Behavior of *Bacillus cereus* under conditions simulating the proximal gut
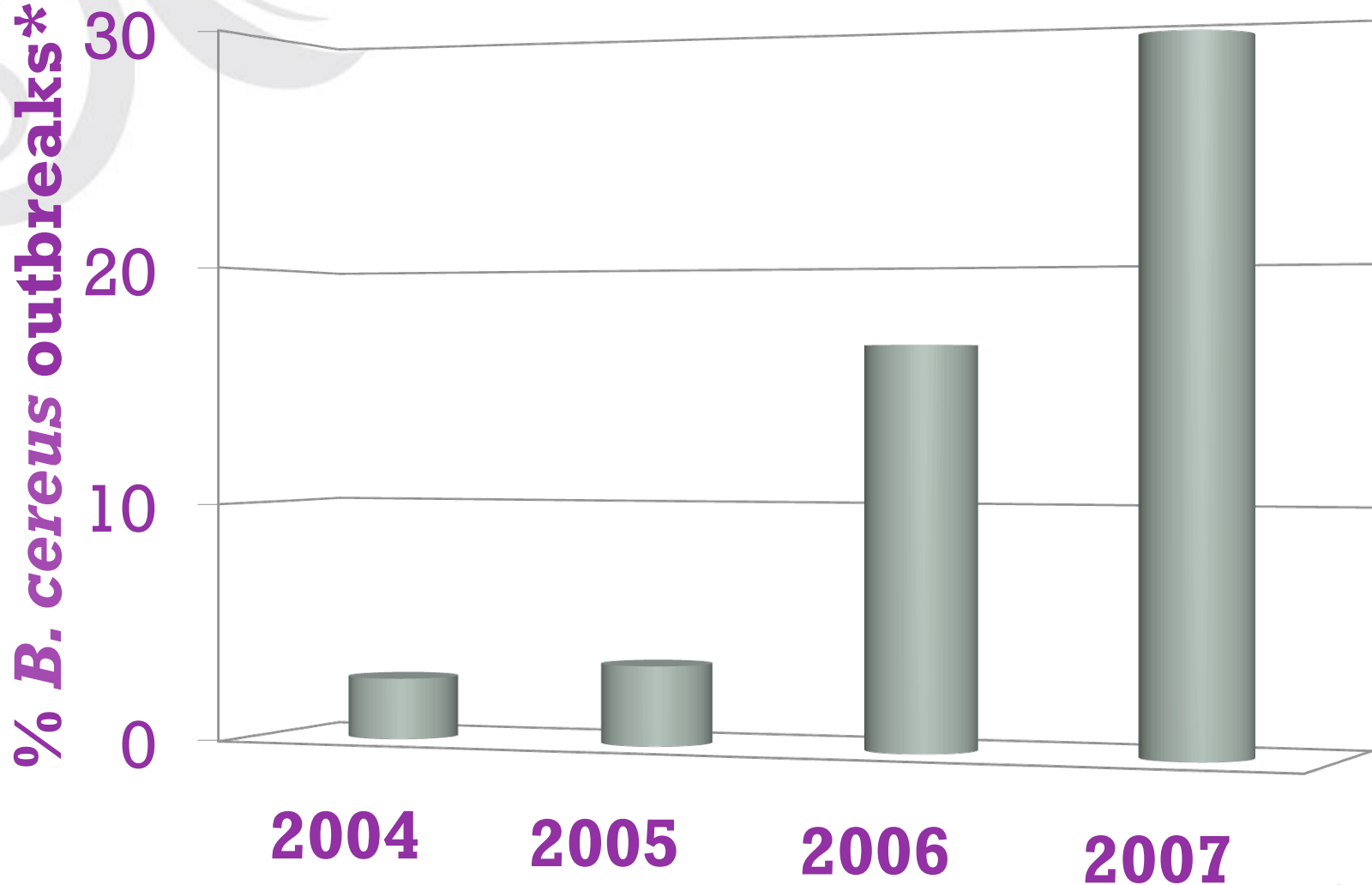
Faculteit Bio-ingenieurswetenschappen
Faculty of Bioscience Engineering

France
MARSEILLE
15–17 May 2013
INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION'S
EUROPEAN SYMPOSIUM ON FOOD SAFETY

M.Sc.Varvara TSILIA

Foodborne Outbreaks – Belgium

% *B. cereus* outbreaks*

30

20

10

0

2004   2005   2006   2007

FASFC, 2004-2007

Foodborne Outbreaks – France

'In France, from 1998 to 2000, *B. cereus* represented 4 to 5 % of foodborne poisoning outbreaks of known origin.'

efsa
European Food Safety Authority

'Strain 391-98 ... was responsible for an outbreak of <u>diarrhoeal syndrome</u> food poisoning in a nursing home for elderly people in <u>France in March 1998</u>. The strain was isolated at a level of 3×10^5 g^{-1} from a vegetable purée, and 44 people were ill. Six of the patients had bloody diarrhoea, and <u>three of them died</u>'.

