



Biotechnology beyond the stars: how *Aspergillus niger* forms the industry of the future



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Introduction

Fungi were found on Mir space station. By improving the robustness of these fungi, substances could be produced in space, making future missions more sustainable.

Citric acid is produced by the fermentation of *Aspergillus niger*.



Developing an *Aspergillus niger* mutant that can produce high concentration of citric acid even in a Mn^{2+} added environment is very important. **CexA** is a **gene** which codes a transporter protein responsible for getting out citric acid into the medium. A *cexA* overproducing mutant created by the Vienna University of Technology was tested by us.

During citric acid production, keeping the concentration of Mn^{2+} under $5 \mu g/L$ in the reactor, 90% specific molar yield can be achieved. However the Mn^{2+} content of stainless steel parts of bioreactors is more than 2% and during corrosion effects, Mn^{2+} leaches out into the medium.



Aims

- Discovering the leaching of Mn^{2+} induced by **low pH** and hot temperature during **sterilization**
- Understanding the effect of Mn^{2+} on citric acid production at **different times** during the fermentation
- Determining the fermentation yield of the **cE cexA mutant** strain

Materials and methods

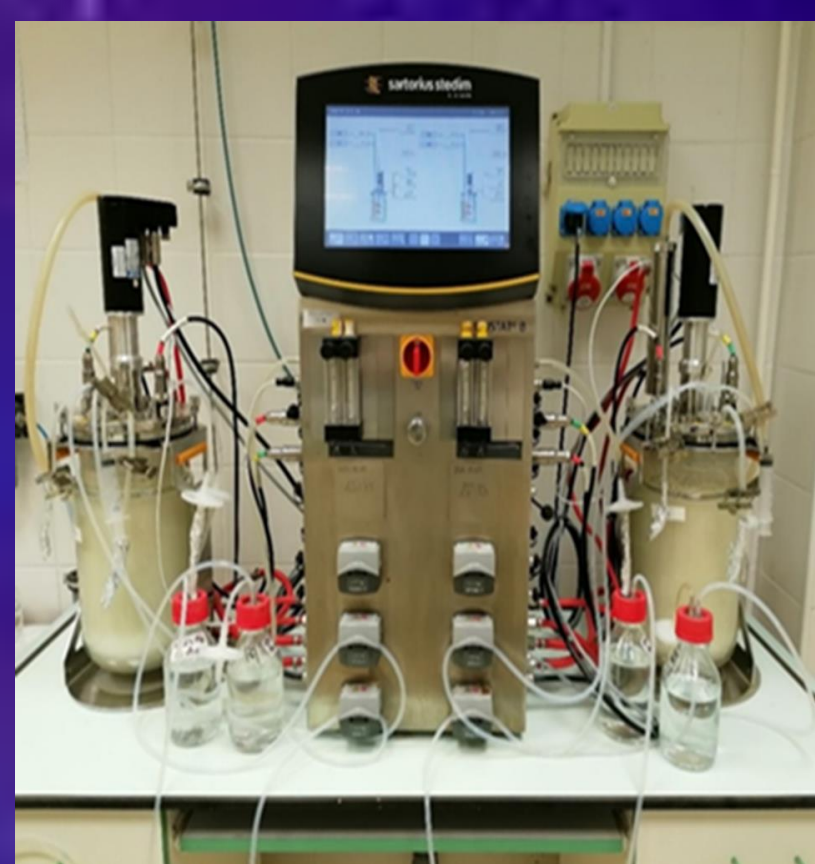
Three *Aspergillus niger* citric acid producing strains:

