

**OPTIMISATION OF CARBON SOURCES IN
FERMENTATION MEDIUM FOR THE PRODUCTION OF
 β -GALACTOSIDASE FROM
LIMOSILACTOBACILLUS FERMENTUM LF08**

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INTRODUCTION

- The β -Galactosidase enzyme can be found in wide variety of sources, including plants, animals and **microorganisms**
- **Industrial sources** are usually obtained from *Aspergillus* sp. and *Kluyveromyces lactis*
- There is a **big diversity** in the **functional properties** of the enzyme **between different sources, even different strains**
- Of high importance is the enzyme produced from **probiotic bacteria** because of **Generally Recognized as Safe (GRAS)** status
- Produced **intracellularly** in the bacteria
- *Limosilactobacillus fermentum* **LF08**

INTRODUCTION AND AIM

- **Industrial application**
 - Production of **lactose-free milk products**
 - **Removing of lactose in the whey** (treatment of whey)
 - Production of **prebiotic galacto-oligosaccharides (GOS)**
- The **medium optimisation** is one of the **crucial aspects** of the **fermentation technologies**, especially in the industrial and biotechnology applications
- It has various **advantageous effects**, such as: **increased productivity, improved strain performance, importance in the scaling up process, reduced fermentation time, cost effectiveness**
- **The objective of our study is to follow how different modifications of the carbon source in the medium can influence the production of the β -Galactosidase**

MATERIALS AND METHODS

PROBIOTIC BACTERIA

Isolate of *Limosilactobacillus fermentum* LF08 obtained from Probiotical S.p.A.

ENZYME FERMENTATION

- Fermentation time 16 hours
- De Man, Rogosa and Sharpe medium
- Optimised with different carbon sources (Glucose, Lactose, Galactose)