Lund et al. (2000) Mol. Microbiol. 38

Disease Mechanism? - Diarrhea

Food Processing/Bad handling
B. cereus spores may survive
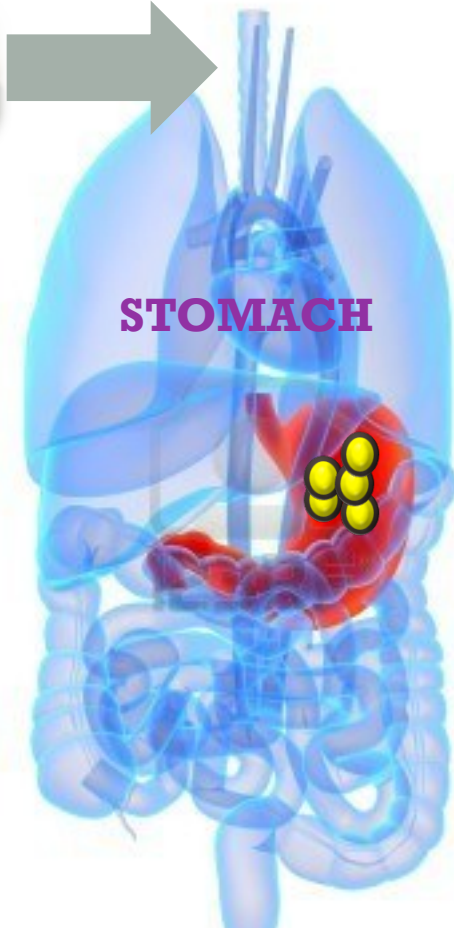Spore germination, growth and toxin

Spores ⬤ *Vegetative cells* ⬭ *Enterotoxins* ★

Disease Mechanism? - Diarrhea

STOMACH

STOMACH (HCl/Pepsin)
Vegetative cell death
Enterotoxin degradation

Spores ● *Vegetative cells* ▬ *Enterotoxins* ★

Disease Mechanism? - Diarrhea

INTESTINE

Duodenum (bile, pancreatin)
Spore survival

Ileum
Spore germination, growth and toxin
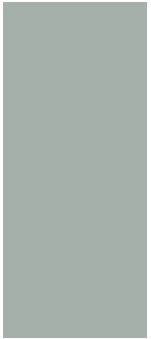
Spores   ◯   *Vegetative cells*   ▬   *Enterotoxins*   ★

Disease Mechanism? - Diarrhea
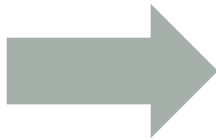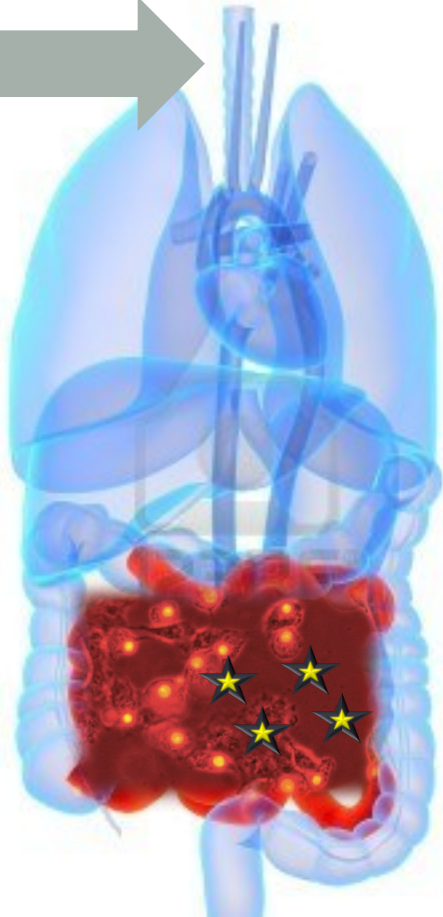
DIARRHOEA

Spores 🟡 *Vegetative cells* ⬭ *Enterotoxins* ⭐

+ Disease Mechanism? - Requirements

1. **Germination/Growth**   ☒   No germination/growth *(Ceuppens et al. 2012a)*

2. **Toxin production**   ☒   Quorum sensing *(Gohar et al. 2002; 2008)*

3. **Functional toxins**   ☒   Instability *(Ceuppens et al. 2012b)*

?. **Adhesion on mucus**   ☐?   Surface proteins *(Sanchez et al., 2009)*

Hypothesis:

