Rica*
[4]*Laboratory of Biotechnology of Milk Processing, Department of Animal Science, Faculty of Animal Sciences, Hasanuddin University, Makassar, Indonesia*

[a)] Corresponding author: mvargas@utn.ac.cr
[b)] rohernandez@uned.ac.cr
[c)] ajimenez@utn.ac.cr
[d)] malaka_ag39@yahoo.co.id

Abstract. The purpose of this research is to evaluate the inhibitory effect produced by the presence of lanthanide ions (Ln^{3+}) at different concentrations on the biochemical process of lactic acid fermentation. Assays were carried out in reconstituted skim milk media with different contents of solids non-fat (SNF) by inoculation with *Streptococcus thermophilus* from commercially available freeze-dried, single-strain starter culture in Costa Rica, currently used in the manufacture of commercial cheese, yogurt, and other dairy products. Lanthanide elements find their main applications as structural components of electronic devices widely used in modern information and communication technologies (ICTs), among which cell phones, flat-screen televisions, hearing aids, desk computers, laptops, household appliances, and energy-saving fluorescent bulbs can be mentioned. In addition, they also play an important role in the manufacture of electric cars and wind turbines contributing to the development of alternative clean energies. Nevertheless, little progress has been made in developing recycling processes for the corresponding electronic waste containing these elements, which are being currently recognized as potential emerging pollutants in the environment. In this study, it was found significative inhibitory effects on the lactic acidification rate in the presence of lanthanides concentrations as low as 10^{-4} mol/L, at different incubation temperatures, incubation periods, and solid non-fat contents (SNF %), which might affect the quality and safety of some agro-industrial products of importance for the economy of the country, as well as some common biological processes such as the natural decomposition of organic matter and silage.

INTRODUCTION

Lanthanides are those chemical elements belonging to the first block f of the periodic table that includes lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm) ytterbium (Yb) and lutetium (Lu) [1], which together with the elements scandium (Sc) and yttrium (Y) constitute a group of chemically similar metals usually recognized by the International Union of Pure and Applied Chemistry (IUPAC) under the name of rare earth elements (REE) [2]. Incomplete 4f orbitals give these elements unique properties related to magnetic susceptibility, radiant energy absorption, and luminescence [3–4]. During the last three decades, these elements have become essential for the development of clean energies and devices for the new information and

Proceedings of the 4th International Conference of Animal Science and Technology (ICAST 2021)
AIP Conf. Proc. 2628, 050034-1–050034-14; https://doi.org/10.1063/5.0144195
Published by AIP Publishing. 978-0-7354-4529-1/$30.00

communication technologies (ICTs), including cell phones, computers, flat-screen televisions, fluorescent tubes, and energy-saving light bulbs [5–6].

With an increasing demand for green and sustainable products for energy, military, and manufacturing use, China has become the global supplier of more than 90 % of rare earth elements [7]. However, in present times, lifecycles of technological devices are becoming noticeably shorter, and ideally, electrical and electronic waste should be recycled in order to recover some of the most common lanthanide elements used, such as cerium (Ce), lanthanum (La), neodymium (Nd), praseodymium (Pr), samarium (Sm), gadolinium (Gd) and dysprosium (Dy) [8].

Nevertheless, due to the current low market prices and the need for high purity of the elements to be reusable in their various applications, global recovery rates are in most cases lower than 12% [9] and usually, less than 1 % of these elements have been effectively recycled; hence, the rest of them have entered the environment with an unknown final destination [10–11]. Recycling lanthanide metals is a technically complex task due to the difficulty of recovering these elements from the structural matrices in which they are occluded [12]. More often than not, current methodologies tend to generate potentially dangerous emissions of secondary and tertiary chemical wastes [13–14].

On the other hand, the global research to date related to the mechanisms by which lanthanide ions (Ln^{3+}) might affect natural living organisms, food industrial processes and human health is comparatively scarce [15]. Therefore, a better knowledge of the physicochemical and biological behavior of Ln^{3+} ions when interacting with the different agricultural, food, and environmental chains is of great interest and concern for many countries [16]. Several investigations have shown that REE at low concentrations and under certain environmental conditions, can cause a strong inhibition to the growth of some algae [15, 17–18], some bacteria [19–20] and some fungi [21–23] and many of these groups of microorganisms are key for agricultural and industrial production of alcoholic beverages, fermented vegetables or meat and dairy products, among others [24]. These elements in an ionic state can efficiently displace calcium ions in cell metabolism and their effect on microbiological processes carried out by lactic acid bacteria (LAB) under different conditions are unpredictable in most cases [5,25]. The European Agency for Safety and Health at Work [26] has declared that at present their disposition as electronic waste represents a serious toxicological threat to the health of workers involved in their use. Therefore, REEs must be recognized as new emerging pollutants with multiple forms of entry into the environment, for example, in wastewater from hospitals and in industrial effluents in soil [27]. Lanthanum and a few other rare earth elements have a specific interest as main pollutants in industrial effluents [28–29]. Some studies have also noticed an increase in the concentration of REEs in drinking water in large urban settlements [14, 30–32]. On the other hand, recently synthesized nanoparticles of cerium oxide and neodymium oxide have shown inhibitory activity on microbial growth of *Staphylococcus aureus*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Escherichia coli*, and *Candida albicans*, among others [33]. Results of an investigation to determine the effect and degree of accumulation of scandium (Sc), yttrium (Y), lanthanum (La), erbium (Er), and lutetium (Lu) in *in vitro* in 69 species of microorganisms, including 22 species of bacteria, 20 species of actinomycetes, 18 species of fungi and 16 species of yeasts have been presented [19]. It was also found that gram-positive bacteria, such as *Streptococcus thermophilus*, accumulate higher concentrations of these elements, particularly samarium (Sm). Soil fungi as *Trichoderma* and others showed great tolerance to the presence of significant concentrations of REE in culture media, although they were inhibited by concentrations higher than 100 mmol/L.

Several other food products owe their production and nutritional characteristics to the biochemical activity of lactic acid bacteria, such as fermented cheeses, pickles, and fermented sausages [34]. In the same way, products such as sourdough bread [35], olives [36], sauerkraut [37], as well as natural processes associated with grass silage for animal feeding [38–40] depend upon this biochemical process. Composting of lignocellulosic biomass residues occur largely by means of lactic acid fermentation and lactic acid can also be used to produce polylactic acid polymers, a biodegradable material gaining high popularity recently [41] and for the post-harvest treatment of agro-industrial biomass waste such as those generated in the production of potatoes [42]. Lactic acid fermentation at room temperature is important for the industrial processing of coffee beans [43] and cocoa [44] because during this phase their precursors of the aroma and flavor are developed.

This research, is intended to understand what is the effect of the presence of several Ln^{3+} ions at various concentrations on the lactic fermentation process carried out by *Streptococcus thermophilus*, a model lactic acid bacteria widely used in Costa Rican dairy food industry. The freeze-dried culture is commercialized in Costa Rica as an STI-14 culture from CHR-Hansen and is currently used for the elaboration of fermented milk and some cheeses.

MATERIALS AND METHODS

Lanthanide ions (La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+}, Lu^{3+}) were tested at three different concentrations in reconstituted skim milk culture media inoculated with *Streptococcus thermophilus*. For the general assays, an equimolar mixed stock solution (1.0 mol/L) containing all the elements was also prepared and diluted as necessary. Because of its radioactive nature [45], promethium (Pm) was excluded from this research. For the preparation of *Streptococcus thermophilus* starter culture, a 500 mL-Erlenmeyer flask with 15.0% reconstituted skim milk was pasteurized at 63 ± 2°C for 30 min (LTLT), cooled to 25°C, inoculated with 0.10 g freeze-dried culture powder and incubated 6 h at 40°C [46].