CELL LYSIS

CTAB (cetyl-trimetyl-ammonium bromide) lysis buffer

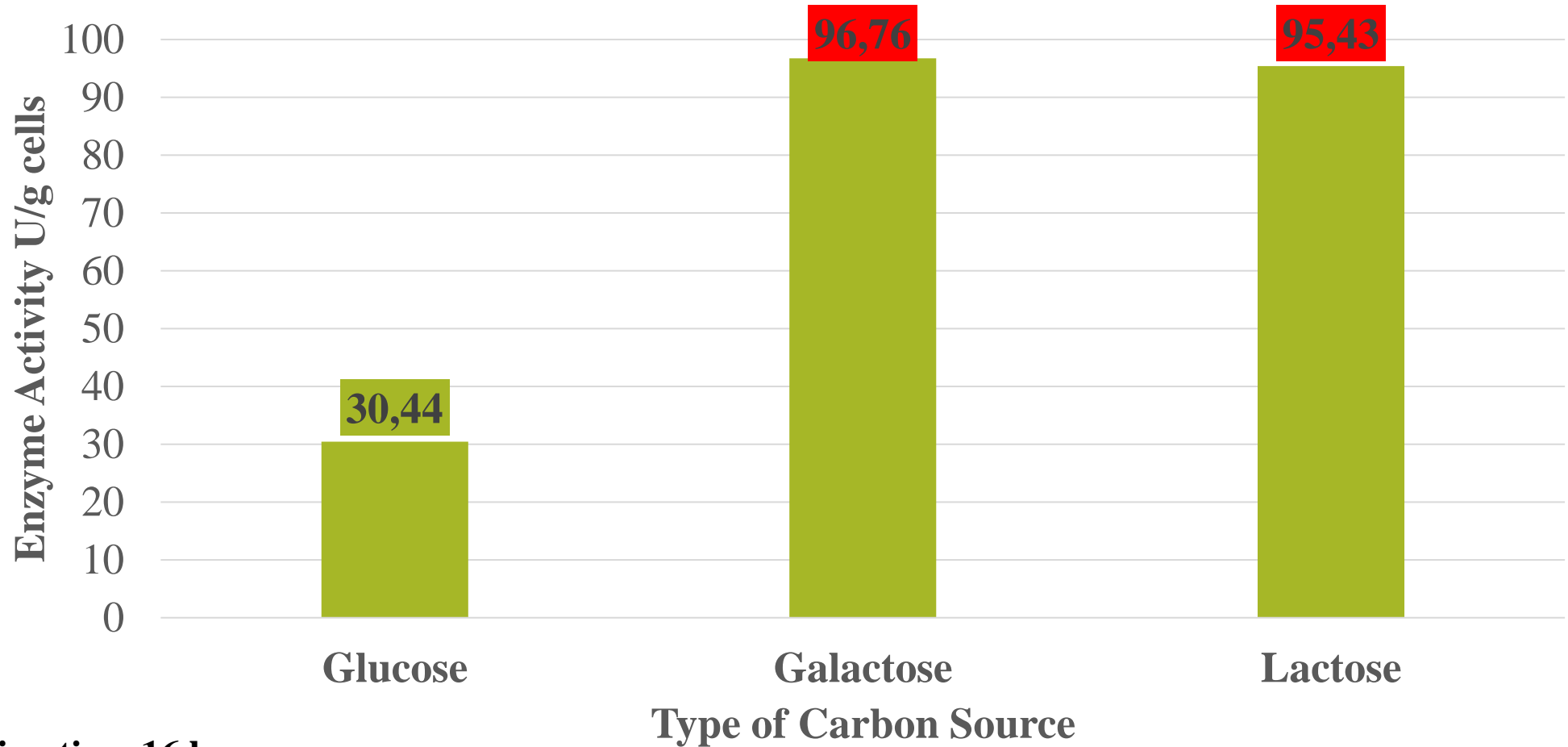
DETERMINATION OF ENZYME ACTIVITY

- Optimal conditions:
 - pH 6.5 (Sorensen buffer)
 - Temperature 50°C
- Na₂CO₃ for stopping enzyme reaction
- 4-nitrophenyl β-D-galactopyranoside substrate



