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Accelerated storage tests for fast product registration?

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Fermentation and formulation technology

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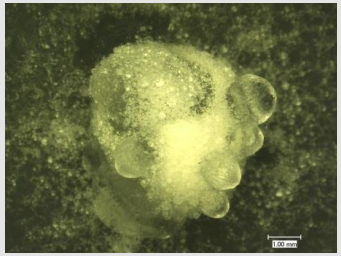
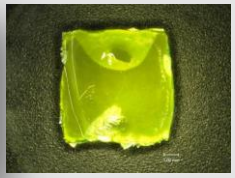
Main research focus: systematic development of materials, methods and production processes to prepare novel formulations (IP available)





- ATTRACT -"Attract & kill" capsules
- Formulation of plant extracts
- Fermentation and formulation of endophytes
- Biotechnology of endophytes
- INBIOSOIL - Innovative biological products for soil pest control
- Co-immobilization of chemo- and biocatalysts
- Novel bioinks for bioprinting
- Immobilization of hydrogen-producing *C. reinhardtii* and light harvesting complex in novel silica gels
- Towards bioactive nano-hybrid membrane systems for efficient and stable photon energy transfer
- Immobilization of Co-nanoparticles in conductive gels

Registration
of biological control agents

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- Microorganisms used for control of pests are subject to registration as a „plant protection product"
- Registration is certainly **the largest barrier** for commercialization of biopesticides

Reasons for these problems are [1]:

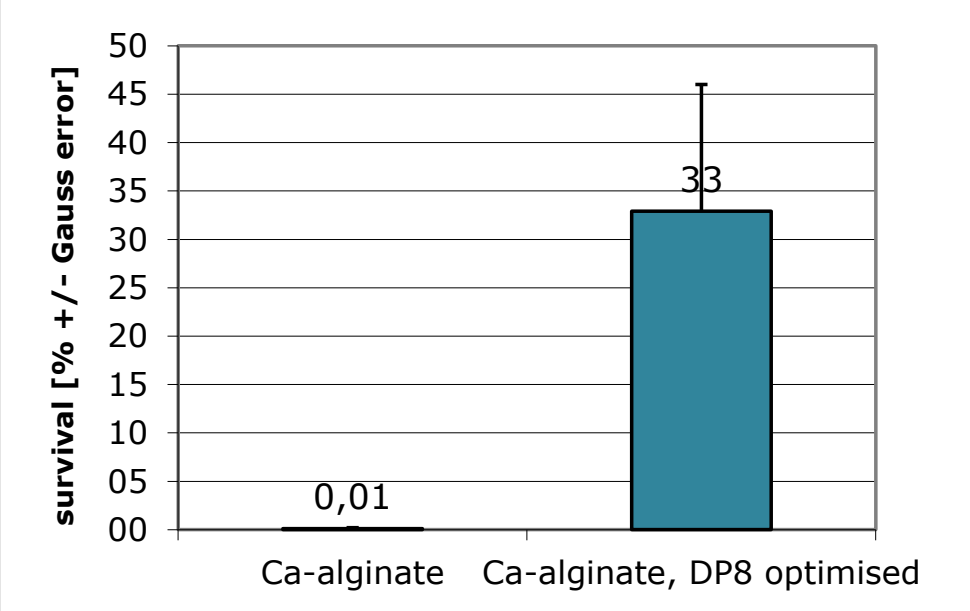
- Requirements are often adapted from requirements for chemicals which are not appropriate for microorganisms
- End-points of risk assessments are not clearly established which allow differences in interpretation and often leads to more data being required
- Procedures are lengthy, non-transparent and costly
- **Evidence of stability and shelf life is required, ideally after drying**

[1] Ravensberg W.J. A Roadmap to the successful development and commercialization of microbial pest control products for control of arthropods, 2010, Springer Verlag

Drying of encapsulated *Pseudomonas fluorescens*
relevant parameters

Influence of…..

- culture age
- osmotic stress
- drying time
- bead material
- drying protectants →
- yeast
- gelatin
- rehydration
- atmospheric conditions during drying
- …



…..on survival of cells.

