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Simulated reprocessing model for flexible endoscopes: assessment of routine cleaning to prevent accumulation of build-up biofilm

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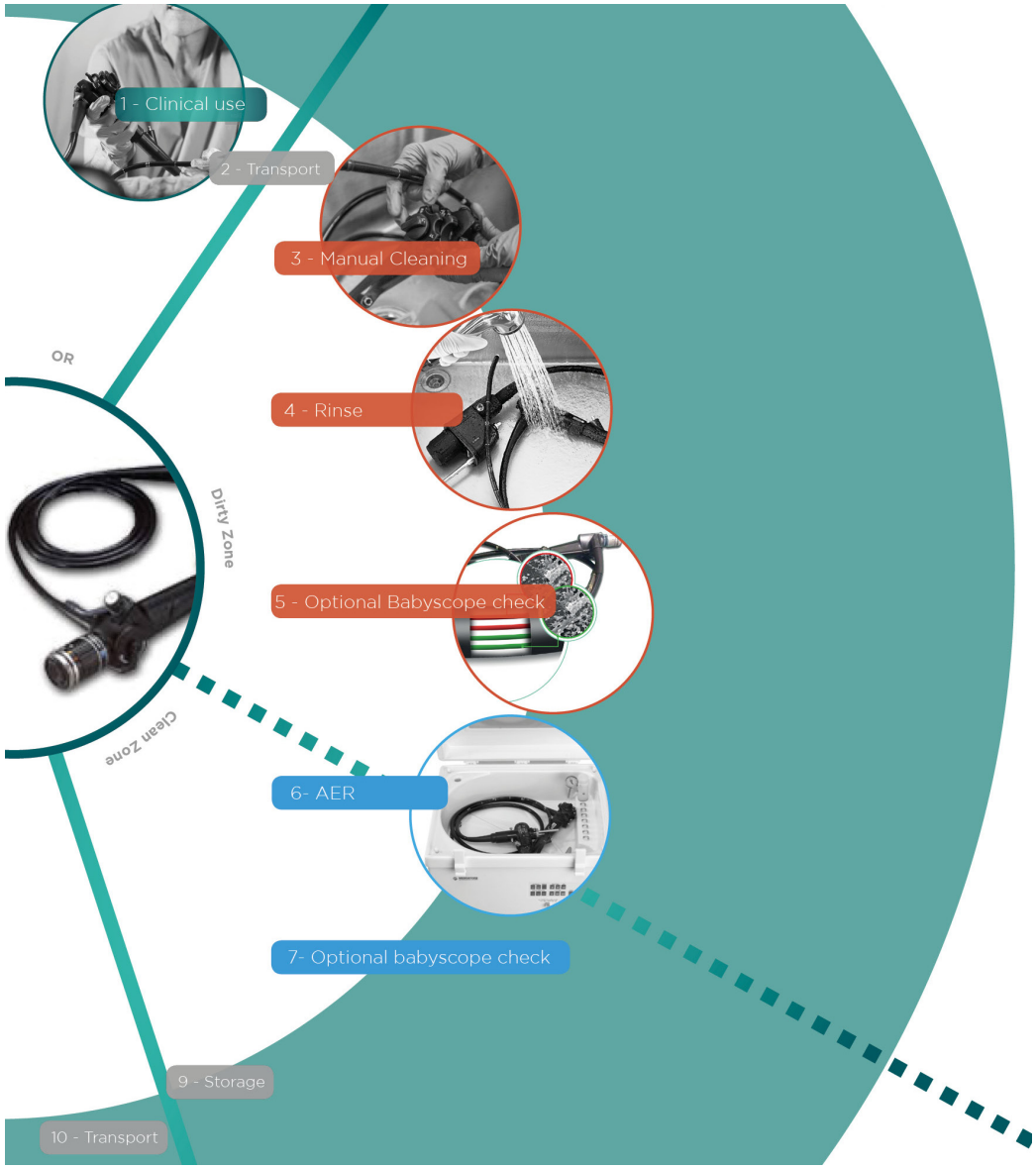
Endoscope reprocessing



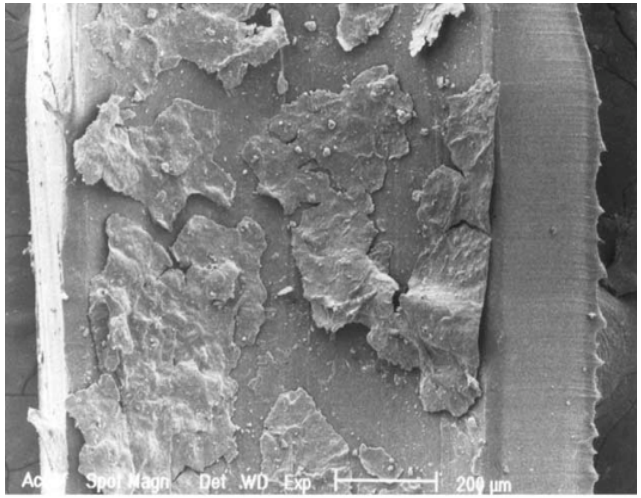
Flexible endoscopes are reprocessed with a **low margin of safety** :

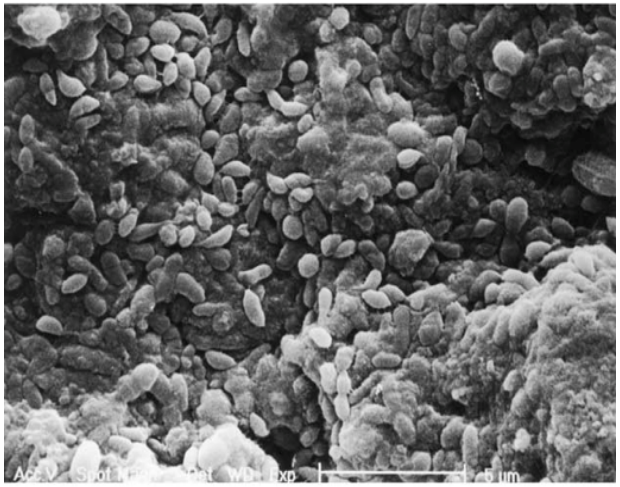
- Process subjected to human error
- Requires good training of staff
- Complex design of endoscopes
- High-Level disinfection is not sterilization
- Biofilms may form in endoscopes

→ Each step must be optimized to deliver endoscopes that are safe for patients

*AER: Automated Endoscope Reprocessors

Biofilms in endoscopes





Pajkos et al., J Hosp Infect. 2004;58:224-9.