Inhibitory Effect as a Function of Solids Non-Fat Content (SNF)

An equimolar mixture of 14 Ln^{3+} ions was added to a final concentration of 0.10 mmol/L; 1.0 mmol/L and 10 mmol/L to pasteurized, reconstituted skim milk culture media, inoculated with 3.0 % of the starter culture and with the following SNF contents: 5%, 7.5%, 10%, 12.5%, 15%, 27.5% and 20% (w/w). Culture media were incubated for 6 h at 40°C and sampled every two hours for the potentiometric determination of titratable acidity.

Inhibitory Effect as a Function of Incubation Temperature

An equimolar mixture of 14 Ln^{3+} ions was added to a final concentration of 0.10 mmol/L; 1.0 mmol/L and 10 mmol/L to pasteurized, reconstituted skim milk culture media (SNF 15%), inoculated with 3% of the starter culture and incubated at 25°C, 35°C, 45°C, 55°C and 65°C for 6 h. A control test was kept at 5°C.

Inhibitory Effect and Fermentation Rates as a Function of Incubation Time

An equimolar mixture of 14 Ln^{3+} ions was added to a final concentration of 0.10 mmol/L; 1.0 mmol/L and 10 mmol/L to pasteurized, reconstituted skim milk culture media (SNF 15%), inoculated with 3% of the starter culture and incubated 12 h at 40°C. Samples were taken every 2 hours and analyzed for pH and titratable acidity.

Inhibitory Effect as a Function of The Lanthanide Element

Forty-seven labeled 250 mL Erlenmeyer flasks were placed in a dry oven, covered with aluminum foil, and sterilized by heating at 180°C. Using a micropipette, 1.00 mL of each of the 14 Ln^{3+} ion solutions at three different concentrations (1.0 mol/L; 0.10 mol/L and 0.010 mol/L) was added for final concentrations of 10 mmol/L, 1.0 mmol/L and 0.10 mmol/L, respectively). Five Erlenmeyer flasks containing no lanthanide ions were used as control. Ninety-nine milliliters of reconstituted skim milk media (15%) inoculated with *Streptococcus thermophilus* starter culture was added to each Erlenmeyer flask and mixed thoroughly. Incubation took place at 40°C for 10 h.

Analytical Methods

The contents of each test tube were poured into a 250 mL beaker, weighed, dispersed with 100 mL of deionized water, and titrated potentiometrically to a pH of 8.00 ± 0.02 at 22 ± 2°C, using NaOH 0.1004 mol/L. pH was measured with a pH-metro HACH HQ440D Multi. All samples were refrigerated at 5 ± 1°C prior to their analysis in order to stop the fermentation process. The acidity value could be calculated and the value was expressed as lactic acid, according to the following formula:

$$\text{Titratable Acidity (\%)} = \frac{V_{NaOH} \times M_{NaOH} \times 90.08}{1000 \times m_S} \times 100 \quad (1)$$

V_{NaOH} : Volume of titrating solution (mL)
M_{NaOH} : Molarity of the titrating solution (0.1004 mol/L)
m_s : Mass of the sample (g)

Fermentation rates were calculated according to:

$$\text{Fermentation Rate} = \frac{AT_n - AT_{n-1}}{t_n - t_{n-1}} \times 1000 \quad (2)$$

$AT_n \; y \; AT_{n-1}$: The titratable acidity for two consecutive hours (t_n y t_{n-1})

The inhibitory effect was expressed as a percentage, as follows:

$$\text{Inhibition (\%)} = \frac{AT_{0t} - AT_{xt}}{A_{0t}} \times 100 \quad (3)$$

A_{0t} : The titratable acidity of a non supplemented blank expressed as lactic acid
A_{xt} : The titratable acidity of samples supplemented with lanthanide ions (x) at different concentrations (0; 0.10; 1.0 and 10 mmol/L), incubated under the same experimental conditions (SNF: 15.0 %, 40°C and incubation time (t): 0 , 2 , 4 , 6 , 8, 10 and 12 h).

RESULTS AND DISCUSSION

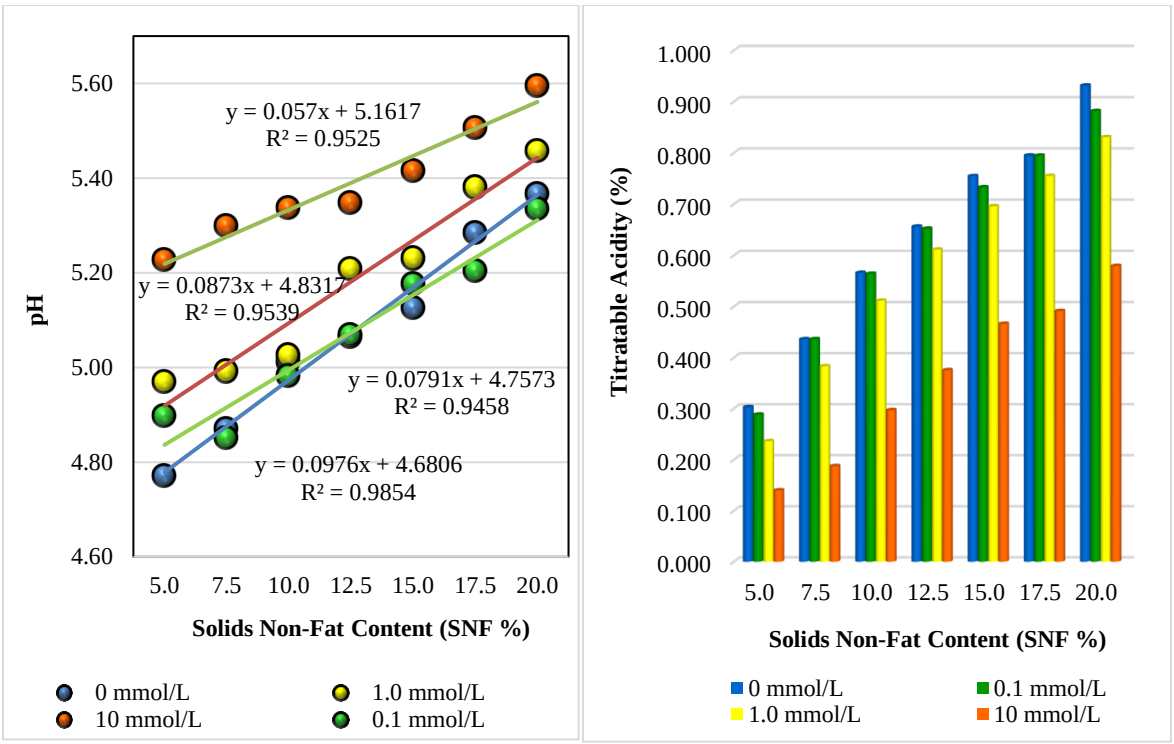
Inhibitory Effect of Lanthanide Ions as a Function of Non-Fat Solids Content

The most abundant metal ions in milk include potassium (K), sodium (Na), calcium (Ca), and magnesium (Mg), and in lower amounts iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), iodine (I), boron (B), fluorine (F), silicon (Si), bromine (Br), nickel (Ni), molybdenum (Mo) and cobalt (Co), in addition to traces of barium, aluminum, titanium, vanadium, rubidium, lithium, strontium, chromium, silver and tin [47]. Therefore, the eventual presence of Ln^{3+} ions is not considered normal in milk and can be originated exclusively by processes of exogenous contamination.

Figure 1a shows the effect of Solids Non-Fat contents (SNF%) on the pH of culture media supplemented with an equimolar mixture of lanthanide ions (Ln^{3+}) at concentrations of 0 mmol/L, 0.1 mmol/L, 1.0 mmol/L, and 10 mmol/L. and incubated for 6 h at 40°C. The corresponding titratable acidity values are shown in Figure 1b. In a previous test, it was found that a concentration of 50 mmol/L was completely inhibitory of the lactic fermentation process, so the value of 10 mmol/L Ln^{3+} value was fixed as the highest experimental concentration for this research.