Adhesion on mucus

- **protect *B. cereus* from the toxic intestinal slurry**

- **preserve/enhance the activity of enterotoxins**

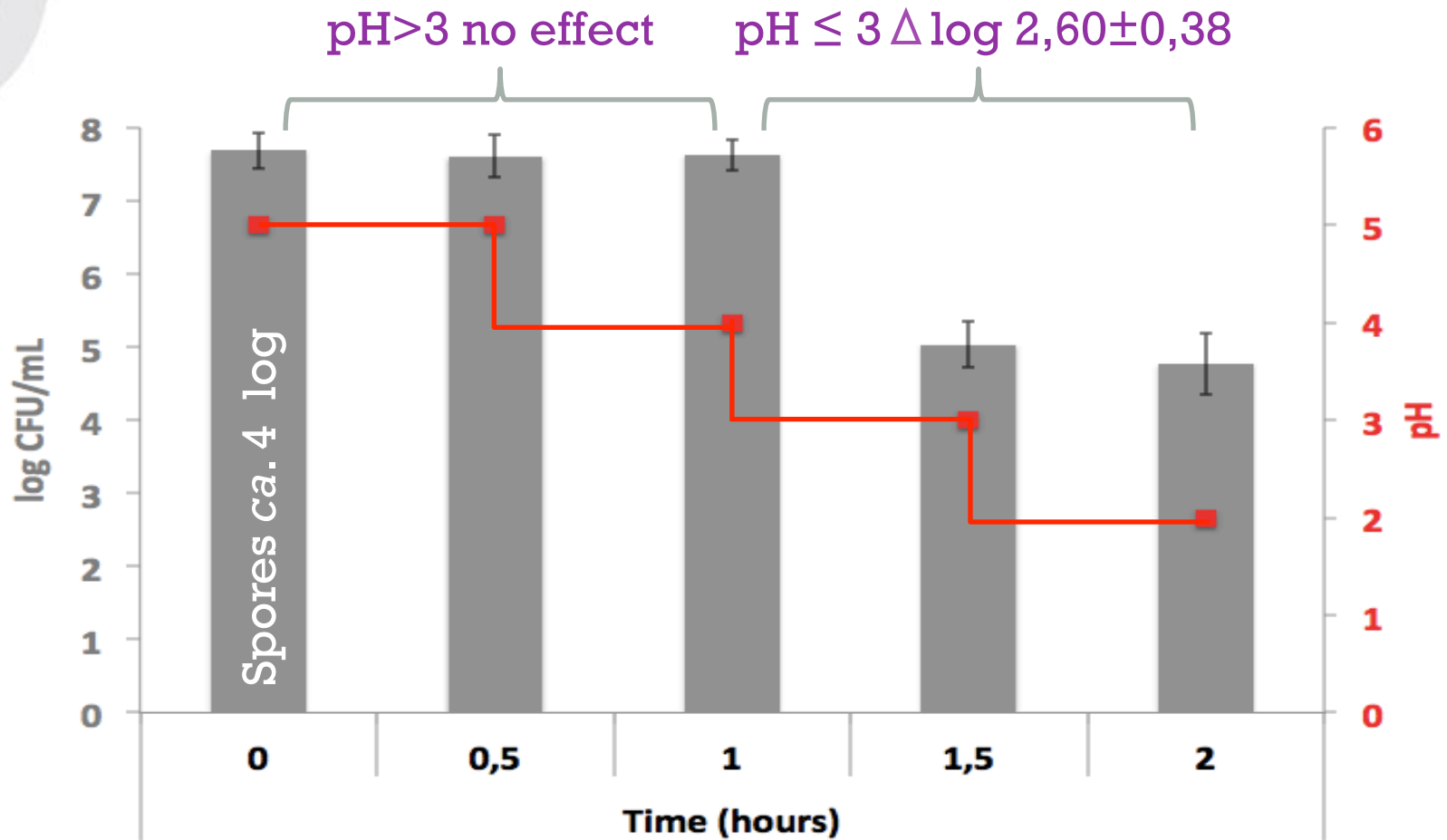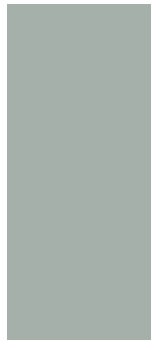# **Methods –** Proximal gut simulation

	Time (h)	Addition	pH	Effect of
STOMACH	0	+ *B. cereus* NVH 0500/00	Stepwise decrease	**pH**
	2			
UPPER INTESTINE	2	+ bile/pancreatin	7	**Host secretions**
	4			
ILEUM	4	+ mucin microcosms	7	**Mucus**
	8			