« Build up biofilm forms due to the repeated exposure to disinfectants and to cycles of wet and dry phases. » (Alfa et al., BMC Infect Dis. 2009; 9: 56)

→ Biofilm-contaminated endoscopes are a permanent source of germs transmitted to patients

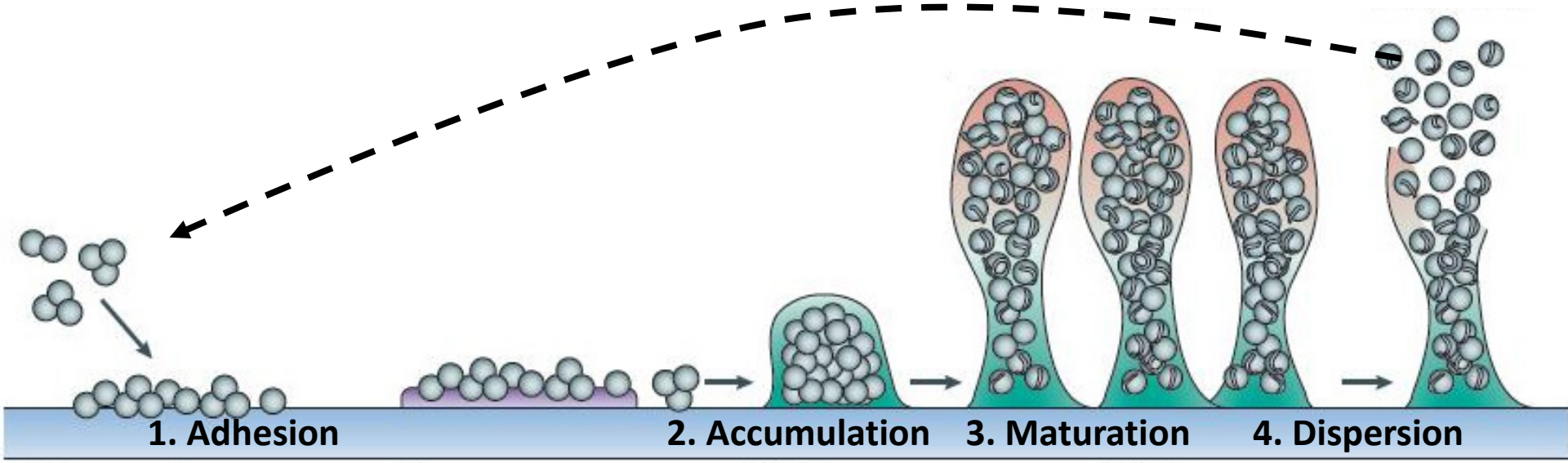
The impact of biofilms on reprocessing

Biofilms are microbial communities, often composed of multiple species, developing on surfaces or at interfaces and encased in a self-produced matrix of polymers (EPS)

- They form where there is water, nutrients and the adequate temperature
  (… all of which can be found in a soiled endoscope)
- They are tolerant to high concentrations of biocides

→ Biofilms increase the cleaning challenge

→ If biofilms persist in endoscopes, they are a threat to patient safety

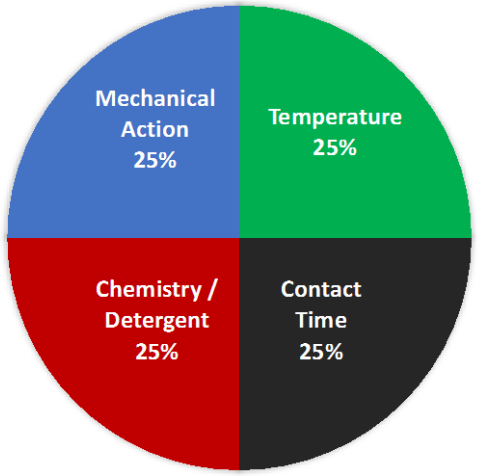


Otto, Nature Reviews Microbiology, 2009; 7: 555–567

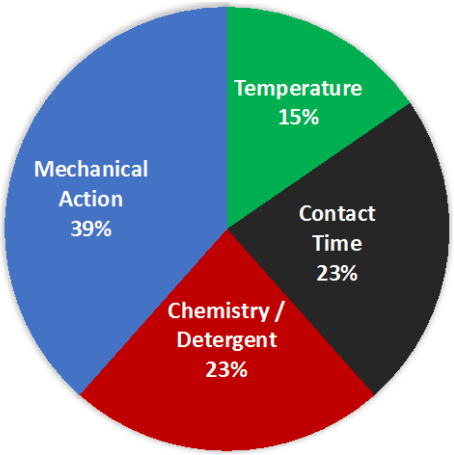
The role of cleaning in endoscope reprocessing

Cleaning is the physico-chemical removal of all soils and bioburden,
it is determined by **Sinner's cycle** :

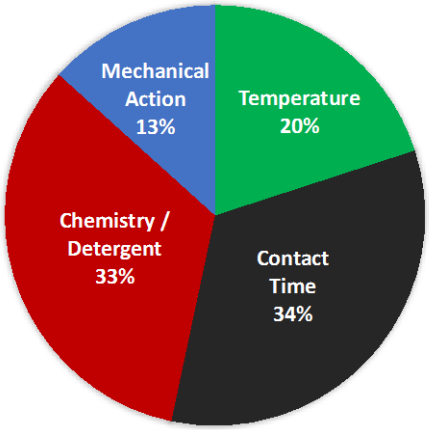
The theory…



The practice?



e.g. Biopsy channel



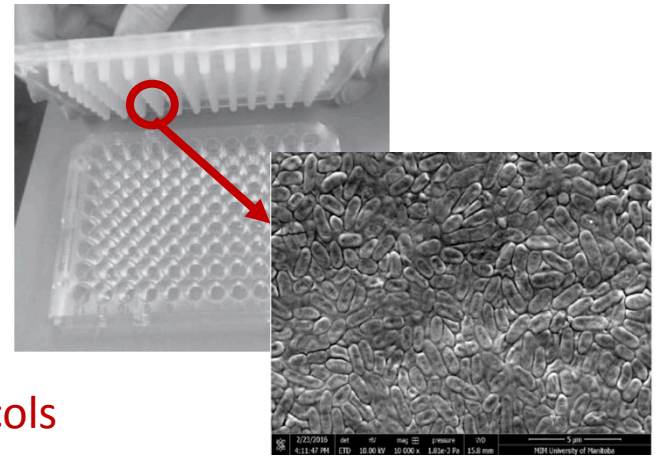
e.g. Air/water channel
(non-brushable)

- How can we make sure that cleaning will be effective?
- What happens when the biofilm is forming – is cleaning still effective?
- Is a more thorough cleaning procedure needed to prepare the endoscope for effective disinfection?