→ The survival of cells could not be enhanced above 30 %

Accelerated storage test
with encapsulated *P. fluorescens*

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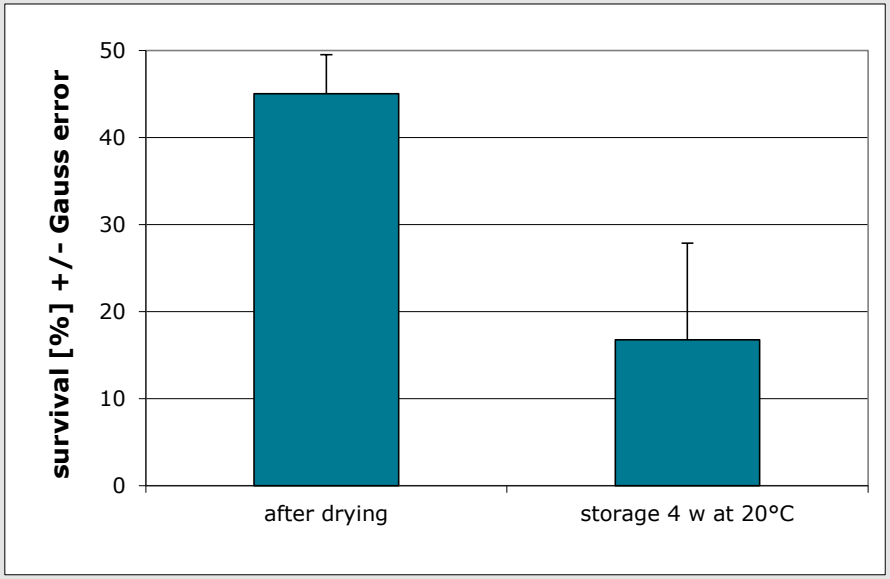
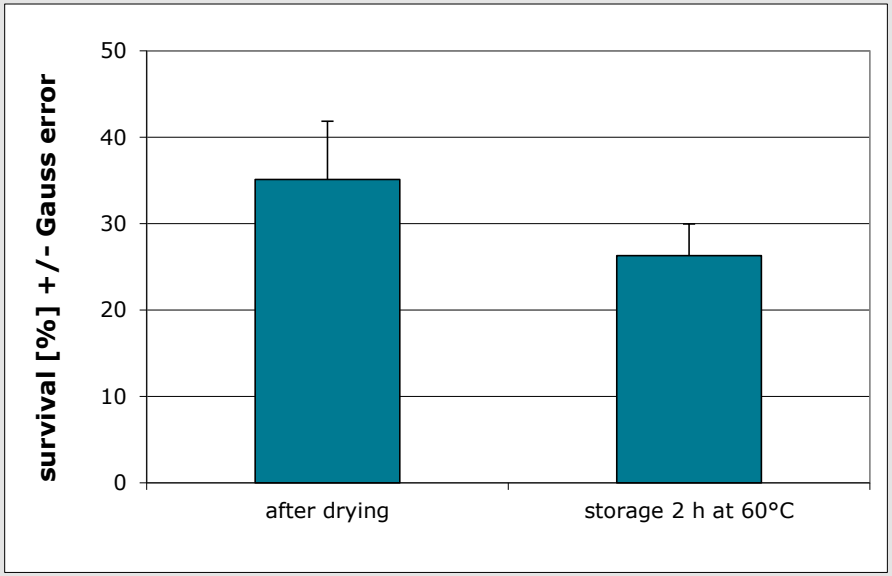
Very quick estimate of shelf life: "thermostability test"

after 4 weeks of storage at 20°C



accelerated storage test (2 h at 60°C)



For a fast estimation of shelf life, incubate formulation 2 h at 60°C?