Results – Stomach

pH>3 no effect   pH ≤ 3 △ log 2,60±0,38
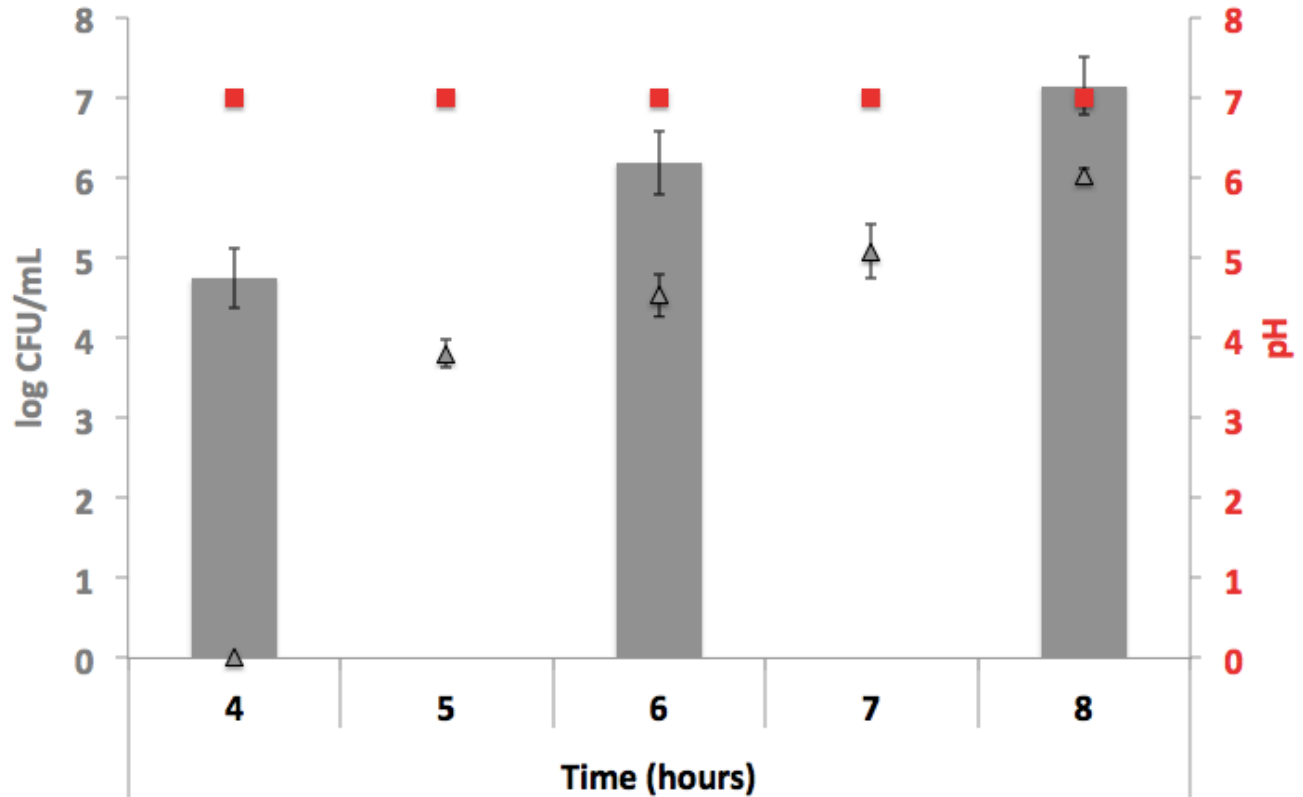
Results – Upper Intestine
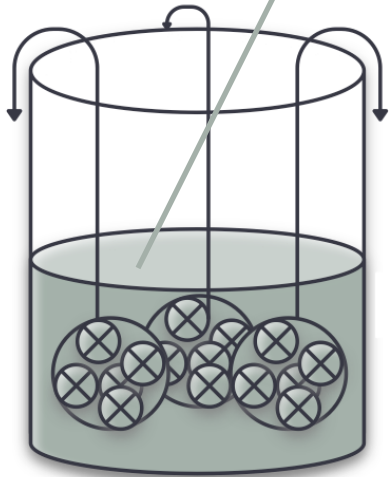
No effect of bile/pancreatin
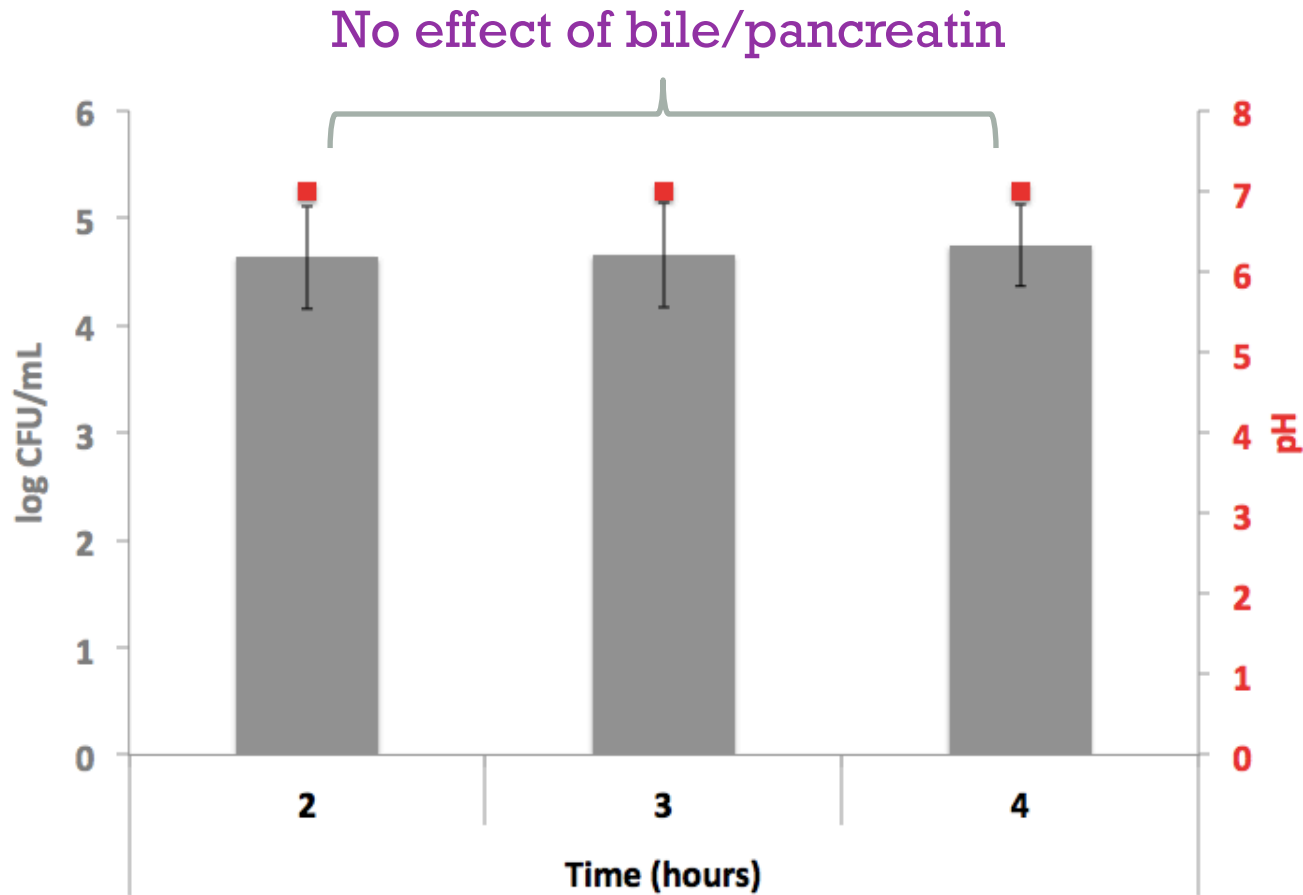
Results – Ileum