In almost all cases, pH values after 6 h incubation were higher as the concentration of Ln^{3+} increased in each culture media. The only exception was observed in culture media with higher concentrations of solids non-fat (SNF%) when pH reached in the presence of 0.10 mmol of Ln^{3+} was compared to the pH value reached by the blank. This was probably due to a hormetic response of Ln^{3+} on lactic acid bacteria at very low concentrations since similar behavior has been reported for other biochemical processes, especially in relation to the growth of plant cells [48].

In more concentrated media, a higher tendency to syneresis could be also observed in the presence of Ln^{3+}, even at concentrations as low as 0.1 mmol/L, so the spontaneous whey separation resulting from the contraction of the milk curd was probably enhanced by the presence of highly charged Ln^{3+} ions without the participation of other external forces such as centrifugation [49].



(a) (b)

FIGURE 1. (a) pH and (b) titratable acidity in culture media supplemented with lanthanide ions (0 mmol/L, 0.10 mmol/L, 1.0 mmol/L, and 10 mmol/L), as a function of Solids Non-Fat (%), incubated 6 h at 40°C.

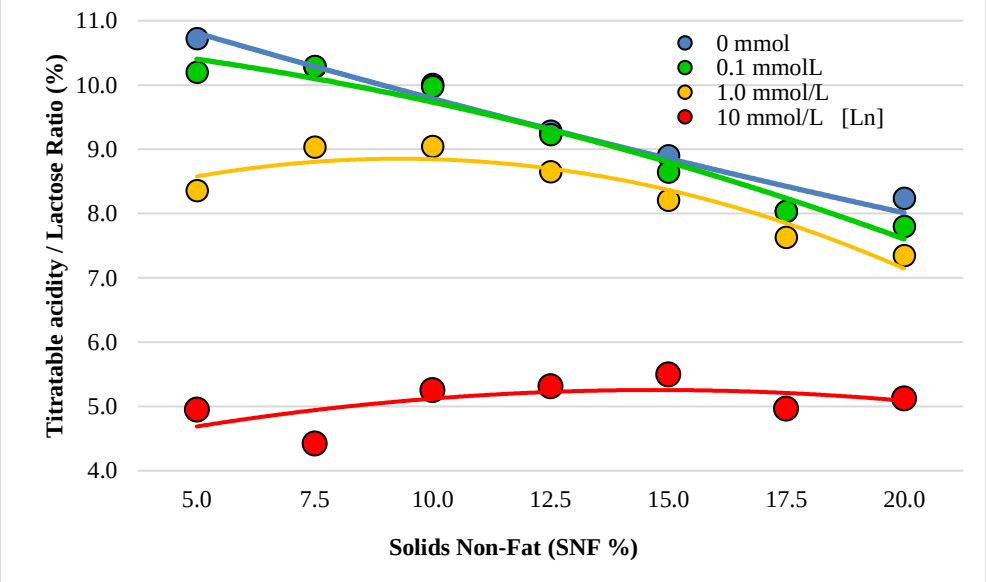


FIGURE 2. Lactic acid ratio as a function of SNF (%), in the presence of Ln^{3+} ions.

On the other hand, for all culture media with Solids Non-Fat (SNF) contents between 5.0% and 20.0%, titratable acidities increased as the SNF contents increased. Since lactose contents in these media varied between 2.83 % and

11.3%, noticeable differences in the productivity of lactic acid from the available lactose was observed as the concentration of Ln^{3+} ions in the culture media increased from 0 to 10 mmol/L.

Under these experimental conditions, inhibitory effects might be a consequence both of higher osmotic pressure of the culture media and of the presence of a higher concentration of Ln^{3+} ions. These effects have been observed on other microorganisms under different laboratory conditions [23]. In some cases, the addition of 0.1 mmol/L had no apparent effect, specifically in the concentration range between 7.5 % and 12.5 % SNF. However, in all cases, the presence of 1.0 mmol/L had a significant effect under these conditions.

Figure 2 shows how much of available lactose is transformed into lactic acid, as a function of the concentration of solids non-fat (SNF) contents, from which lactose represents around 56.5% (w/w).

In culture media with an addition of Ln^{3+} ions up to 0.10 mmol/L, a significant decrease in the acidity/lactose ratio could be observed as the concentration of lactose increased, decreasing from 10.5% to values around 8.0%. In culture media with concentrations higher than 1.0 mmol/L of Ln^{3+}, the effect becomes less noticeable, showing the highest transformation ratio (~9%) between 7.5% to 10.0% SNF content and the lowest transformation ratio (~7%) as SNF% in the culture media increases up to 20.0%. In culture media supplemented with 10 mmol/L of Ln^{3+}, the lactose transformation ratio in lactic acid was rather low, oscillating between 5.0 to 5.5%. High carbohydrate contents in culture media tend to reduce the rate of lactic acid fermentation due to a decrease in the entry rate of fermentable carbohydrates into the bacterial cell since they may become saturated with the excessive availability of substrate [50].

Figure 3 shows the inhibitory effect (%) produced by the Ln^{3+} ions on the fermentation rate as a function of the concentration of solids non-fat (SNF%).

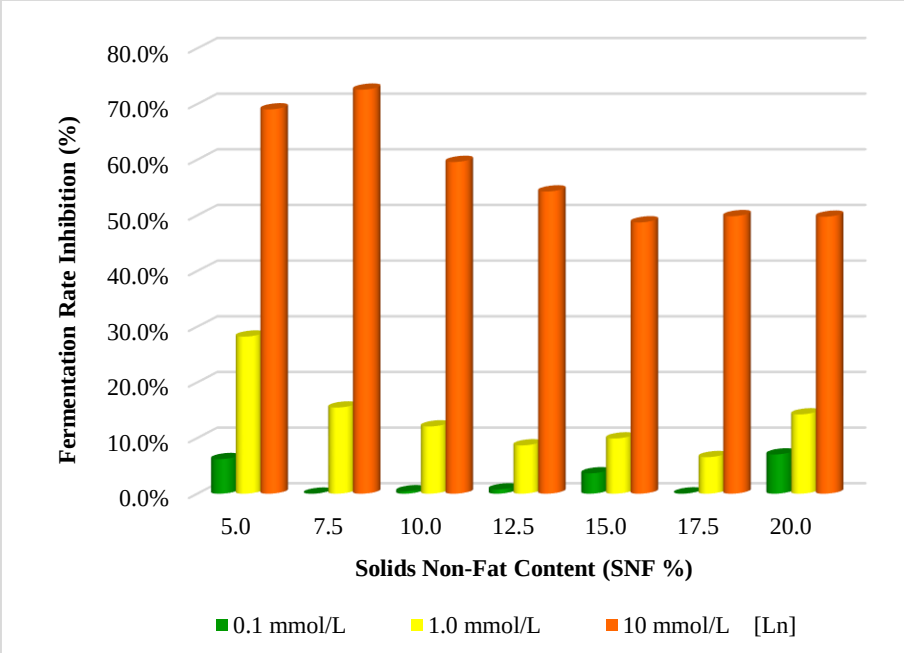


FIGURE 3. Fermentation rate inhibition in the presence of Ln^{3+} as a function of SNF (%).

It is evident that the inhibition was significantly higher as the concentration of Ln^{3+} increased in the culture media. However, the highest inhibitory effect did not take place at the same Ln^{3+} concentration in all media. The effect of the addition of 10 mmol/L of Ln^{3+} was maximal (72.6%) in the medium with 7.50% SNF and gradually decreased to a minimum (48.7%) in the culture medium with 15.0 % SNF. From there, it increased very slightly up to 49.8% in the culture media with 20.0% SNF. In this case, the inhibitory effect caused by a higher concentration of carbohydrates could be related to the increased osmotic pressure of the media [50]. In comparison, it has been reported that the growth of other common lactic acid bacteria is completely inhibited when the osmotic pressure reaches 2416 mOsm/kg, due to the continuous accumulation of several metabolites in the culture media [51].