Accelerated storage test
Basic idea

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- Quick and objective determination of storage stability
- Samples are stored at three or more different temperatures for hours or few days
- Arrhenius relationship obtained permits predicting the rate of death at **any** storage temperature and time

loss of cells N during storage follows:

$$\log N = \log N_0 - k*t$$

where k = f(1/*T*) specific rate of degradation according to Arrhenius equation:

$$\log k = -(\Delta H_a/2.303*R)*1/T$$

ΔH_a : „heat of inactivation“ [J/mole] R : universal gas constant

- Other models: WLF, Eyring-Polanyi, WeLL; exponential model (Peleg, 2012)

Accelerated storage test

State of the art

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microorganism		reference	Comparison estimated/experimental	Survival/Proliferation
Plant viruses		Yordanova, A. et al. (2000)	+	
Archaebacteria		Sakane, T. et al. (1992) [2]	+	
Lactobacillus	*brevis*	Desmons, S. et al. (1998)	+	
		Tsen J-H. et al. (2007)	+	
	acidophilus	Mitic, S. et al. (1974)	+	Survival
		King, V. A. et al. (1998)	+	
	bifidus	Damjanivic V. et al. (1986)	-	
Lactococcus spp.		Achour, M. et al. (2001)	-	
Pseudomonas spp.		Kim, W. et al. (2012)	-	
		Bruckner S. et al. (2013)	+	Proliferation
		Cardenas F. C. et al. (2008)	-	

- At times accelerated storage tests without real storage tests in comparison
- Actual - prognosis for microorganisms in dairy products or contaminations in meat
- For meat *Gombertz model*, modified Arrhenius kinetic

Accelerated storage test
Examples

Example I – Desmons 1998 [4] Storage of *Lactobacillus brevis*
Comparison of survival

Time of storage (4°C)	Survival estimated by accelerated storage test [%]	Survival experimentally measured [%]
50 days	58	59
137 days	23	16

Example II – Tsen 2007 [5] Storage of immobilized *Lactobacillus acidophilus*
Comparison of degradation rate k (h^{-1}) values

	Temperature (°C)	Free cell	Immobilized cell	
			Ca-alginate	κ-Carrageenan
Predicted value	4	0.0085	0.0019	0.0030
	25	0.527	0.0176	0.0252
Experimental value	4	0.0082	0.0018	0.0029
	25	0.0531	0.0179	0.0254

Accelerated storage test
with encapsulated *P. fluorescens*

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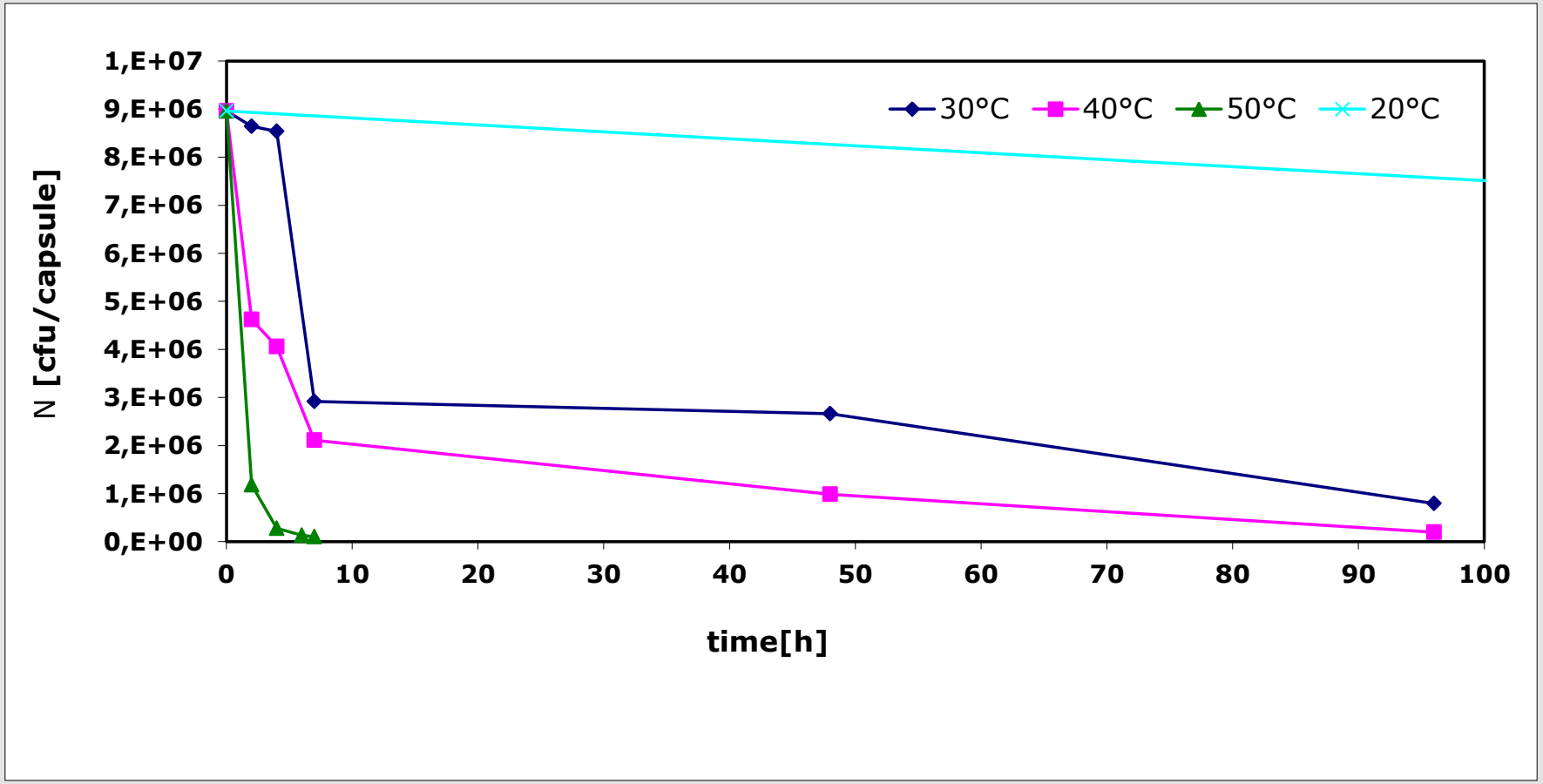
N as a function of time for different temperatures