Lumen

Growth recovery within 4 hours
No enterotoxin production
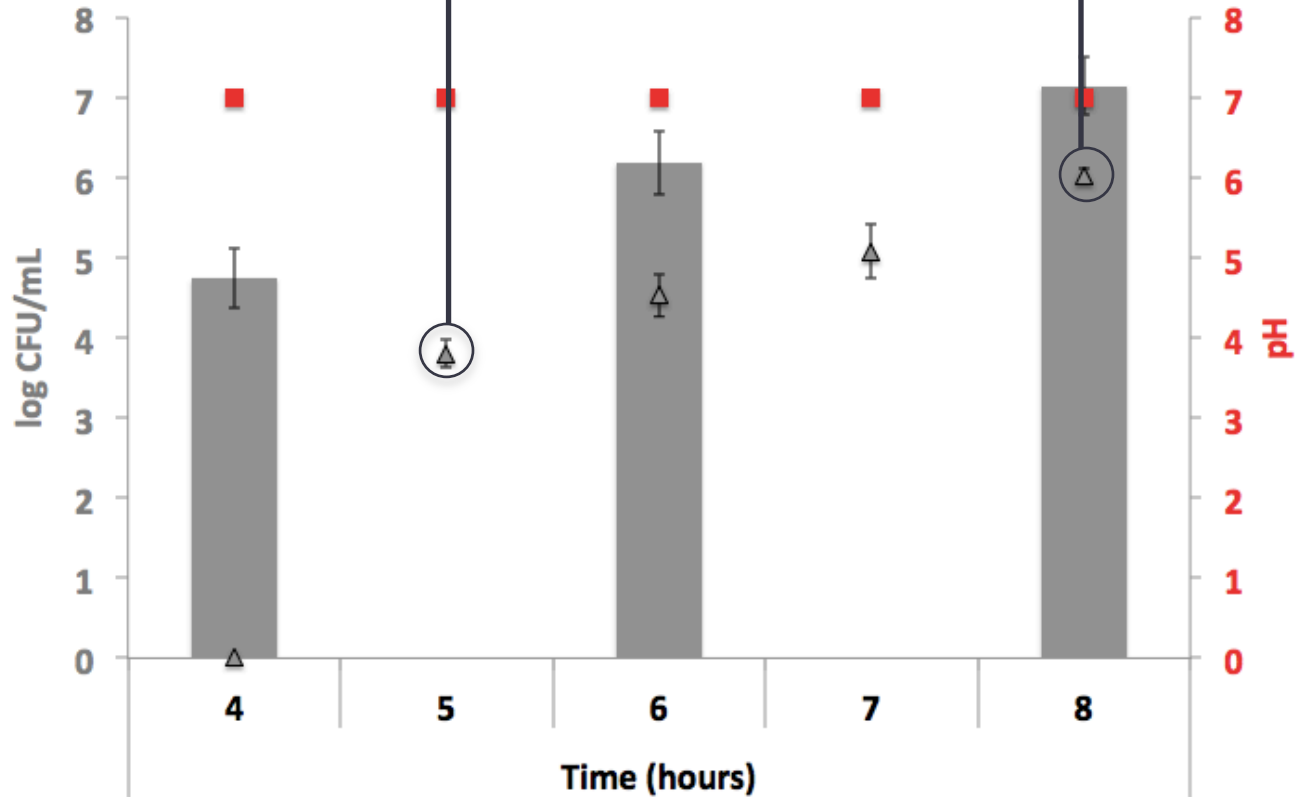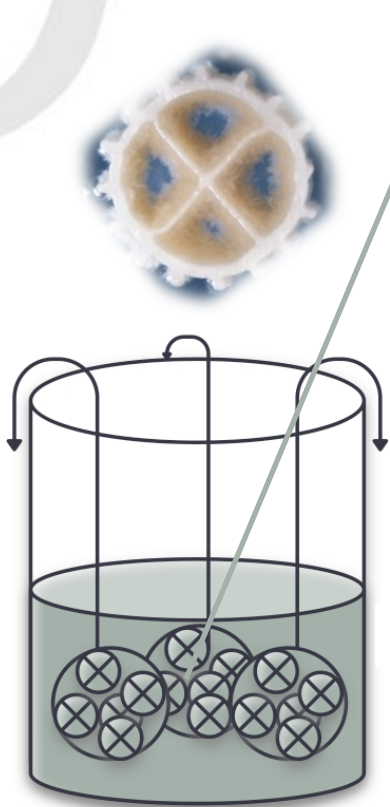
Results – Ileum