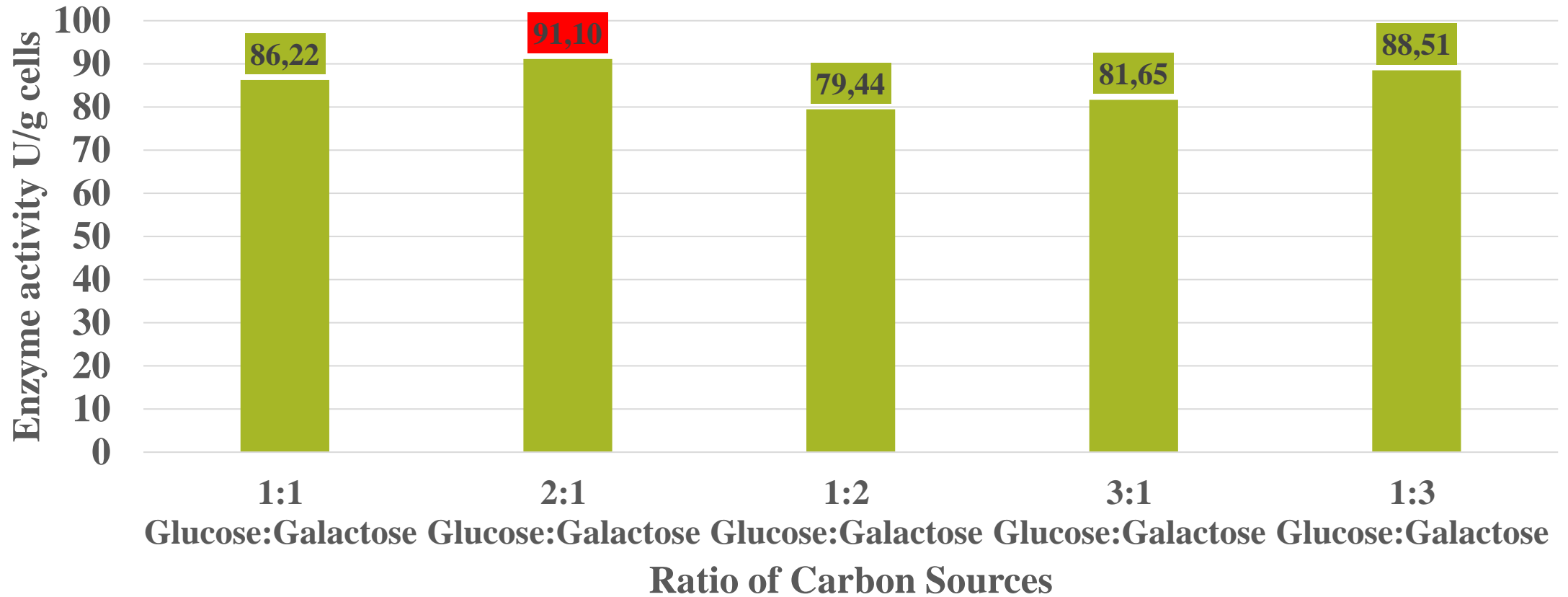
RESULTS

Effect of Different Carbon Source



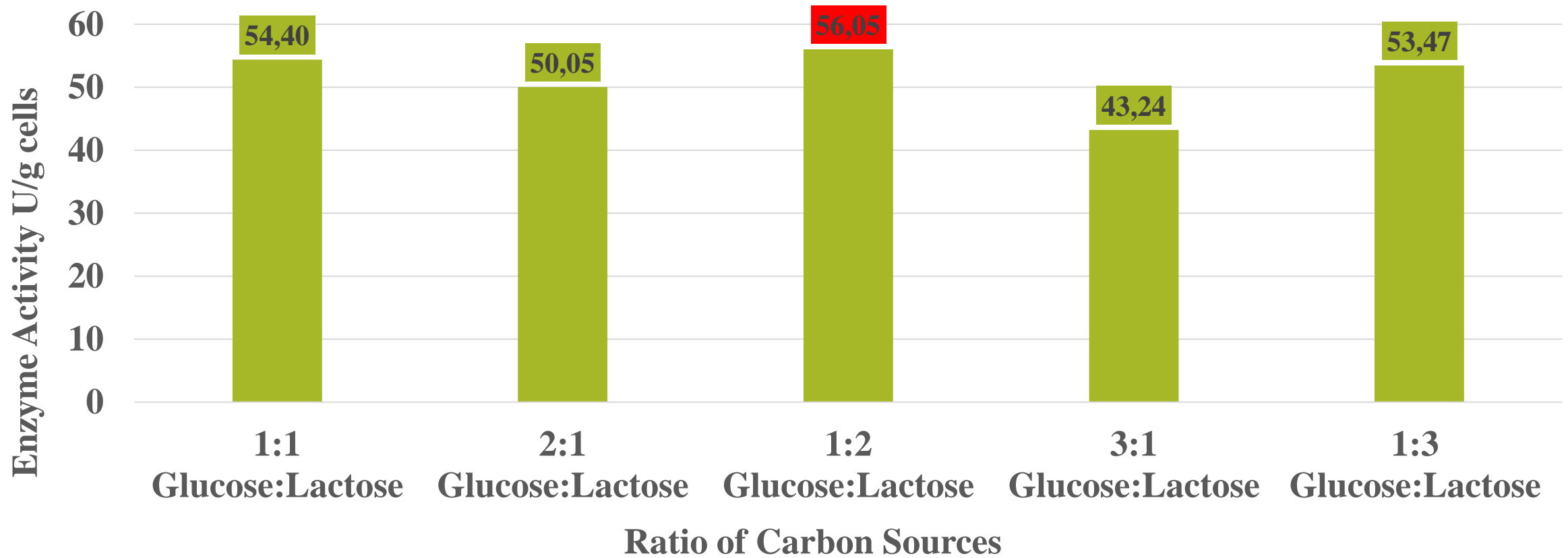
- Fermentation time 16 hours
- Inoculum size 1%
- Maximum concentration of sugar 1%