Also, Ln^{3+} ions are capable of joining active sites that are normally linked with Fe^{3+} ions of transferrin and ferritin, Mn^{2+} ions of pyruvate kinase (EC 2.7 .1.40) and glutamine synthetase (EC 6.3.1.2) or ions Mg^{2+} in pyruvate

kinase and alkaline phosphatase [52]. Furthermore, the ionic radius of hexacoordinated Ca^{2+} ions is reported to be 1.00 Å, while the average ionic radius of all Ln^{3+} ions in chemical environments with coordination numbers of 6, 7, 8 and 9 is about 1.02 ± 0.02. This indicates that the size of Ln^{3+} ions is virtually the same as that of Ca^{2+} ions under normal physiological conditions. Therefore, Ln^{3+} can partially compete with Ca^{2+} ions and other elements such as Mg, Fe, Mn, and Zn for active sites in functional biomolecules and then inhibit biochemical processes such as active transport, cellular respiration, oxidation of cytochromes, and permeability of acetyl coenzyme A, among others [53].

On the other hand, the similarity of Ln^{3+} ion spectra united to certain enzymes suggests that the ion charge rather than the ionic radius, could be the factor that most favors the configuration of the active sites and the structural stability of the complexes formed when protein and enzymes bind with Ln^{3+} ions [54].

Inhibitory Effect of Lanthanide Ions as a Function of Incubation Temperature

Figure 4 shows the effect of the incubation temperature (15.0% SNF, 6 hours, 100 mL) in the production rate of lactic acid (mg/h), in the presence of 0.10, 1.0, and 10 mmol/L of an equimolar mixture of 14 Ln^{3+} ions.

Between 25°C and 65°C, the addition of Ln^{3+} to the culture media showed an inhibitory effect on the fermentation rate. At 35°C, the production of lactic acid dropped from 0.660% to 0.442%, which is 33.1% less in the presence of 10 mmol/L of Ln^{3+} ions. At 45°C, the fermentation rate dropped from 0.858% to 0.647%, that is, 24.6% less with the same Ln^{3+} addition. Finally, at 55°C, the percentage in which the fermentation rate decreased 22.9%, falling from 0.776% to 0.598%. This indicates that around the optimal growth temperature zone of *Streptococcus thermophilus* (35°C–55°C), the inhibitory effect caused by the addition of Ln^{3+} tended to be very high.

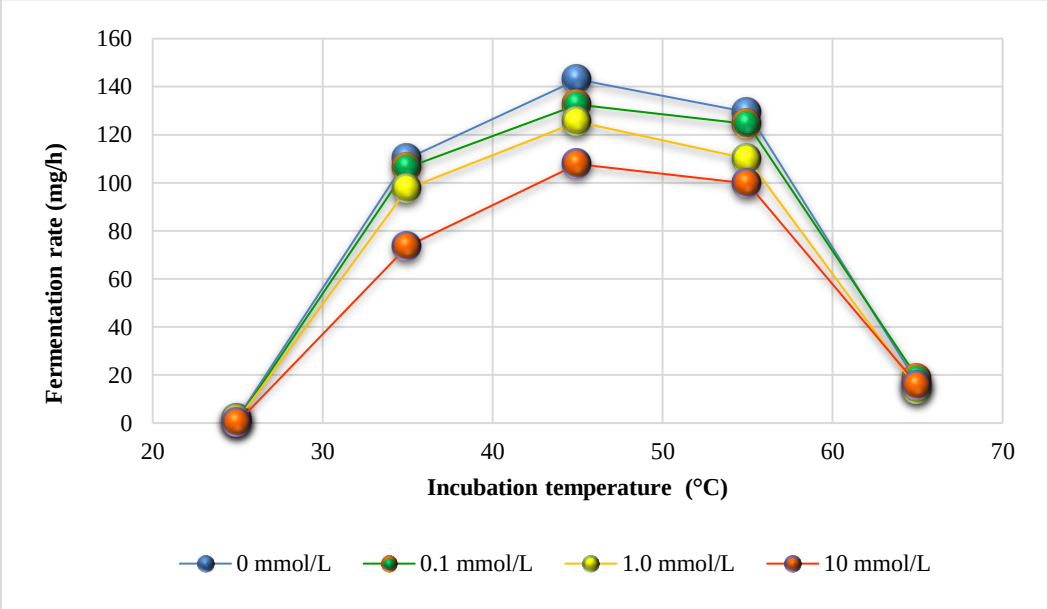


FIGURE 4. Fermentation kinetics based on the incubation temperature in the presence of Ln^{3+} (15.0 % SNF, 6 h).

At temperatures of 25°C and 65°C, there is no statistical evidence ($p \geq 0.05$) for the occurrence of an inhibitory effect of Ln^{3+} ions on lactic acid fermentation under the experimental conditions applied. This is important to consider in the case of lactic acid fermentation processes that occur slowly at room temperatures such as the silage of forage species, acidification of vegetables or composting [38–40].

The maximum production rate of lactic acid in 100 mL culture media was 143 mg/h and took place in the absence of Ln^{3+} ions at 45°C. In contrast, 10 mmol/L Ln^{3+} ions reduced this rate to 108 mg/h, a decrease around 25 %. The addition of 0.1 mmol/L and 1.0 mmol/L reduced the production of lactic acid by 7 % and 13 %, respectively.

On the other hand, the higher the incubation temperature, the higher the susceptibility of the milk curd to syneresis, especially at temperatures above 45°C when milk curds tend to form more compact gels. This is

especially remarkable at samples with a pH close to 4.6, the isoelectric point of milk [46]. From these results and taking into account the tendency to syneresis at temperatures above 45°C, the temperature of 40°C was chosen for subsequent inhibition tests in the present study.

Effect of Lanthanide Ions on Lactic Acid Fermentation Rates

Figure 5 shows the change of pH and the titratable acidity of a culture media with 15.0% SNF, incubated 12 hours at 40°C in the presence of 0.1 ; 1.0 and 10.0 mmol/L of an equimolar mixture of Ln^{3+} ions. As can be seen, pH curves tend to reach a limit approaching a minimum pH value of around 4.50.

Under these conditions, low pH values of culture media due to high concentrations of non-dissociated lactic acid constitute the most inhibitory component of the growth of lactic acid bacteria and analysis can be done using mathematical models similar to those that describe the lag phase, the exponential growth phase, the stationary phase and the exponential death phase. However, due to the complexity of biological phenomena, the use of non-linear mathematical models is required to identify some changes in processes caused by the presence of inhibitory or stimulating agents [55]. Figure 6 presents an approximation of the bacterial activity by calculating the derivative of the acidity produced (dA/dt) as a descriptive parameter for the fermentation process. As the substrate required for the production of lactic acid decreases in time, a reduction of the fermentation rate occurs and is intensified by the gradual increase in the concentration of lactic acid in the culture media.