Mucus

△ Mucus

3,8 log in 1h   10% adhered

1. Why could we not detect enterotoxins in the **lumen**?

    1.1. Toxin degradation by host secretions?
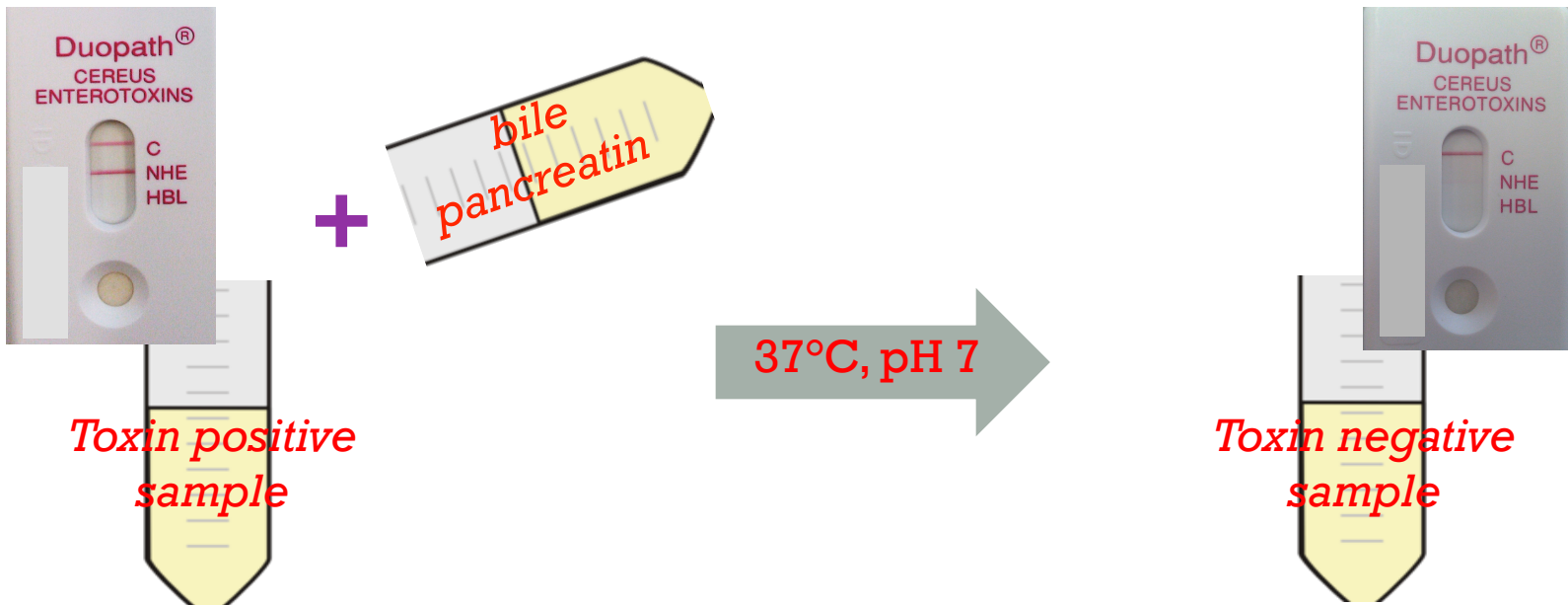   1.2. Quorum sensing not activated?

2. What is the role of the **mucus**?

    2.1. Could we detect enterotoxin in mucus?
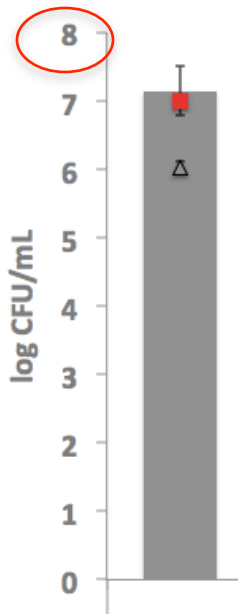   2.2. Are enterotoxins protected by mucus?
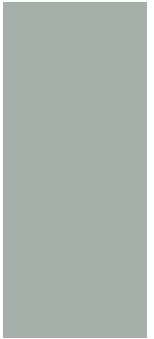
1.1. Toxin degradation by host secretions?

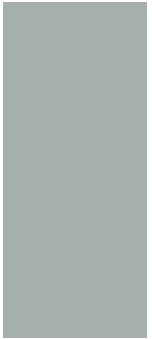**Toxin is sensitive to bile/pancreatin mixture**