The added value of models

Models can simulate the clinical reality and can be used to gather valuable information

For surgical instrument cleaning, the Fibrin PCD [Process challenge devices] model[1] provides the means to evaluate and compare detergents and cleaning processes on realistic but **worst-case soils**



A build-up biofilm model was developed[2] to evaluate the efficacy of cleaning on **mature 8-days biofilms**

The goal of the present study is to optimize a model to simulate the effectiveness of endoscope cleaning protocols **in conditions where early biofilm develops**

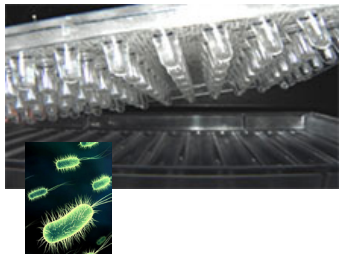
→ Use of ATS (Artificial test Soil)[3] containing blood, serum, mucin, bile, endotoxins, etc… and inoculated with 10^8 CFU of *Klebsiella pneumoniae* ATCC 700603

[1] *Wehrl et al., ZentralSterilization 2018; 26:382-396*
[2] *Da Costa et al., J Microbiol Methods 2016;127:224-229*
[3] *Alfa et al., ZentralSterilization 2005; 13:387-402 - US patent 6,447,990*

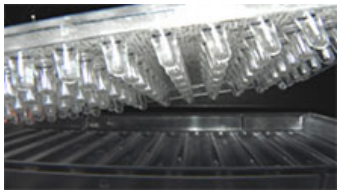
The in vitro model

One cycle

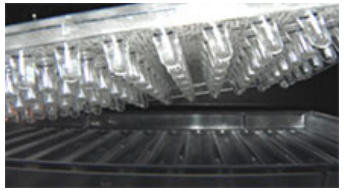
Simulated endoscope use -
Incubation in ATS* (30 min, 4h, 24h)
at room temperature



Cleaning with detergent
(10 ml/l, 5 min, 20 or 40°C)

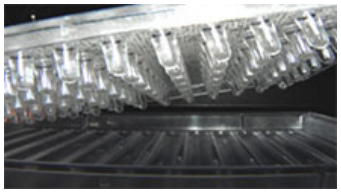


Rinsing 2 x

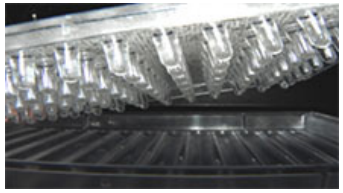


2 x

Disinfection in peracetic acid
(40°C, 3 min, 900 ppm)



Rinsing 2 x



Quantification of residual biomass/bioburden by crystal violet staining

* Artificial Test Soil

The in vitro model: tested cleaning agents

4 detergents representing different types of commercialized cleaning agents were tested:

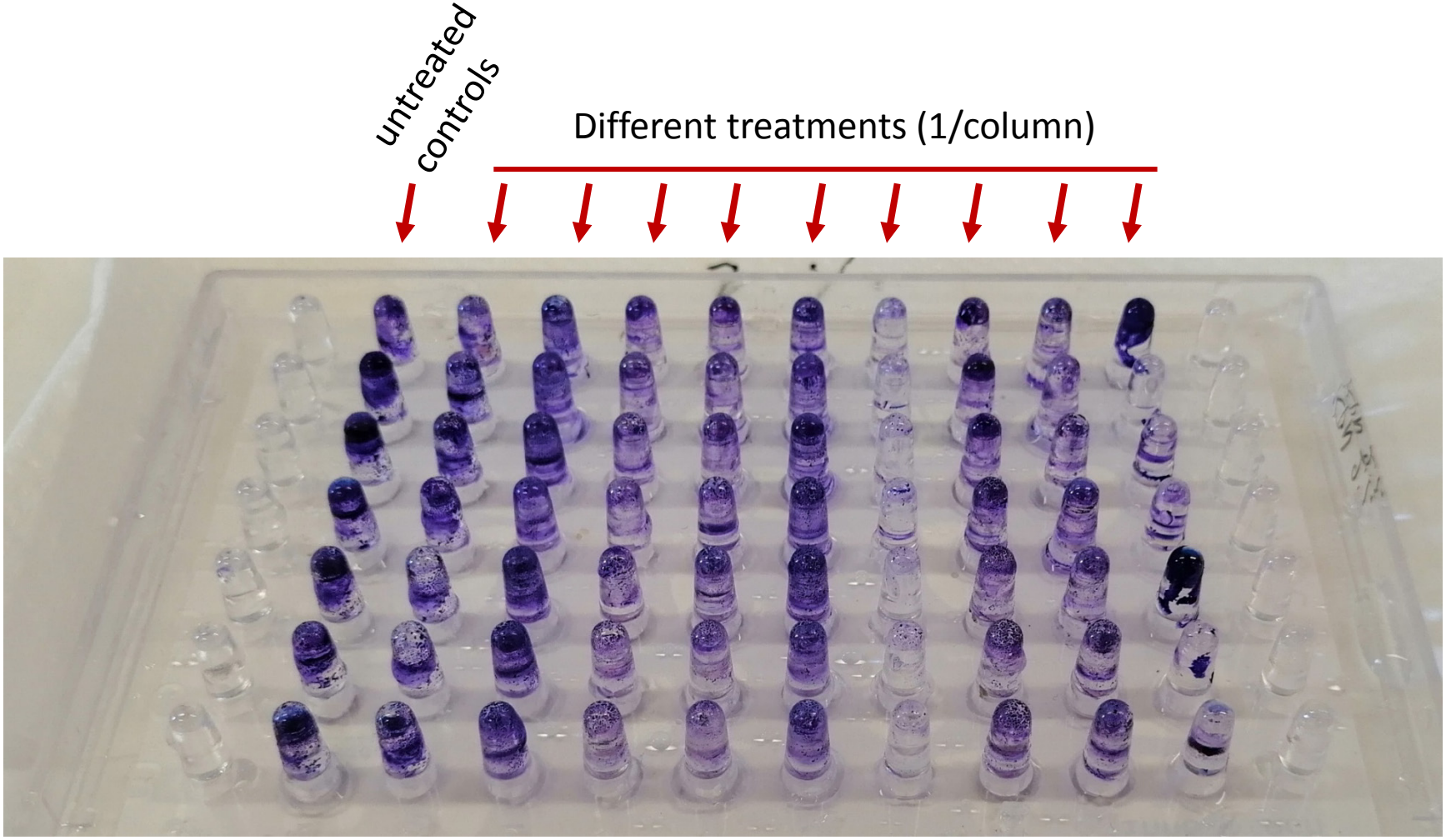
- **M** = neutral detergent (pH = 8.5 in concentrate)
- **N** = enzymatic detergent (M + 5 % w/w protease)
- **O** = multi-enzymatic detergent (M + 5 % w/w protease + 2% w/w of amylase, cellulase and lipase)
- **P** = alkaline detergent (M adjusted to pH 12.5 with KOH)