- NRRL 2270
- ATCC 1015
- cE cexA



D-glucose and citric acid
HPLC

Mn^{2+}
ICP-QMS



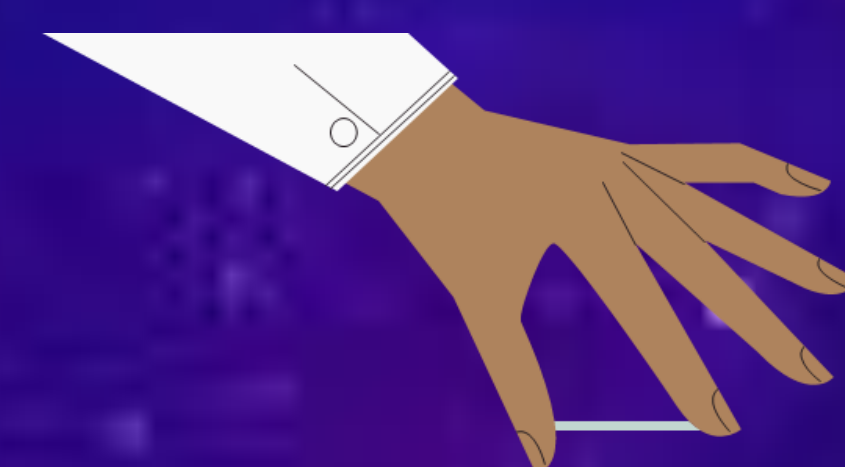
A bioreactor



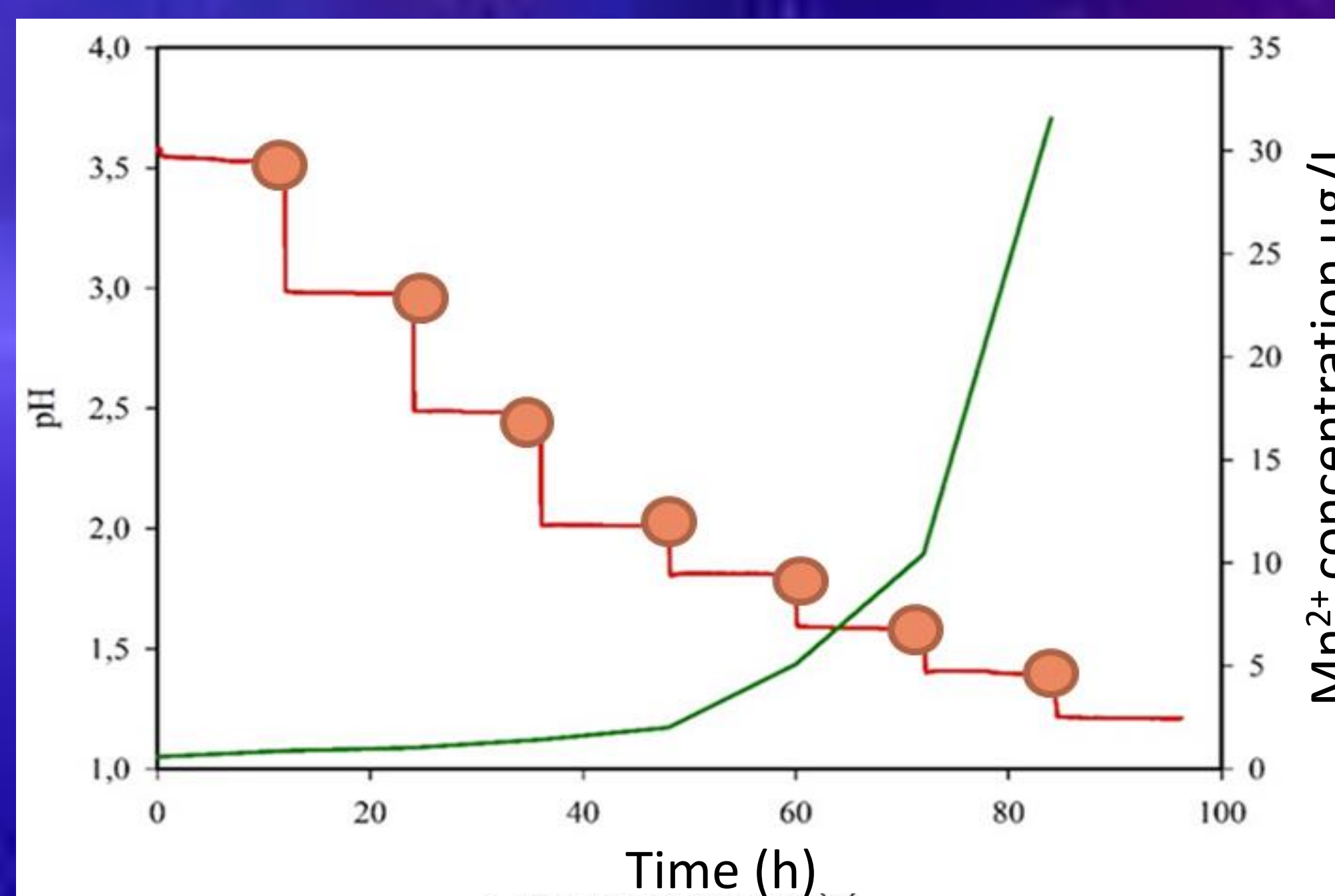
B bioreactor

Results

The leaching of Mn^{2+} in the older bioreactor (B) induced by hot temperature during sterilization was more significant.



	Before sterilization	After sterilization	
		30 min	60 min
Bioreactor A	2.05 ± 0.10	2.90 ± 0.5	2.95 ± 0.4
Bioreactor B	2.01 ± 0.18	20.30 ± 2.48	25.98 ± 3.02



The decrease of pH affects the leaching of Mn^{2+} in a significant way.

Mn^{2+} addition (h)	$5 \mu g/L$ $Y_{p/s}$	$30 \mu g/L$ $Y_{p/s}$	$100 \mu g/L$ $Y_{p/s}$
0	0.73 ± 0.05	0.66 ± 0.04	0.35 ± 0.02
6	0.71 ± 0.04	0.63 ± 0.03	0.39 ± 0.04
24	0.72 ± 0.06	0.64 ± 0.03	0.44 ± 0.04
72	0.83 ± 0.05	0.77 ± 0.02	0.71 ± 0.04
172	0.88 ± 0.04	0.89 ± 0.02	0.87 ± 0.04

The presence of Mn^{2+} is critical at the early stage of fermentation

Conclusion

We investigated the leaching of Mn^{2+} caused by low pH and sterilization. We introduced the use of empty steam sterilization as a solution to Mn^{2+} leaching during sterilization. We also discovered that the presence of Mn^{2+} is critical at the early stage of the fermentation. We determined a direct connection between Mn^{2+} concentration and *cexA* gene expression. If we could map the interactions of the genes in *Aspergillus niger* even more thoroughly, we could create an even more robust fungus that could be used in space exploration.

References

- Cortês et al. (2020), Fungal Biotechnology in Space: Why and How? In Grand Challenges in Biology and Biotechnology (p.: 501–535).
- Fekete et al. (2022), Bioreactor as the root cause of the “manganese effect” during *Aspergillus niger* citric acid fermentations. Frontiers in Bioengineering and Biotechnology, (10) 935902.



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	ATCC 1015 100 mg/L Mn^{2+}	cE cexA 100 mg/L Mn^{2+}
Initial volume	5 L	
pH	Initial pH = 2.5 not controlled	Initial pH = 2.5 not controlled
Molar yield	1.3%	25.8%
Final concentration (g/L)	1.51	36.83
DCW (g/L)	39.14	37.66

Comparing the fermentation in Mn^{2+} added environment, the cE cexA mutant strain produced much more citric acid.