1.2. Quorum sensing not activated?
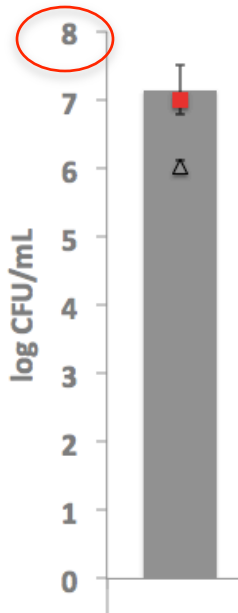

Probably not, stationary phase in not reached

1.2. Quorum sensing not activated?

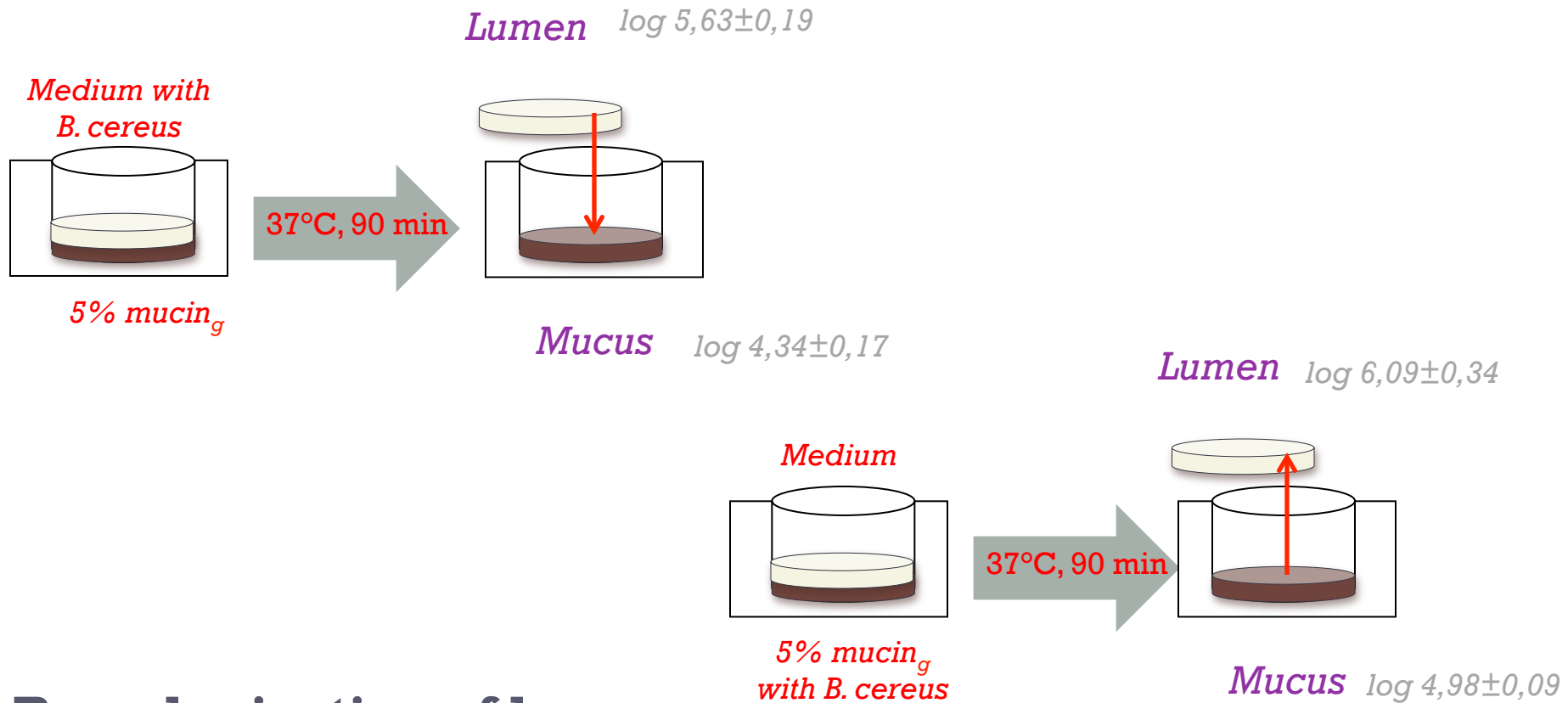Run the system longer?

Luminal Bacteria

Adhered Bacteria

What do the adhered bacteria do?

What do the adhered bacteria do?