Detergent M		
Category	**Raw material**	**Mass (%)**
Solvents	Distilled water	50,0
Stabilizer	4-Phenylboronic acid	2,5
Stabilizer	Sodium formate	3,0
Builder	GLDA	2,0
Non-ionic Surfactant	Fatty alcohol ethoxylated - CAS : 27458-92-0	5,0
Solvents	Glycerol	15,0
Preservative	Methylisothiazolinone	0,5
pH adjustment	Phosphoric acid	To pH 8.5
Solvents	Distilled water	up to 100 %

Disinfectant used: Soluscope PAA
(5% peracetic acid solution) diluted
at 900 ppm active peracetic acid
in the final solution
according to manufacturer's instructions

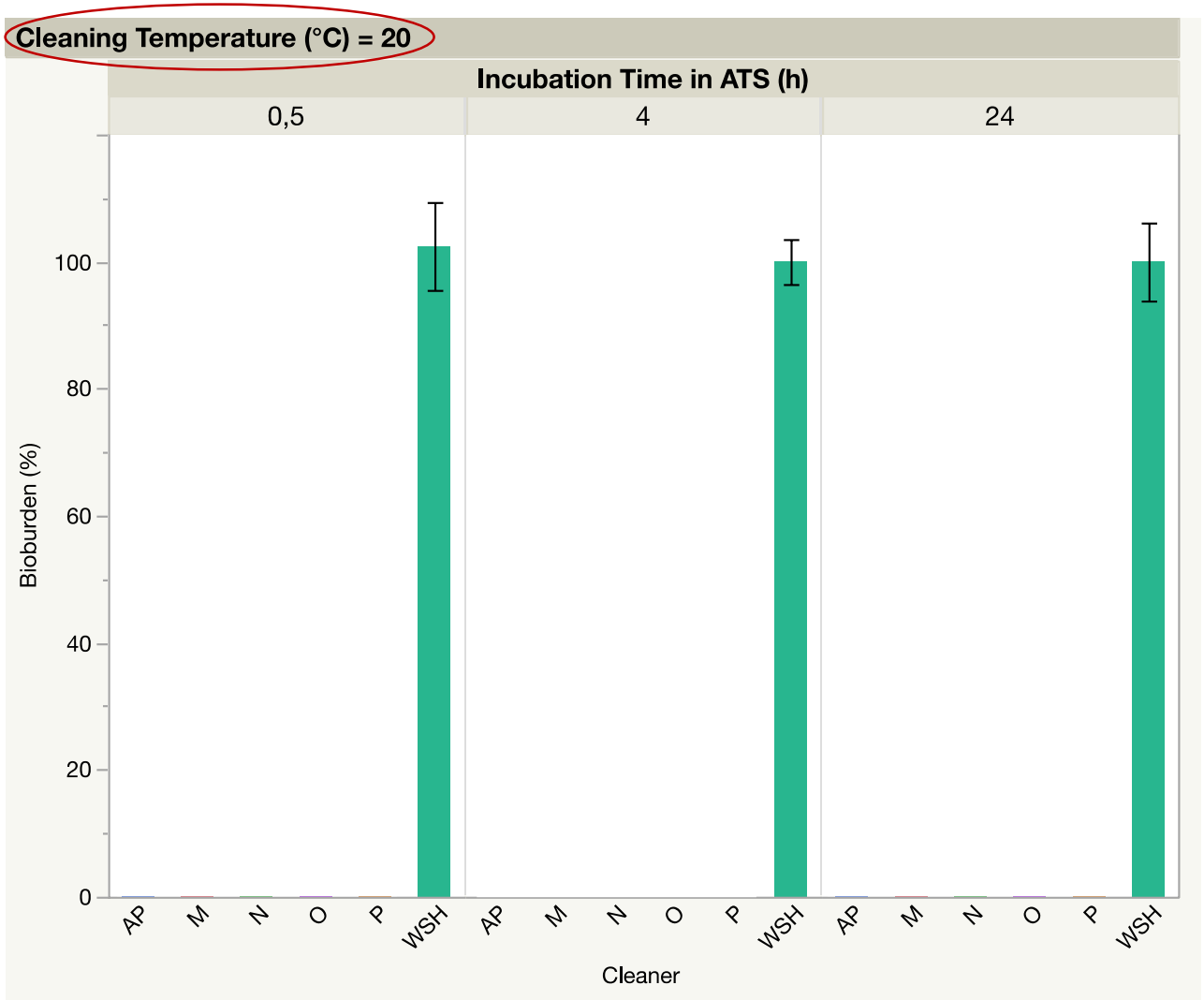
Results: biofilm formation on PEGs

Example of the crystal violet colored substrates at the end of a cycle



Results: influence of the duration of incubation in ATS

30 min, 4 h and 24 hours incubation in ATS (1 cycle – 5 min cleaning time at 20°C)