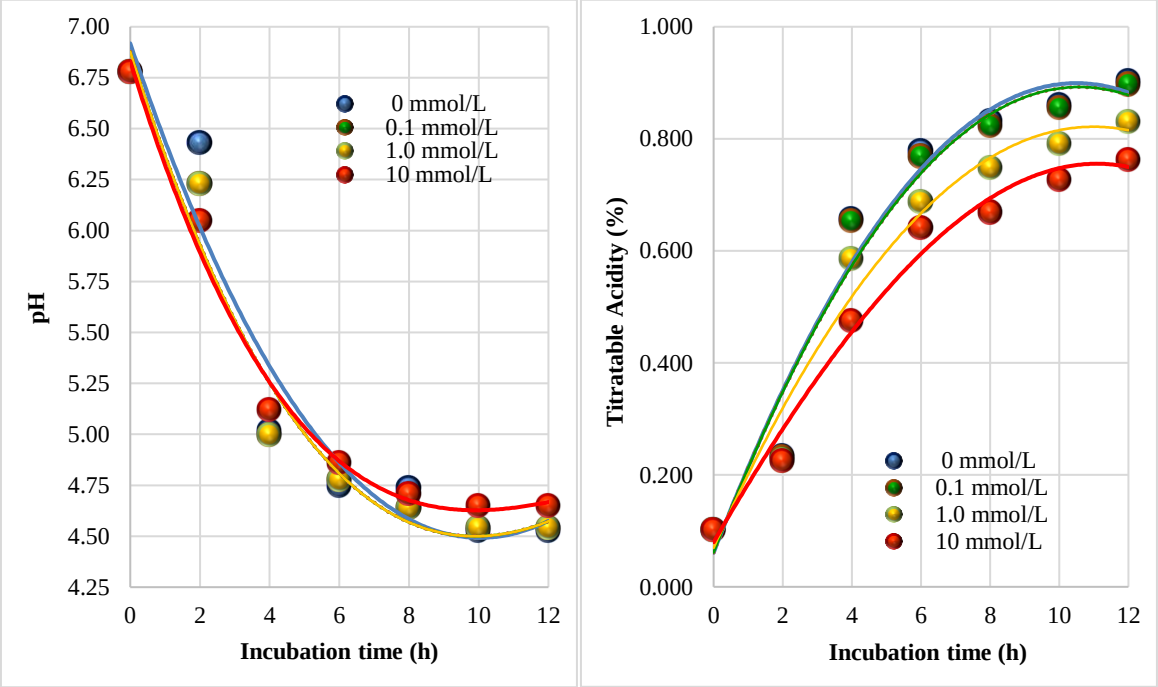


FIGURE 5. Time variation of a) pH and b) titratable acidity in inoculated media with *Streptococcus thermophilus* in the presence of Ln^{3+} ions (12 h, 40°C, 15.0 % SNF).

Keeping all factors under control (40°C, 15.0% SNF), the only factor responsible for the difference of the curves observed in Figure 6 was the presence of the Ln^{3+} in the culture media. As can be observed in Figure 5, the inhibitory effect caused by the addition of 0.10 mmol/L of Ln^{3+} was quite slight but detectable in relation to the development of acidity in the culture medium that did not contain Ln^{3+} ions. However, in terms of lactic acid production rate shown in Figure 6, both curves were practically indistinguishable throughout the entire test. Nevertheless, by increasing the concentration of Ln^{3+} ions to 1.0 mmol/L, the effect on fermentation rates became much more pronounced.

On the other hand, the presence of 10.0 mmol/L of Ln^{3+} showed a very remarkable effect on both the intensity of the inhibition and the shape of the curves. An important characteristic of the curve with 10.0 mmol/L of Ln^{3+} was

that it did not show the symmetry of the curves with lower Ln^{3+} concentrations, suggesting that the maximum production rate of lactic acid had shifted slightly to the right, which could be due to a longer delay in the lag phase of *Streptococcus thermophilus*. After 6 hours incubation all curves tended to be similar and the fermentation process practically stopped after 12 h in all culture media.

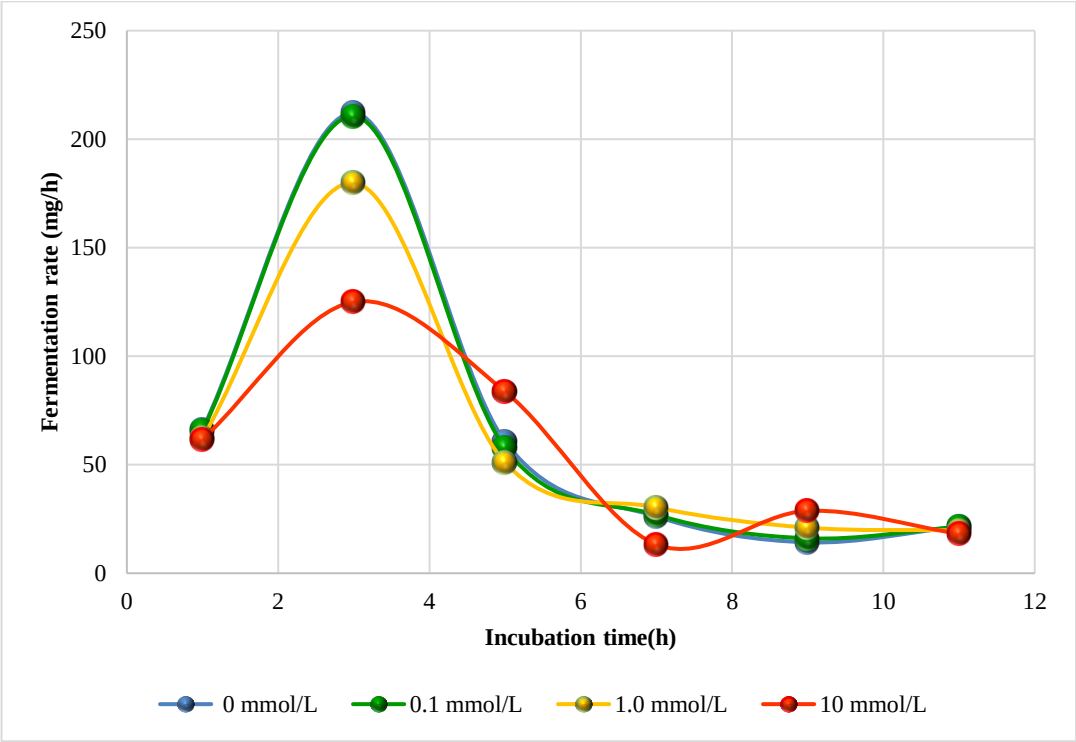


FIGURE 6. Lactic Fermentation of *Streptococcus thermophilus* in the presence of Ln^{3+} (12 h, 40°C, 15.0 % SNF).

In those cases in which it is required to optimize the generation of lactic acid for industrial purposes, such as for the production of polylactic acid to be used as a biodegradable polyester [56, 57], it is recommended to control pH by using buffered systems and supplement culture media with carbon and nitrogen sources, since lactic acid bacteria are unable to use the compounds released by lysis of dead cells [55].

The inhibition of the formation of lactic acid is due to the solubility of non-dissociated acid and the insolubility of dissociated lactate ions within the cytoplasmic membrane, causing acidification and failure of the proton motor forces [58]. This phenomenon affects the transmembrane pH gradient and decreases the amount of energy available for cell growth. In the industrial field, to avoid the inhibition of the fermentative process, extractive recovery techniques can be applied such as the addition of calcium hydroxide that neutralizes the acid produced precipitating the respective insoluble calcium salts [59]. It is important to note that Ln^{3+} ions form complexes in which three or four carboxylate groups bind to each Ln^{3+} ion, as the concentration of the lactic acid in the media increases, so their ability to interact and inhibit lactic acid bacteria decreases at lower pH values [52].

Individual Effect of Lanthanide Ions on Lactic Acid Fermentation

Figure 7 shows the individual effect of Ln^{3+} ions (La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+}, Lu^{3+}) at different concentrations (0.10 mmol/L, 1.0 mmol/L and 10 mmol/L) on the titratable acidity of culture media inoculated with *Streptococcus thermophilus* incubated at 40°C during 10 h.

As can be seen, without exception, all the Ln^{3+} ions inhibited the fermentation process to a certain degree, what was more remarkable as their concentration increased from 0.10 mmol/L to 10 mmol/L. The average acidity developed in culture media supplemented with 0 mmol/L, 0.1 mmol/L, 1.0 mmol/L and 10 mmol/L were 1.17 (± 0.01) %; 1.13 (± 0.02%, 1.07 (± 0.02) % and 0.72 (± 0.06) % at a 95 % confidence level ($\alpha = 0.05$).

Unexpectedly, no important correlation was found between the intensity of the inhibitory effect of each lanthanide ion and the atomic number of the corresponding element. Pearson correlation coefficients between the results of titratable acidity in the media with 0.10 mmol/L, 1.0 mmol/L, and 10.0 mmol/L Ln^{3+} ions and their atomic number were +0.484, +0.795, and -0015, respectively.