Schönwandt et al. (in prep.) [13]

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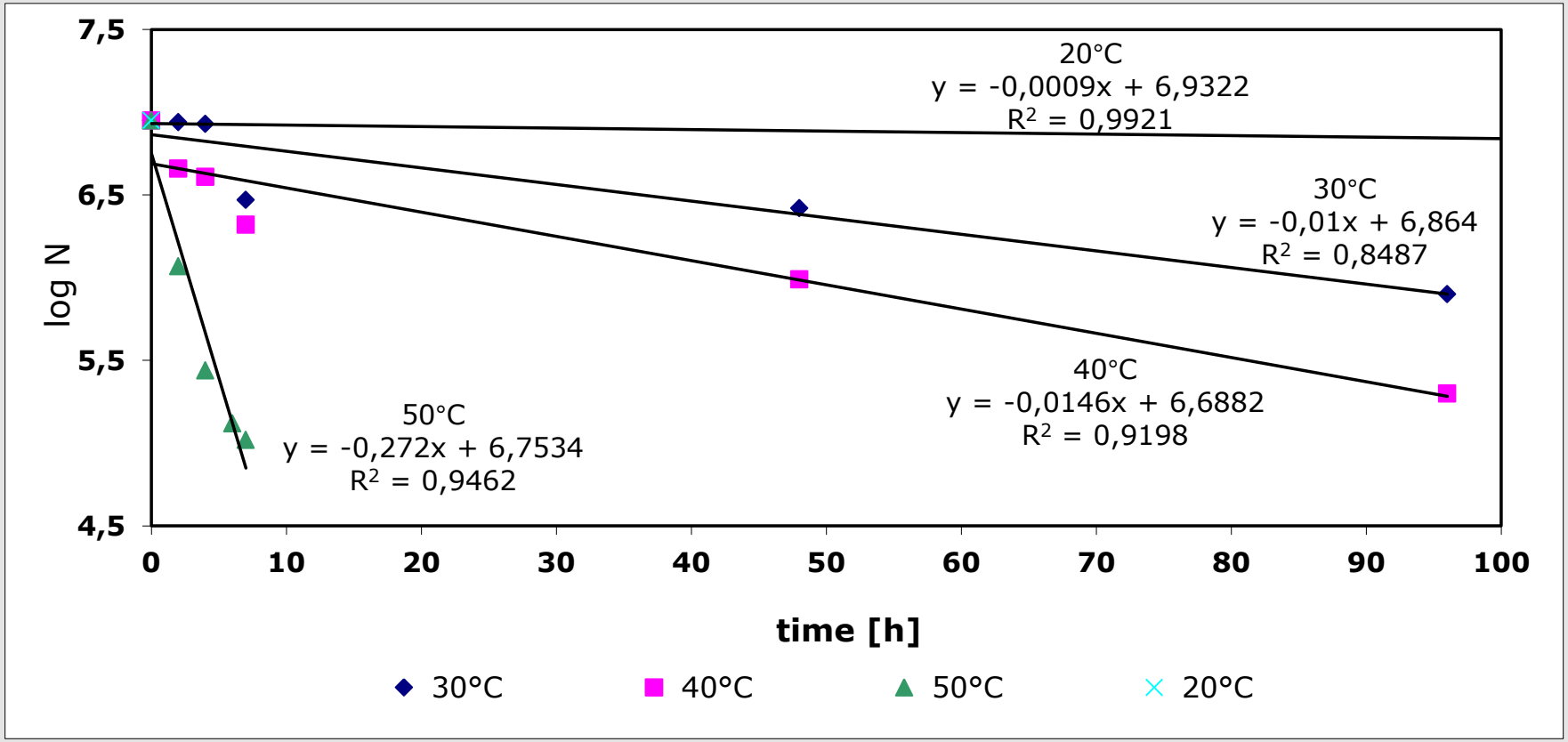
Plot of logN as function of time for different temperatures