Error bars are standard errors

* AP stands for peracetic acid

@ WSH stands for water of standard hardness

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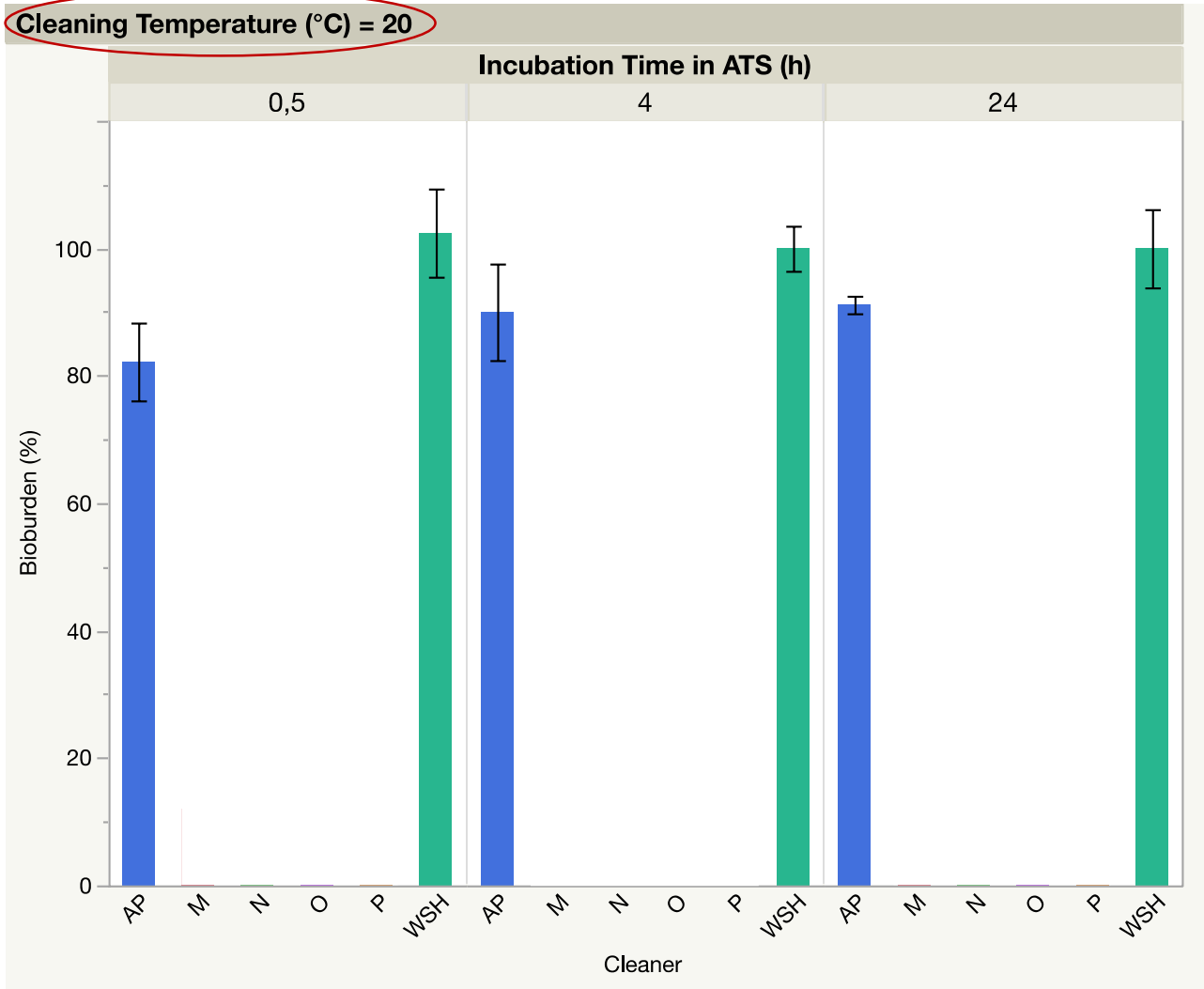

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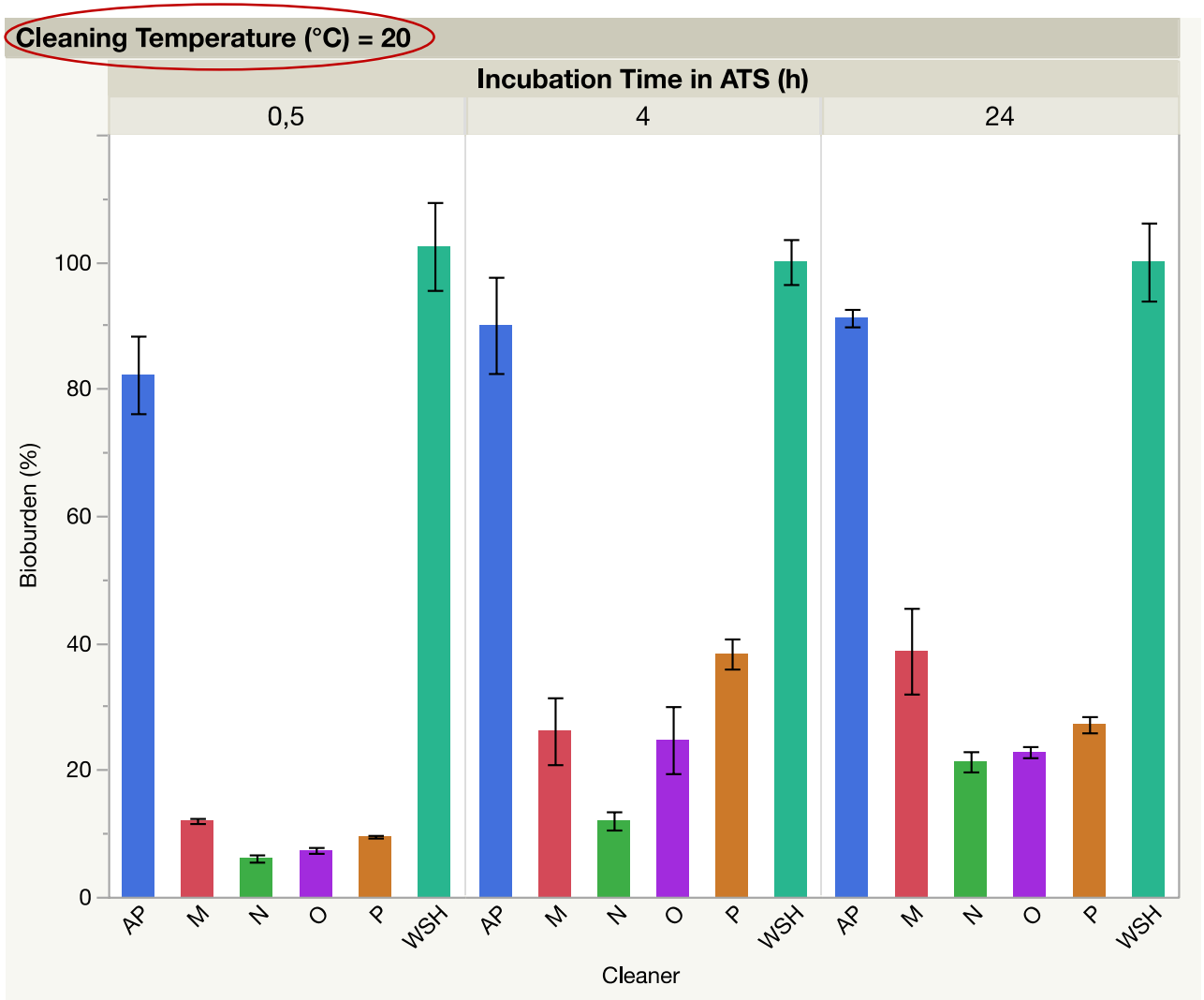