The physiological properties of the lanthanide elements can be explained based on adsorption mechanisms on the surface of the cells through the phosphate groups in the lipid membrane, resulting in alterations in the cellular transport of transmembrane metal ions, although it is said that only the 7% of phosphate groups in lipid bilayer interacts with Ln^{3+} ions. This effect is especially important in the case of Ce^{3+} and La^{3+} in which cellular walls act as barriers for these ions [52].

However, it has recently been proven that a limited number of Ln^{3+} ions can go through the plasma membrane. Even so, the effect of the Ln^{3+} ions in the biochemical processes at intracellular level related to inhibitory and stimulatory effects (Hormesis) of the interactions is still uncertain [23] although it is known that in the case of cerium nanoparticles damage occurs due to disruption of the membrane and the release of free radicals that directly affect cellular DNA [14].

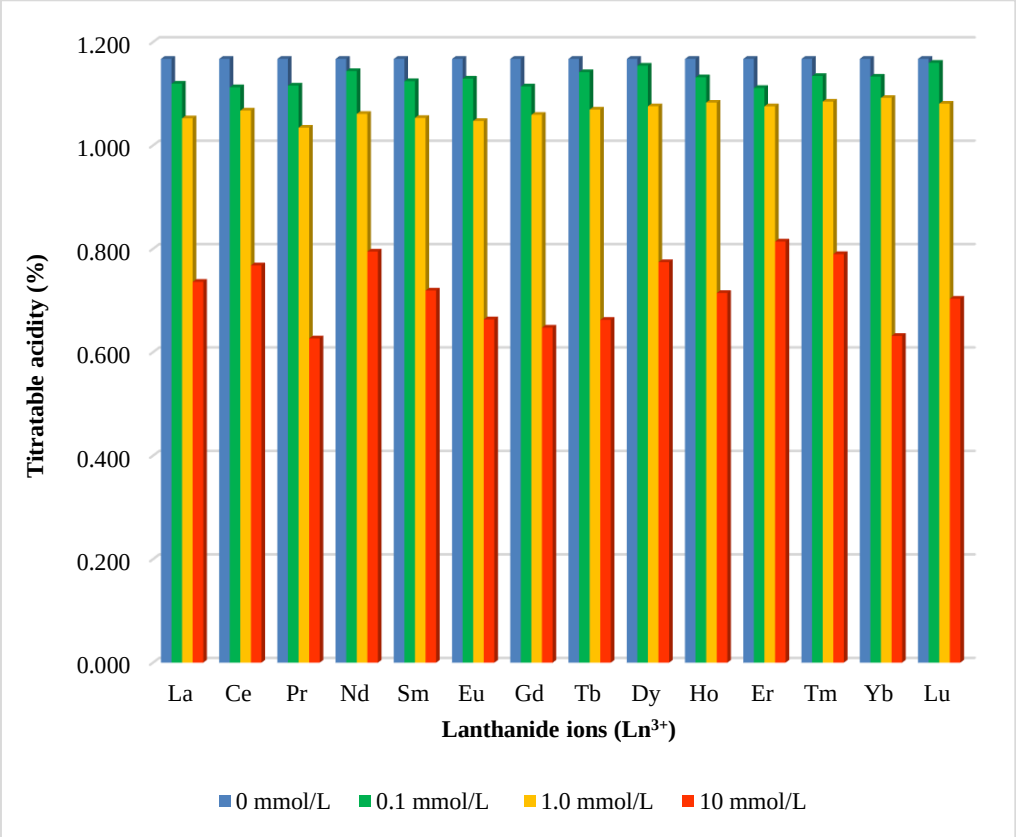


FIGURE 7. Acidity developed by *Streptococcus thermophilus* in the presence of individual Ln^{3+} (10 h, 40°C, 15.0 % SNF).

According to the first observations of electronic microscopy, Ln^{3+} ions adhere passively to the cell surface, but they are not capable of penetrating the cytoplasm of the living cells belonging to bacteria, fungi or algae, because they have a high electron density, unless processes such as phagocytosis or pinocytosis or very prolonged incubation times are given [52].

However, more recent studies have shown that they are capable of entering some cells and producing toxicity associated with mitochondria and cellular core [14]. Some authors have reported that doses between 1 x 10^{-4} and 1 x 10^{-2} mol/L of Ln^{+3} ions are required to inhibit the growth of bacteria, fungi and yeast. On the other hand,

concentrations less than 1 x 10^{-5} have shown in some special cases stimulatory effects on cell growth. This phenomenon is known as hormesis [48].

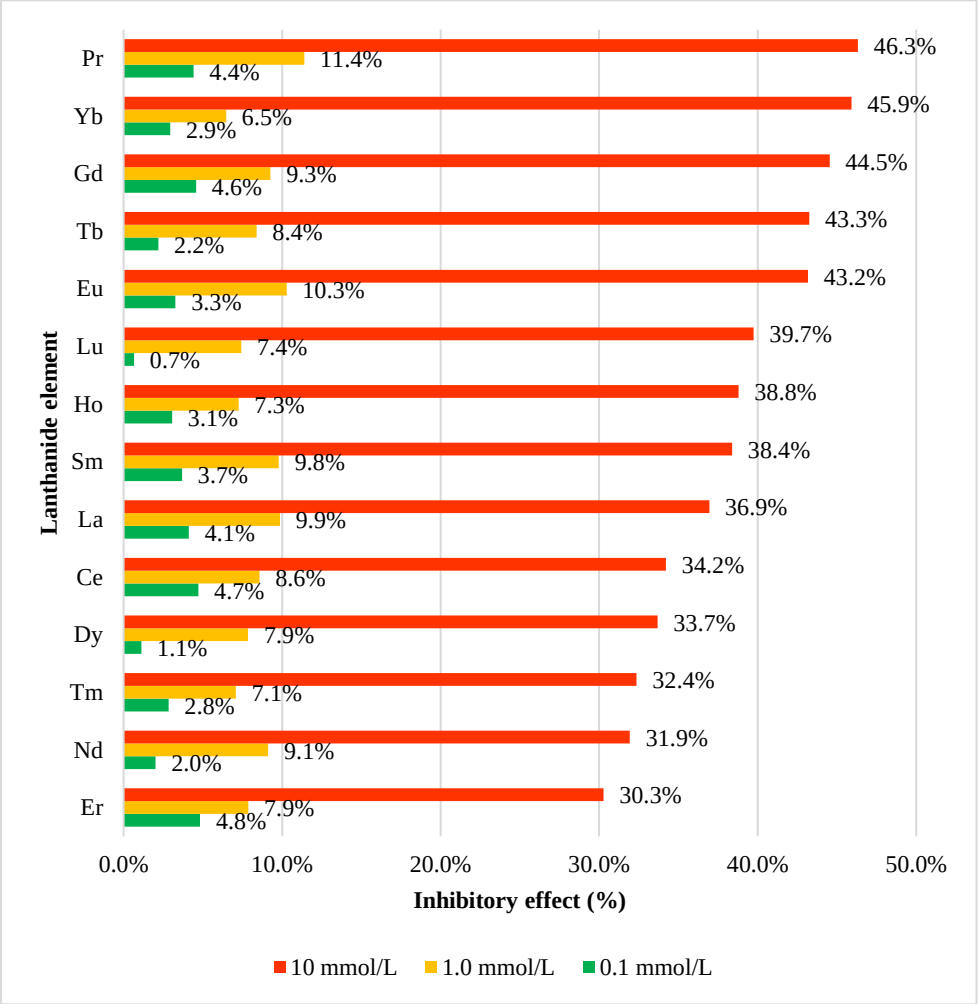


FIGURE 8. Inhibitory effects of lanthanide ions on lactic acid fermentation at different Ln^{3+} concentrations (*Streptococcus thermophilus*, 10 h, 40°C, 15.0 % SNF).