slope of the curves = k values acc. to $\log N = \log N_0 - k*t$

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with encapsulated *P. fluorescens*

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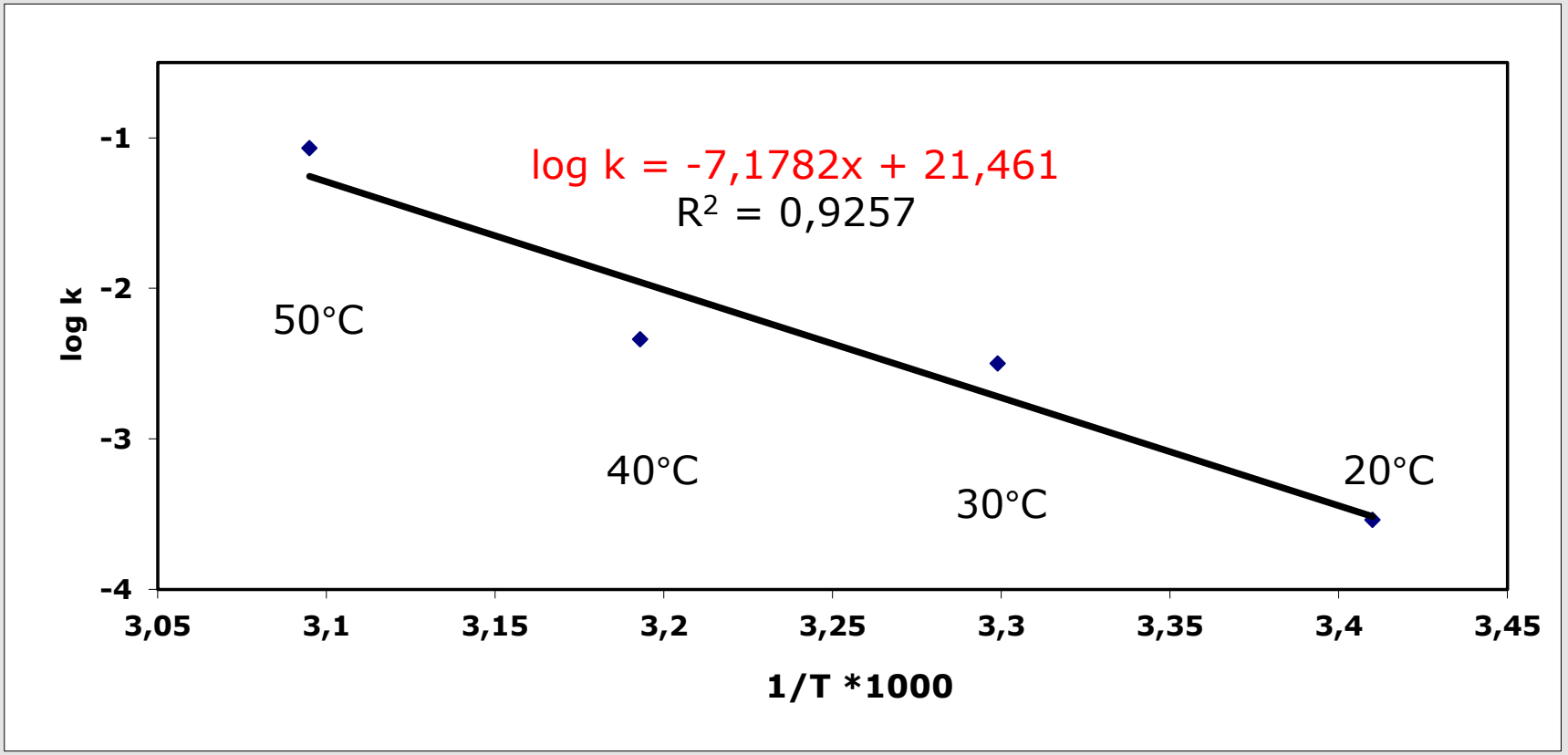
Plot of log k as function of *T* acc. to Arrhenius