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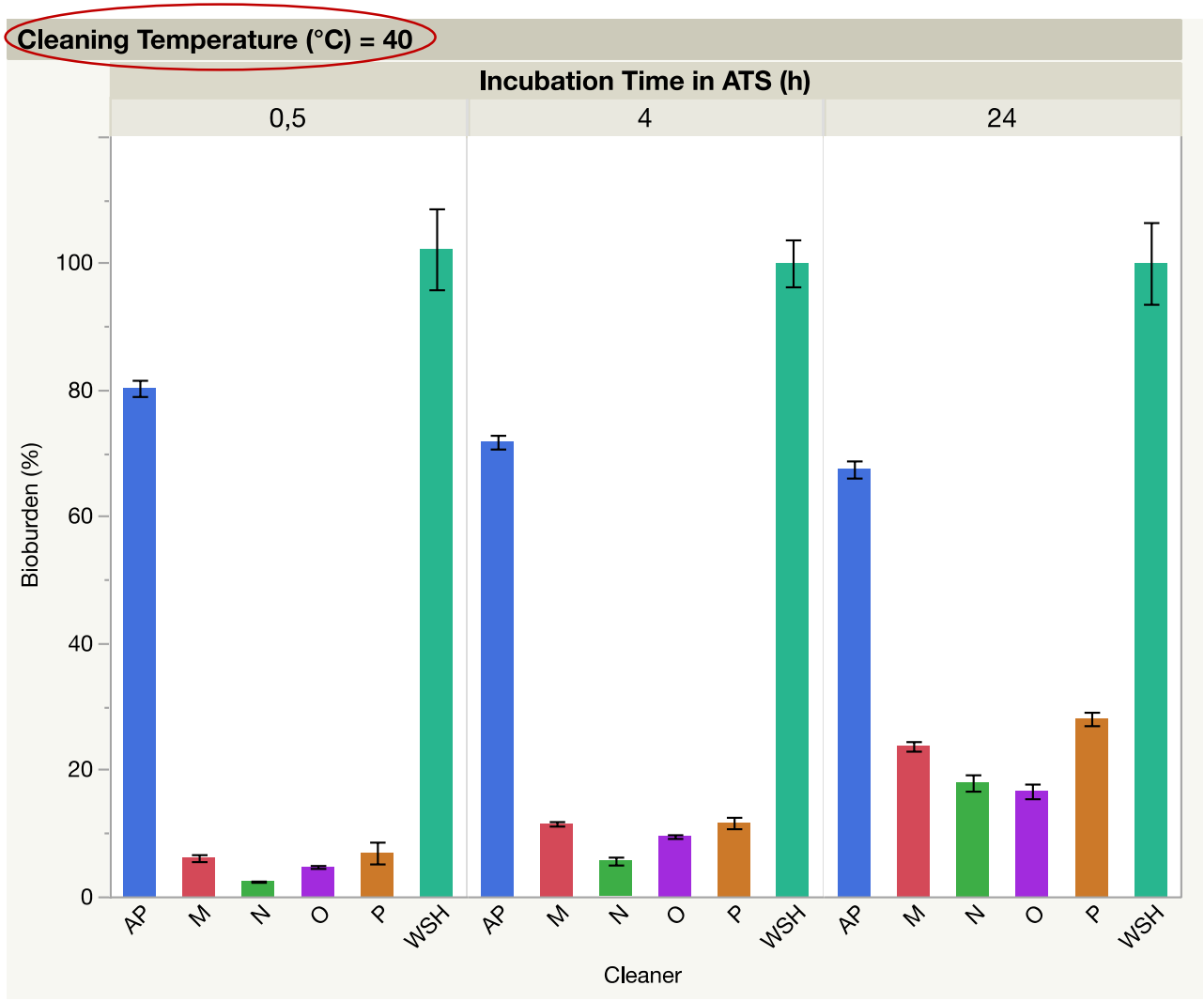
Results: influence of the duration of incubation in ATS

30 min, 4 h and 24 hours incubation in ATS (1 cycle – 5 min cleaning time at 40°C)