Keeping all factors under control (40°C, 15.0% SNF), the only factor responsible for the difference of the curves observed in Figure 6 was the presence of the Ln^{3+} in the culture media. As can be observed in Figure 5, the inhibitory effect caused by the addition of 0.10 mmol/L of Ln^{3+} was quite slight but detectable in relation to the development of acidity in the culture medium that did not contain Ln^{3+} ions. However, in terms of lactic acid production rate shown in Figure 6, both curves were practically indistinguishable throughout the entire test. Nevertheless, by increasing the concentration of Ln^{3+} ions to 1.0 mmol/L, the effect on fermentation rates became much more pronounced.

On the other hand, the presence of 10.0 mmol/L of Ln^{3+} showed a very remarkable effect on both the intensity of the inhibition and the shape of the curves. An important characteristic of the curve with 10.0 mmol/L of Ln^{3+} was that it did not show the symmetry of the curves with lower Ln^{3+} concentrations, suggesting that the maximum production rate of lactic acid had shifted slightly to the right, which could be due to a longer delay in the lag phase of *Streptococcus thermophilus*. After 6 hours incubation all curves tended to be similar and the fermentation process practically stopped after 12 h in all culture media.

CONCLUSION

The presence of the lanthanide elements (Ln) in an ionic form exerts an inhibitory effect in the biochemical process of lactic acid fermentation, in skim milk culture media with SNF contents in the range from 5% to 20%. The phenomenon was observed throughout the range of incubation temperatures from 5°C to 65°C, being more pronounced between 35°C and 55°C, presenting the highest inhibitory effect around 45°C with concentrations of Ln^{3+} ions between 0,10 mmol/L and 10 mmol/L. Under these experimental conditions, it could be verified that the inhibitory effect was detectable even with concentrations of Ln^{3+} ions in the order of 1×10^{-4} mol/L. At lower concentrations, it was not possible to measure any effect, but the possibility of hormesis cannot be ruled out unless more sensitive analytical methods are implemented. On the other hand, the inhibitory effect of each element was slightly different among them, having the elements praseodymium, ytterbium and gadolinium the strongest effect, and erbium, neodymium and thulium the weakest effect. It was not possible to verify any correlation between the atomic number of the element and the intensity of its inhibitory effect on lactic acid fermentation.

In summary, it was verified that the agro-industrial processes related to lactic fermentation done with commercially available freeze-dried culture starter of *Streptococcus thermophilus*, which is relevant for the Costa Rican dairy food industry, might be affected by the presence of very low levels of these chemical elements in the environment, recently introduced as emerging pollutants through an inappropriate handling and disposition of e-waste generated from modern electrical and electronic devices, widely used in the country.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support from the Laboratory of Chemistry and Applied Biosciences in the National Technical University (UTN: Universidad Técnica Nacional de Costa Rica) and the Distance State University (UNED: Universidad Estatal a Distancia de Costa Rica).

REFERENCES

1. A. Bettencourt, "The electronic structure of the lanthanides," in *The Rare Earths Elements: Fundamentals and Applications*, edited by D.A. Atwood (WILEY, Lexington, 2012), pp. 27–34.
2. A. Golloch, *Handbook of Rare Earths Elements* (De Gruyter Analytics. Aachen, Germany, 2017).
3. J. Andres and A. S. Chauvin, "Luminescence applications," in *The Rare Earths Elements: Fundamentals and Applications*, edited by D.A. Atwood (WILEY, Lexington, 2012), pp. 135-152.
4. B. W. Wang and S. Gao, "Magnetism", in *The Rare Earths Elements: Fundamentals and Applications*, edited by D.A. Atwood (WILEY, University of Kentucky, Lexington, 2012), pp. 153-159.
5. H. R. El-Ramady, "Ecotoxicology of rare earth elements: I. Effects on soils and plants," Ph.D. thesis, Kafr El-Sheikh University, 2010).
6. T. Lorenz and M. Bertau," Recycling of Rare Earth Elements," *Physical Sciences Reviews* **2**, 1–24 (2017)
7. G. Pagano, F. Aliberti, M. Guida, R. Oral, A. Siciliano, M. Trifuoggi and F. Tommasi, "Rare earth elements in human and animal health: state of art and research priorities," *Environmental Research* **142**, 215-220 (2015).
8. A. Brumme, *Wind Energy Deployment and the Relevance of Rare Earths* (Best Masters, Springer Fachmedien, Wiesbaden, 2014).
9. H. Royen, and U. Fortkamp, *Rare earth elements: Purification, separation and recycling* (Swedish Environmental Research Institute, Sweden, 2016).
10. K. Binnemans, P.T. Jones, B. Blanpain, T van Gerven, Y. Yang, A. Walton and M. Buchert, "Recycling of rare earths: a critical review," *Journal of Cleaner Production* **51**, 1–22 (2013).
11. K. Binnemans, P.T. Jones, B. Blanpain, T van Gerven and Y. Pontikes, "Towards zero-waste valorization of rare earth containing industrial process residues: A Critical review," *Journal of Clearer Production* **99**, 17–38 (2015).
12. V. González, D. Vignati, C. Leyval, and L. Giamberini, "Environmental fate and ecotoxicity of lanthanides: Are they a uniform group beyond chemistry ?," *Environmental International* **71**, 148-157 (2014).
13. USEPA, *Rare earth elements: a review of the production, processing, recycling, and associated environmental issues* (EPA 600/R-12/572, United States, 2012).