Allows estimation of k values below 20°C

Place k function into $\log N = \log N_0 - k*t$

k_i : specific rate of degradation, t: time

Accelerated storage test
with encapsulated *P. fluorescens*

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Model for prognostication of cells alive after storage of formulation MF+PA5 at defined temperatures *T*

$$\log N = \log N_0 - 10^{-7{,}1782\,(1/T \times 1000) + 21{,}461} \times t$$

k_i : specific rate of degradation, T : Temperature

Model **verification**: Prognosticated and real cfu in formulation stored at **20°C**)

MF+PA5 capsules	cfu / capsule	cfu / g capsules
N_0 (Cfu at t=0 h)	$8{,}96*10^6$	$4{,}19*10^{10}$
2 weeks storage	$3{,}66*10^6$	$1{,}71*10^{10}$
2 weeks (prognosticated)	$4{,}28*10^6$	$1{,}97*10^{10}$
4 weeks storage	$1{,}88*10^6$	$8{,}78*10^9$
4 weeks storage (prognosticated)	$1{,}78*10^6$	$8{,}22*10^9$

Storage time	temperature	cfu/capsule	cfu/g capsules
6 months	20°C	$5{,}52*10^2$	$2{,}58*10^6$
12 months	20°C	$3{,}00*10^{-2}$	$1{,}40*10^2$
6 months	4°C	$6{,}16*10^6$	$2{,}87*10^{10}$
12 months	4°C	$4{,}24*10^6$	$1{,}98*10^{10}$

storage of cells suspended in NaCl resulted in 75 % cell loss.