Effect of the Ratio of Different Carbon Sources Glucose and Galactose



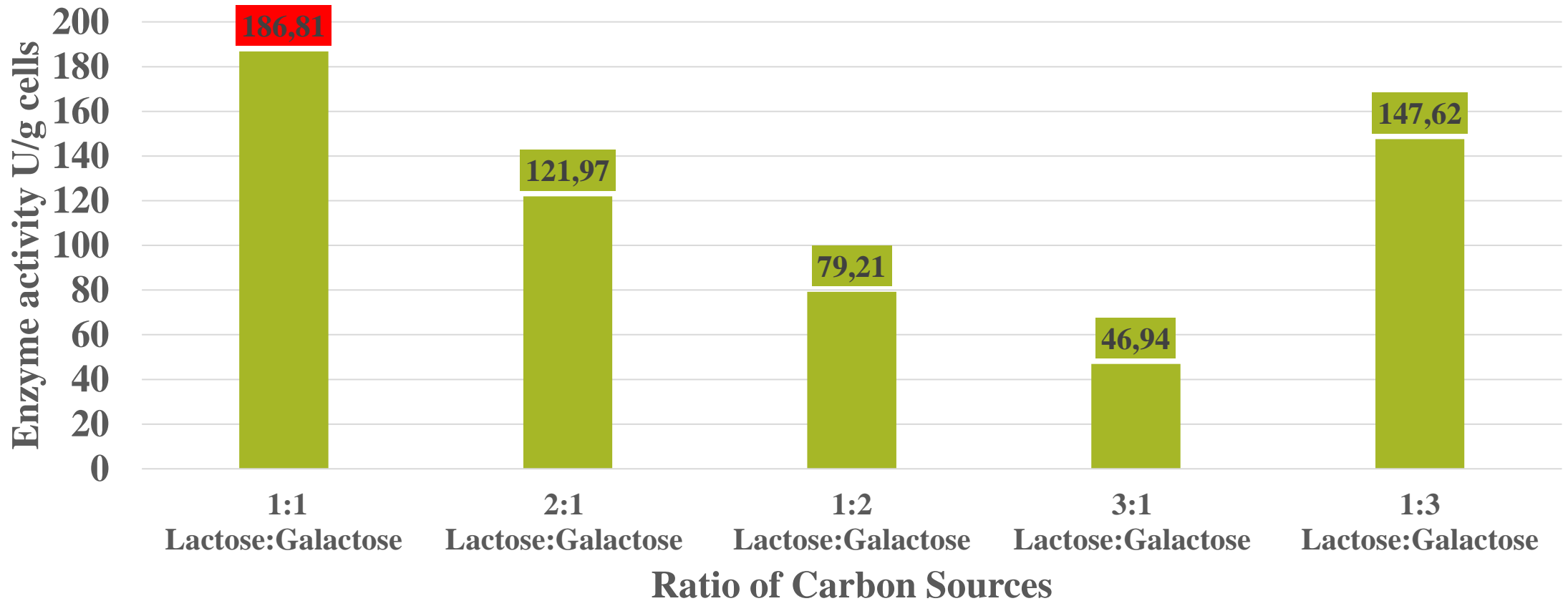
- Fermentation time 16 hours
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Effect of the Ratio of Different Carbon Sources Glucose and Lactose