14. K.T. Rim, K.T, "Trends in occupational toxicology of rare earth elements," in *Rare earth elements in human and environmental health*, edited by G. Pagano (Pan Stanford Publishing, Danvers, Massachusetts, 2017), pp.11-46.
15. M. Trifuoggi, E. Vasca and C. Manfredi, "Rare Earth Elements Equilibria in Aqueous Media," in *Rare Earth Elements in Human And Environmental Health*, edited by G. Pagano (Pan Stanford Publishing, Massachusetts, 2017), pp.127– 141 .
16. J. C. Bünzli, *Ullmann's Encyclopedia of Industrial Chemistry. Rare Earths Elements* (Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2018).
17. P. Tai, Q. Zhao, D. Su, Li D, and F. Stagnitti, "Biological toxicity of lanthanide elements on algae," *Chemosphere* **80**, 1031–1035 (2010).
18. M. Guida, A. Siciliano and G. Pagano, "Rare earth toxicity to marine and freshwater algae," in *Rare Earth Elements in Human and Environmental Health*, edited by G. Pagano (Pan Stanford Publishing, Danvers, Massachusetts, 2017), pp.47–67 .
19. T. Tsuruta,"Accumulation of rare earth elements in various microorganisms," *Journal of Rare Earths* **25**, 526–532 (2007).
20. X. Mou, R. Yang, W. Zhang and B. Yang", Effect of Tb(III) on activity and stability of nattokinase," *Journal of Rare Earths*, **35**, 510–516 (2010).
21. K. Mu, C. Zhang, X. Zhao, J. Cui, W. Zhang, and L. Hu, "Effect of lanthanum on mycelium growth and some pathogenic factors," *Journal of Rare Earths* **24**, 485–490 (2006).
22. A. Aragón and D. Polo, "Synthesis, characterization, thermal behavior, and antifungal activity of La(III) complexes with cinnamates and 4-methoxyphenylacetate," *Journal of Rare Earths* **31**, 1106–1113 (2013).
23. L. d'Aquino, L. and F. Tommasi, "Rare Earth Elements and Microorganisms," in *Rare Earth Elements in Human and Environmental Health*, edited by G. Pagano (Pan Stanford Publishing, Danvers, Massachusetts, 2017), pp. 127–141 .
24. G. Vinderola, A. C. Ouwehand, S. Salminen and A. von Wright, *Lactic Acid Bacteria. Microbiological and Functional Aspects*. 5th ed. (Taylor & Francis, United States, 2019).
25. W. A. Rambeck, *Rare Earth Elements in Agriculture with Emphasis in Animal Husbandry* (Munich, Germany, 2006).
26. EU-OSHA, *Priorities for Occupational Safety and Health Research in Europe 2013-2020* (The European Union, Luxembourg, 2013).
27. J. Li, E.A. Verweij and C. Gestel, "Lanthanum toxicity to five different species of soil invertebrates in relation to availability in soil," *Chemosphere* **193**, 412–420 (2018).
28. H. Hermann, J. Nolde, S. Berger and S. Heise, "Aquatic ecotoxicity of lanthanum – A review and an attempt to derive water and sediment quality criteria," *Ecotoxicological Environmental Safety* **124**, 213–238 (2016).
29. N. Tepe, M. Romero, and M. Bau, "High-technology metals as emerging contaminants: Strong increase of anthropogenic gadolinium levels in tap water of Berlin, Germany, from 2009 to 2012," *Applied Geochemistry* **45**, 191–197 (2014).
30. S. Ali, "Social and environmental impact of the rare earth industries," *Resources* **3**, 123–134 (2014).
31. F. Al-Rimawi, K. Kanan and M. Qutob, "Analysis of different rare metals, rare earth elements, and other common metals in groundwater of South West Bank/Palestine by ICP/MS-Data and Health Aspects," *Journal of Environmental Protection* **4**, 1157–1164 (2013).
32. V. Hatje, K.W. Bruland, and A.R. Flegal, "Increases in anthropogenic gadolinium anomalies and rare earth element concentrations in San Francisco Bay over a 20-year record," *Environmental Science and Technology* **50**, 4159– 4168 (2016).
33. H. Yuliani, R. D. Mayasari, E. Kalembang, Y. Deni, D. R. Santi, P. W. Pangestika, S. Purwanto, B. Sugeng and M. Setyadji, "Analysis of structure and antimicrobial activity of CeO_2 and Nd_2O_3 nanoparticles," *Spektra: Jurnal Disika dan Aplikasinya* **4**, 105–112 (2019).
34. A. M. González, "Establecimiento de una curva de acidificación del yogur Zamorano," MSc.Thesis, Escuela Agrícola Panamericana Zamorano, Honduras, 2006.
35. A. León, K. Motato, D. Granda, O. I. Montoya, S. Echeverri, L. Quinchía, C. Rodríguez, J. Lopera, A. Caro, M. Restrepo and J. Valencia, "Programa cubano de pesquisaje auditivo," *CENIC: Ciencias Biológicas* **36**, 0253-5688 (2005).
36. M. C. Durán, C. Romero, P. García, M. Brenes and A. Garrido, "Lactic acid bacteria in table olive fermentations", *Grasas y aceites* **48**, 297–311 (1997).

37. M. J. Loja, "Determinación de la cinética de fermentación láctica de hojas frescas de col (*Brassica oleracea* l. var. *capitata* l.) en condiciones óptimas de temperatura, tiempo y concentración de NaCl," MSc. thesis, Universidad de Cuenca, 2019.
38. E. A. Silveira and R. Franco. "Estratégias utilizadas no combate a resistência bacteriana," *Revista electrónica de Veterinaria* **2**, 1–37 (2006).
39. A.F. Villa, A.P. Meléndez, J.E. Carulla, M.L. Pabón, M.L. and E.A. Cárdenas, "Study of microbiological and nutritional quality of corn silage in two Colombian ecosystems," *Revista colombiana de ciencias pecuarias* **23**, 65–77 (2010).
40. J.F. Cubero, A. Rojas and R. Wing-Ching, "Uso del inóculo microbial elaborado en finca en ensilaje de maíz (Zea mays) valor nutricional y fermentativo," *Agronomía Costarricense* **34**, 237–250 (2010).
41. J. Clark and F. Deswarte, *Introduction to Chemicals from Biomass* (Wiley Series, Singapore, 2015).
42. L. M. Borras, C. E. Rodríguez and A. Rodríguez, "Evaluation of calcium carbonate (CaCO3) inclusion in solid-state kinetic fermentation of Solanum tuberosum postharvest waste", *Revista colombiana de ciencias hortícolas* **11**, 143–150 (2017).
43. G. I. Puerta, "Fundamentos del proceso de fermentación en el beneficio del café," Programa de Investigación Científica, Fondo Nacional del Café, 2010).
44. L. Ortiz, L. Graziani, and R. Gervaise, "Influence of various factors on characteristics of fermented cocoa beans and sun-dried," *Agronomía tropica* **l59**, 19–127 (2009).
45. K. Broderick, R. Lusk, J. Hinderer, J. Griswold, R. Boll, M. Garland, L. Heilbronn and S. Mirzadeh, "Reactor production of promethium-147," *Applied Radiation and Isotopes* **144**, 54–63 (2019).
46. M. McWilliams, *Foods: Experimental Perspectives*. 4th ed (Prentice-Hall Inc, New Jersey, 2001).
47. J. Amiot, *Cienciay Tecnología de La Leche* (Editorial Acribia, Zaragoza, 1991)
48. M. A. Nascarella, M.A. and E.J. Calabrese, "Hazzard assessment and the evaluation of rare earth element dose-response relationships", in *Rare earth elements in human and environmental health*, edited by G. Pagano (Pan Stanford Publishing, Danvers, Massachusetts, 2017), pp. 183–194.
49. M.A. Coronel, "Estudio de las características fisicoquímicas y sensoriales de yogurt enriquecido con quinua," Ph.D. Thesis, Universidad de Extremadura, Spain, 2018.
50. A. Von Wright and L. Axelsson, "Lactic Acid Bacteria: An Introduction," in *Lactic Acid Bacteria: Microbiological and Functional Aspects*, edited by S. Lahtinen, A.C. Ouwenhand, S. Salminen and A. Von Wright (CRC Press, Taylor and Francis Group, 2012), pp 17–39 .
51. S. Cui, J. Zhao, H. Zhang and W. Chen, "High-density culture of Lactobacillus plantarum coupled with a lactic acid removal system with anion-exchange resins," *Biochemical Engineering Journal* **115**, 80–84 (2016).
52. C. H. Evans, *Biochemistry of The Lanthanides* (Plenum Publishing Corporation, New York, 1990).
53. J. A. Cotruvo, "The chemistry of lanthanides in biology: recent discoveries, emerging principles, and technological applications," *ACS Central Science* **5**, 1496–1506 (2019).
54. S. C. Edington, A. González, T. R. Middendorf, D. B. Halling, E. W. Aldrich and C. R. Baiz, Coordination to lanthanide ions distorts binding site conformation in calmodulin," in *Proceedings of the National Academy of Sciences* (2018), pp 3126–3134 .
55. A. Bouguettoucha, B. Balannec and A. Amrane, "Unstructured models for lactic acid fermentation–A review", *Food Technology Biotechnol* **49**, 3–12 (2011).
56. L. Serna, A. Rodríguez and F. Alban, "Ácido poliláctico (PLA): Propiedades y aplicaciones", *Ingeniería y Competitividad* **5**, 16–20 (2003).
57. X. Montané, J. M. Montornes, A. Nogalska, M. Olkiewicz, M. Giamberini, R. Garica-Valls, M. B. Fabregat, I. Jubany, and B. Tylkowski, "Synthesis and synthetic mechanism of Polylactic acid," *Physical Sciences Reviews* **5**, 1–15 (2020)
58. M. Othman, A.B. Ariff, L. Ríos-Solís and M. Halim, "Extractive fermentation of lactic acid in lactic acid bacteria cultivation: a review," *Microbiol* **20**, 2285 (2017).
59. M. Patel, A.S. Bassi, J.J.X. Zhu and H. Gomaa, "Investigation of a dual-particle liquid-solid circulating fluidized bed bioreactor for extractive fermentation of lactic acid," *Biotechnol. Prog* **24**, 821–831 (2008).