Accelerated storage test
Reproducibility

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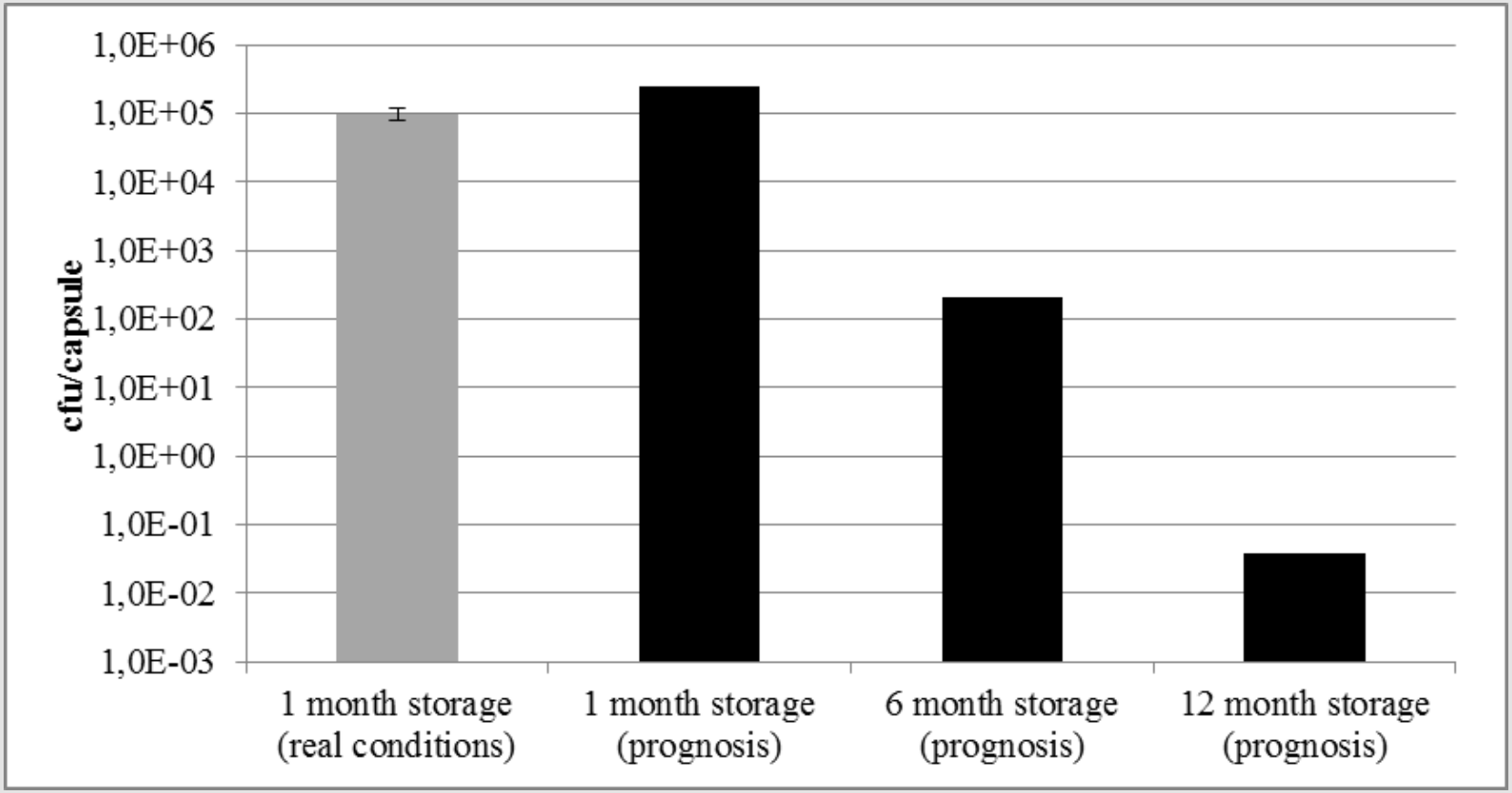
$$\log N = \log N_0 - 10^{-6{,}8557\ (1/T * 1000) + 20{,}31} xt$$




Modeling, Simulation and Optimization of Biological Processes

Dr. rer.-nat. Sabrina Proß, Prof. Dr. phil. Bernhard Bachmann

MODELICA

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Research & Development Focus:

- ✓ Object-oriented modeling and simulation using the hybrid Petri Net formalism
- ✓ Graphical (hierarchical) modeling and hybrid simulation and animation using Modelica
- ✓ Sensitivity analysis of hybrid dynamical systems
- ✓ Model based process optimization and steering/control
- ✓ Successfully applied to biological systems

Conclusion

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Accelerated storage tests for fast biocontrol product registration?

Yes, but…

Proposition to IBMA:

- Fund our working team to develop accelerated storage protocols that stand up to registration authorities
- Either direct funding or within Horizon2020

Conclusion

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Open questions

- How to dry wet formulations for the test?
- How to pack formulations for the incubation?
- How many samples are needed? how many temperatures, what range, how long?

- What is „a mole of cells“?
- How can the Universal Gas Constant be linked to a multi-step degradation process in solids?
- Can the energy of inactivation really be temperature-*independent*?
- Can the unnecessary compression and inversion of the temperature scale be avoided?

- How accurate can and must the model be for registration?
- Differences between biocontrol microorganisms resp. products?
- How to measure shelf life (viability, pathogenicity, thermal markers?)
- Can this approach be used for volatile plant extracts?
- Can this approach be used to investigate the influence of drying protection on shelf life?

Thank you for your attention!

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