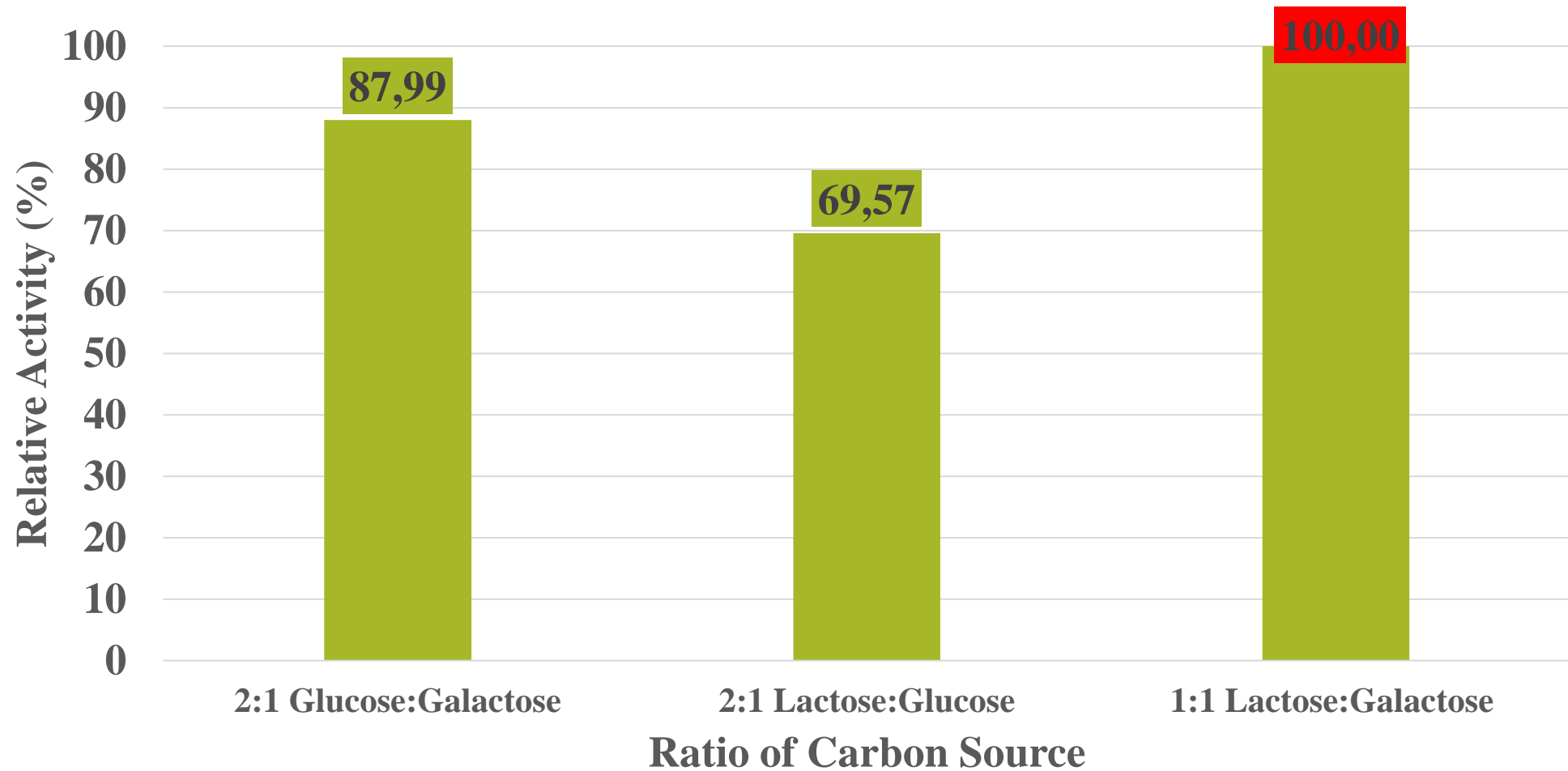
- Fermentation time 16 hours
- Inoculum size 1%
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Effect of the Ratio of Different Carbon Sources Lactose and Galactose