Lumen *log 5,63±0,19*

Medium with B. cereus

37°C, 90 min

5% mucin$_g$

Mucus *log 4,34±0,17*

Lumen *log 6,09±0,34*

Medium

37°C, 90 min

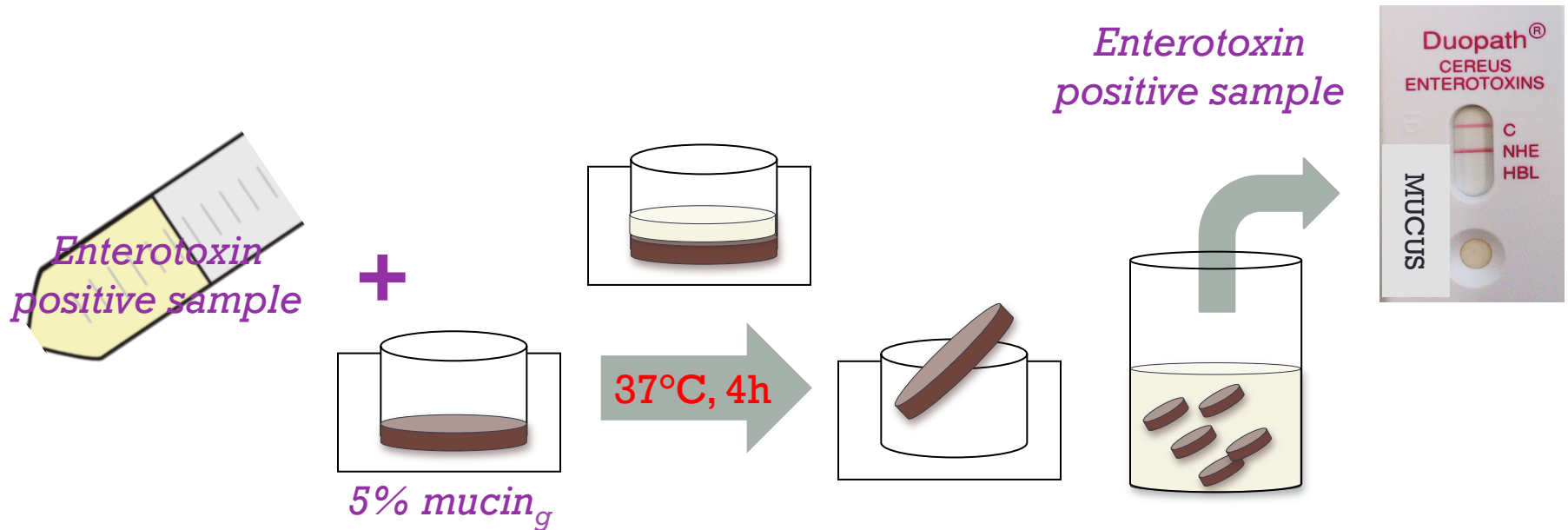*5% mucin$_g$ with B. cereus*

Mucus *log 4,98±0,09*

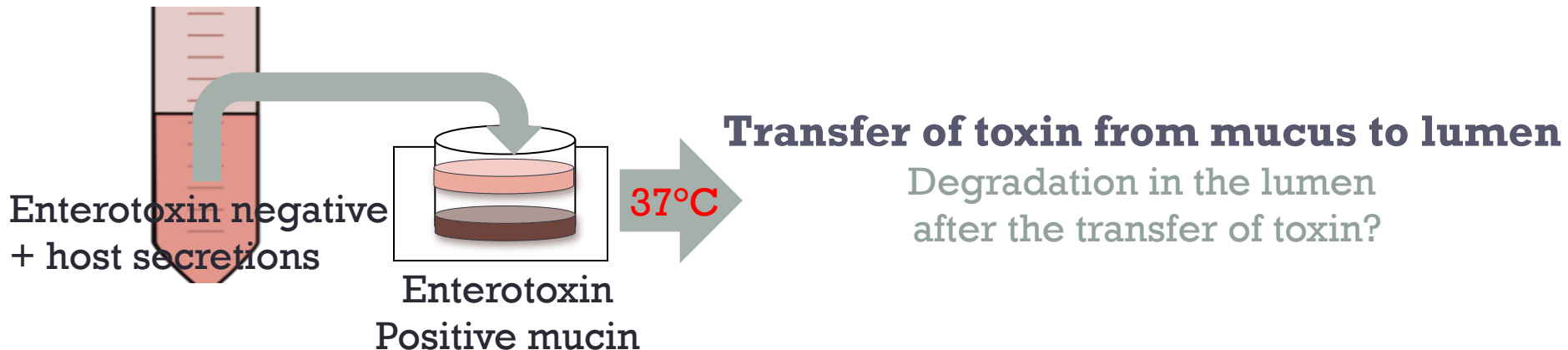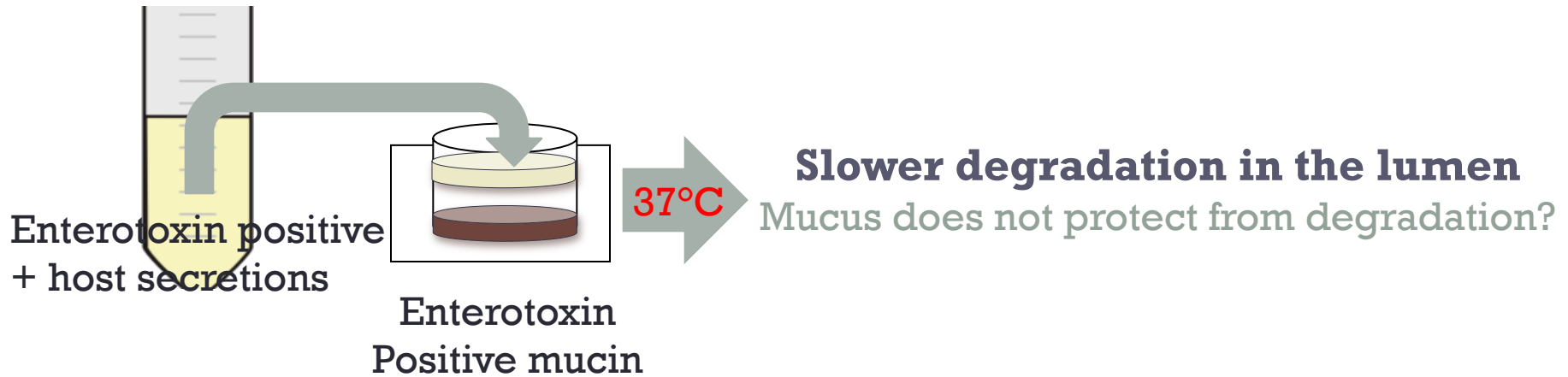**Re-colonization of lumen –
Increase of mucosa and luminal bacteria**

2.1. Could we detect enterotoxin in mucus?

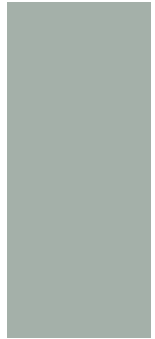**Enterotoxin can be detected in mucin agar**

2.2. Are enterotoxins protected by mucus?

Enterotoxin positive + host secretions

Enterotoxin Positive mucin

37°C

Slower degradation in the lumen
Mucus does not protect from degradation?

Enterotoxin negative + host secretions

Enterotoxin Positive mucin

37°C

Transfer of toxin from mucus to lumen
Degradation in the lumen after the transfer of toxin?

Toxins are maintained in the system longer due to mucus?

Thank you for your attention

Promotors

Prof. dr. ir. Van de Wiele
Prof. dr. ir. Uyttendaele

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Collaborators

ILVO

LabMET