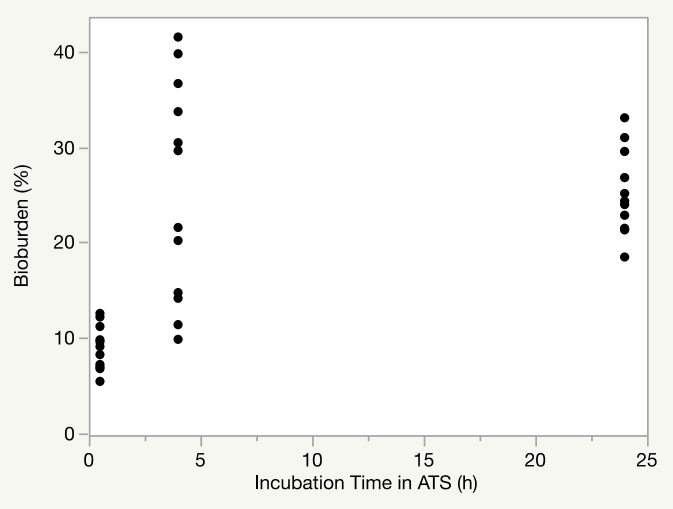


Error bars are standard errors

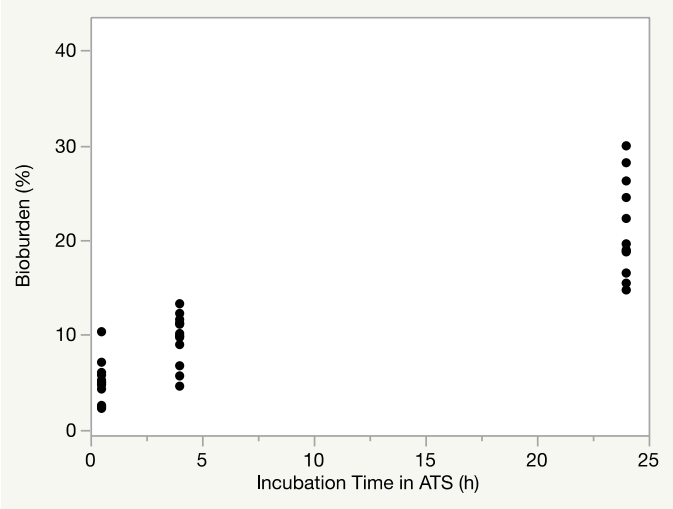
Influence of incubation time in ATS and of cleaning t°

- Relation between the ATS incubation time and the bioburden residue (controls excluded) :

At 20°C / All cleaners : p-value = 0.0018



At 40°C / All cleaners : p-value = 0.0001

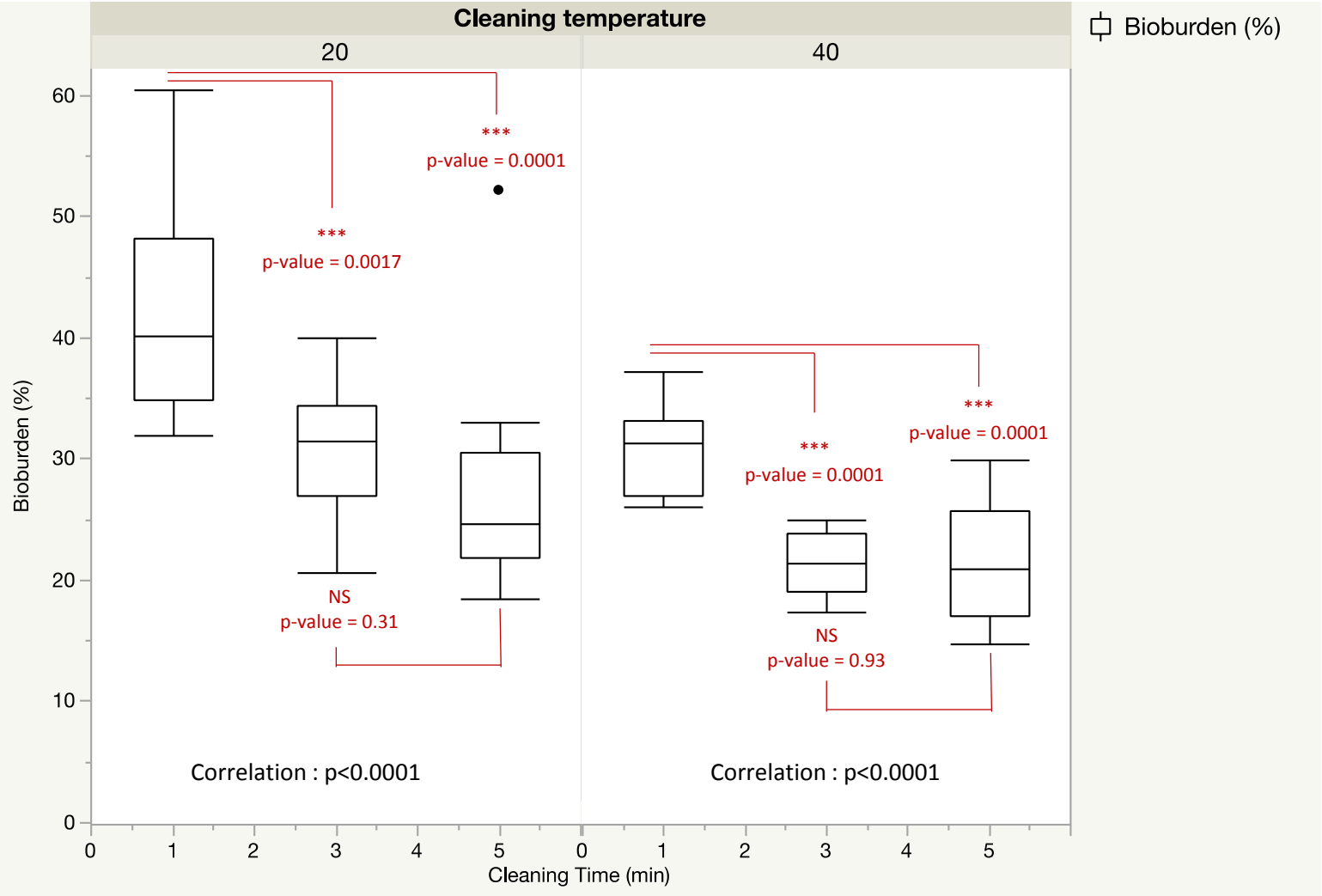


- Effect of cleaning temperature – all detergents pooled (controls excluded) :

ATS incubation duration	Residual bioburden (%) @ 20°C	Residual bioburden (%) @ 40°C	P-value of Student T test
30 min	8.7	5.0	*** 0.0004
4 h	25.3	9.6	*** 0.0003
24 h	27.5	21.6	* 0.0305

Influence of cleaning time

Different cleaning times (1 cycle – 24 hours incubation in ATS – all cleaners pooled – controls excluded)



Statistical comparisons (One-way ANOVA)