- Fermentation time 16 hours
- Inoculum size 1%
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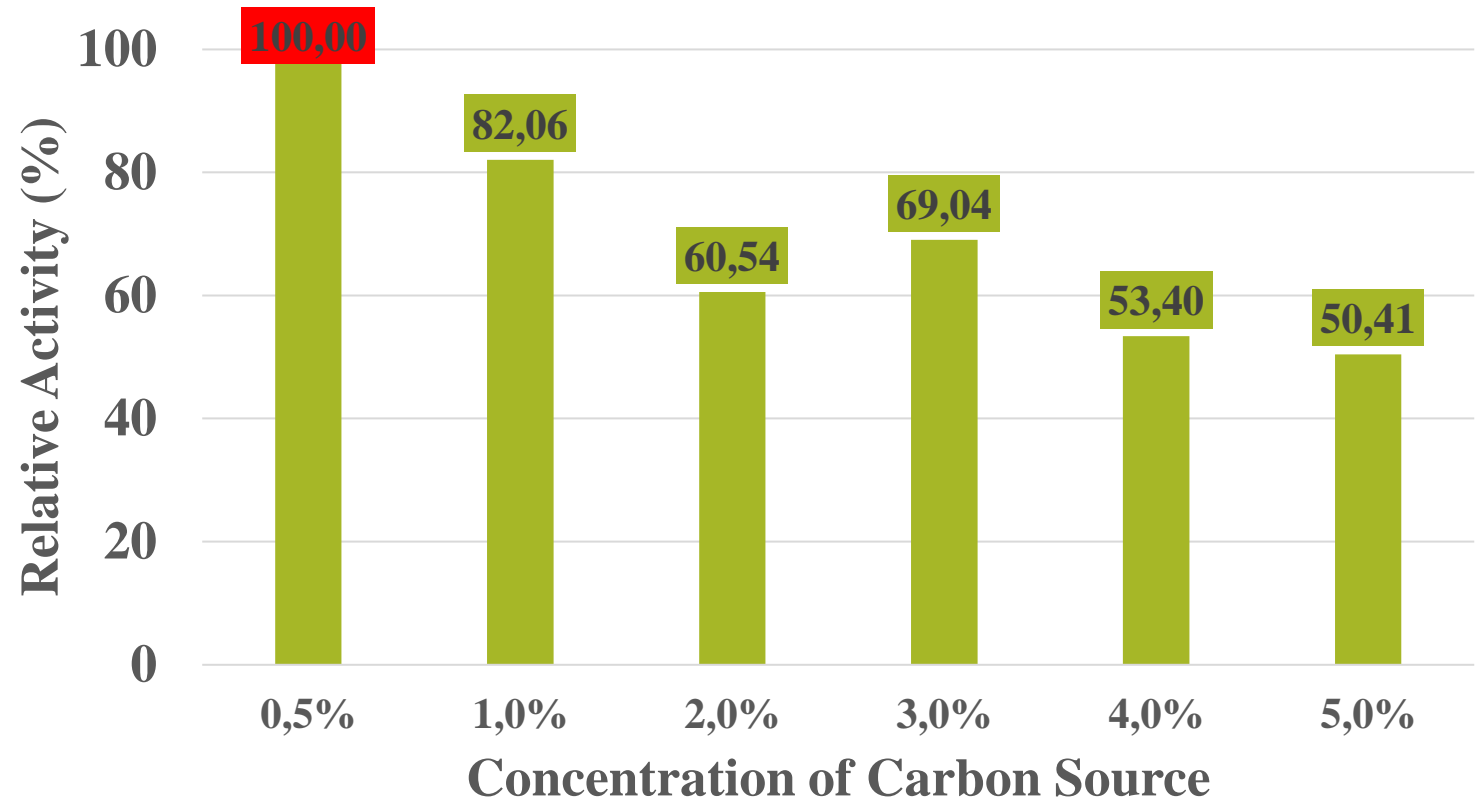
Effect of the Ratio of Different Carbon Sources



- **Fermentation time 16 hours**
- **Inoculum size 1%**
- **Maximum concentration of sugar 1%**

Effect of Increased Concentration of Carbon Source 1:1 ratio of Lactose and Galactose

- Similar results were observed in previous experiments when the activity of β -Galactosidase was tested from *L. casei* 01 and *L. acidophilus* La-5
- In case of the *L. acidophilus* La-5 it was determined that further increasing of the concentration of carbon source (lactose) above 0,5% did not result in significant increase of the enzyme activity



- **Fermentation time 16 hours**
- **Inoculum size 1%**

CONCLUSION

- The **importance** of the effect of the **carbon sources** supplemented in the medium is well documented in wide variety of research
- The **effect of the carbon source** on the production of β -Galactosidase **may differ between different strains**
- Our goal was to study how it affects the **biosynthesis of the enzyme from *Limosilactobacillus fermentum* LF08**
- Our studies confirm that there is a **connection between the chosen type of the carbon source and final obtained enzyme activity**
- Among the different tested ratios, it was determined that ratio of **2:1 Glucose:Galactose, 2:1 Lactose:Glucose and 1:1 Lactose:Galactose** showed the best effect, from which more detailed emphasis was given on the combination between lactose and galactose
- Performed experiments also suggest that **concentration of the carbon source** is also relevant for the enzyme production
- We observed that **maximal concentration of 0,5%** (mixture of lactose and galactose) was most suitable
- Our experiment will furthermore continue with **following the effect of the supplemented sugar, while also considering the optimisation of the fermentation time**



THANK YOU FOR YOUR ATTENTION