Detergent efficacy comparison pooling data from 20°C and 40°C cleaning during 5 minutes
(comparison of least squared means), treatments with different letters are statistically different)

ATS incubation time 30 min

Cleaner				Mean Bioburden (%)
WSH	A			102.3
AP		B		81.2
M			C	9.0
P			C	8.2
O			C	6.0
N			C	4.2

ATS incubation time 4 h

Cleaner						Mean Bioburden (%)
WSH	A					100.0
AP		B				80.9
M			C	D		18.8
P			C			25.0
O				D		17.1
N					E	8.8

ATS incubation time 24 h

Cleaner					Mean Bioburden (%)
WSH	A				100.0
AP		B			79.3
M			C		31.2
P			C		27.6
O				D	19.7
N				D	19.6

Observations:

- After 30 min of incubation in ATS : No discrimination between cleaners
- After 4 hours of incubation in ATS : detergent N (enzymatic) performs best
- After 24 hours of incubation in ATS : detergent N & O (enzymatics) perform best

Conclusions

- The proposed in vitro model enables simulation of reprocessing of soiled medical devices (e.g. endoscopes) taking into account biofilm formation
- Longer incubation time in ATS resulted in overall reduction of cleaning efficacy probably due to biofilm buildup
- A cleaning time of 3 minutes seems to be as effective as 5 minutes (all data together) but it may be cleaner dependent
- When biofilm starts to form (4 h and 24 h incubation) enzymatic formulations were performing better than neutral and alkaline cleaners in this model
- A higher cleaning temperature was found to provide better cleaning efficacy at all incubation times (all cleaners pooled)

Perspectives

- Monitor the levels of cultivable bacteria (CFU) surviving reprocessing on top of bioburden

- Use the model to understand how commercially available products behave with respect to cleaning temperature, contact time and concentration

  → Valuable information to optimize cleaning phases in reprocessing

- Repeat the ATS incubation / reprocessing cycles 2, 3 and 4 times to determine whether further accumulation of bioburden occurs
  (Preliminary data suggest otherwise)

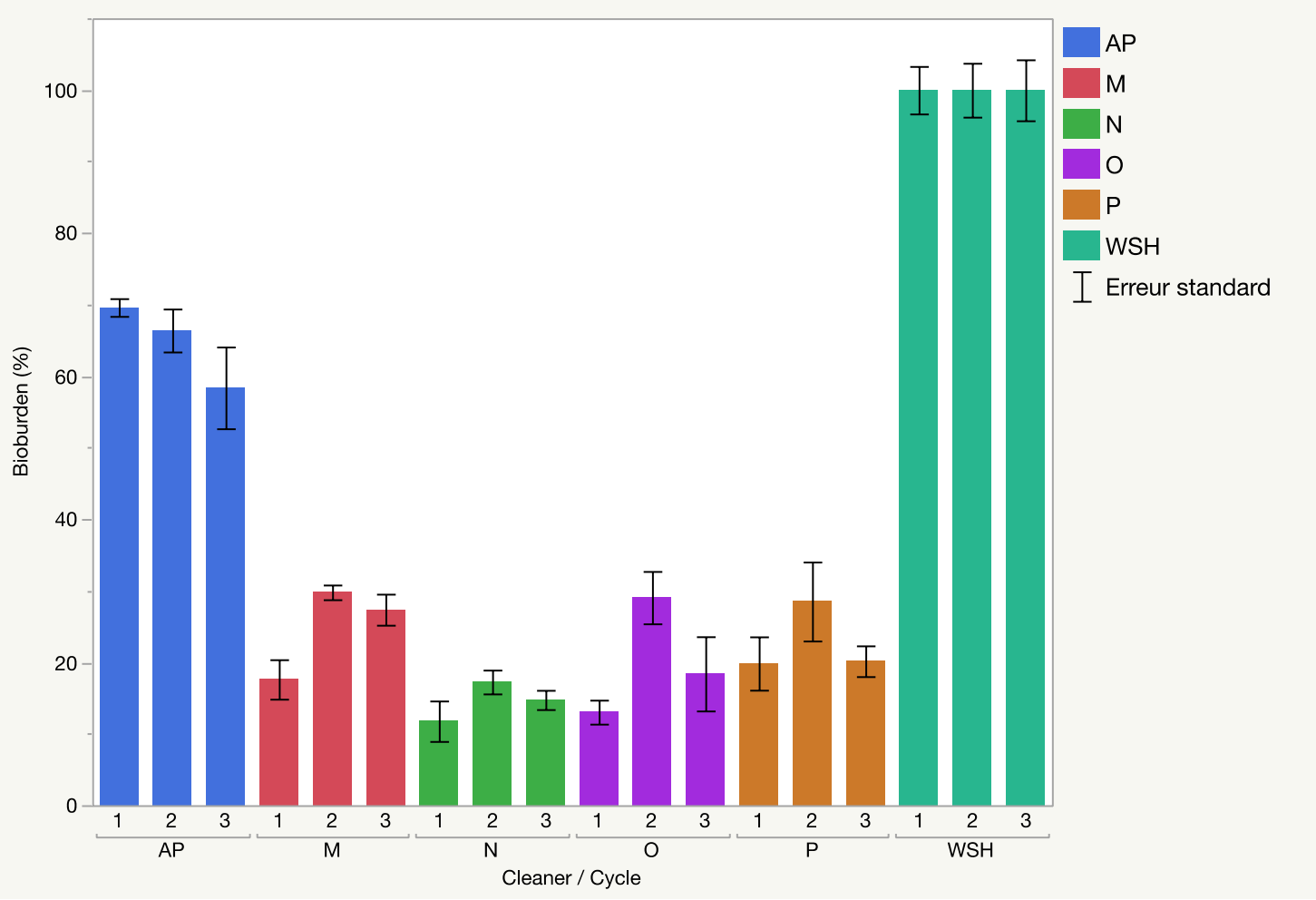
- Use other bacterial species or multi-species inoculum in ATS

- Validate model reproducibility with other laboratories

Appendix – Repeated cycles

1, 2 or 3 cycles or use-reprocessing with 4 hours incubation in ATS (5 min cleaning time at 40°C)

→ No marked trend of biofilm buildup or reduction



AP
M
N
O
P
WSH

Appendix – Comparison of commercial cleaners

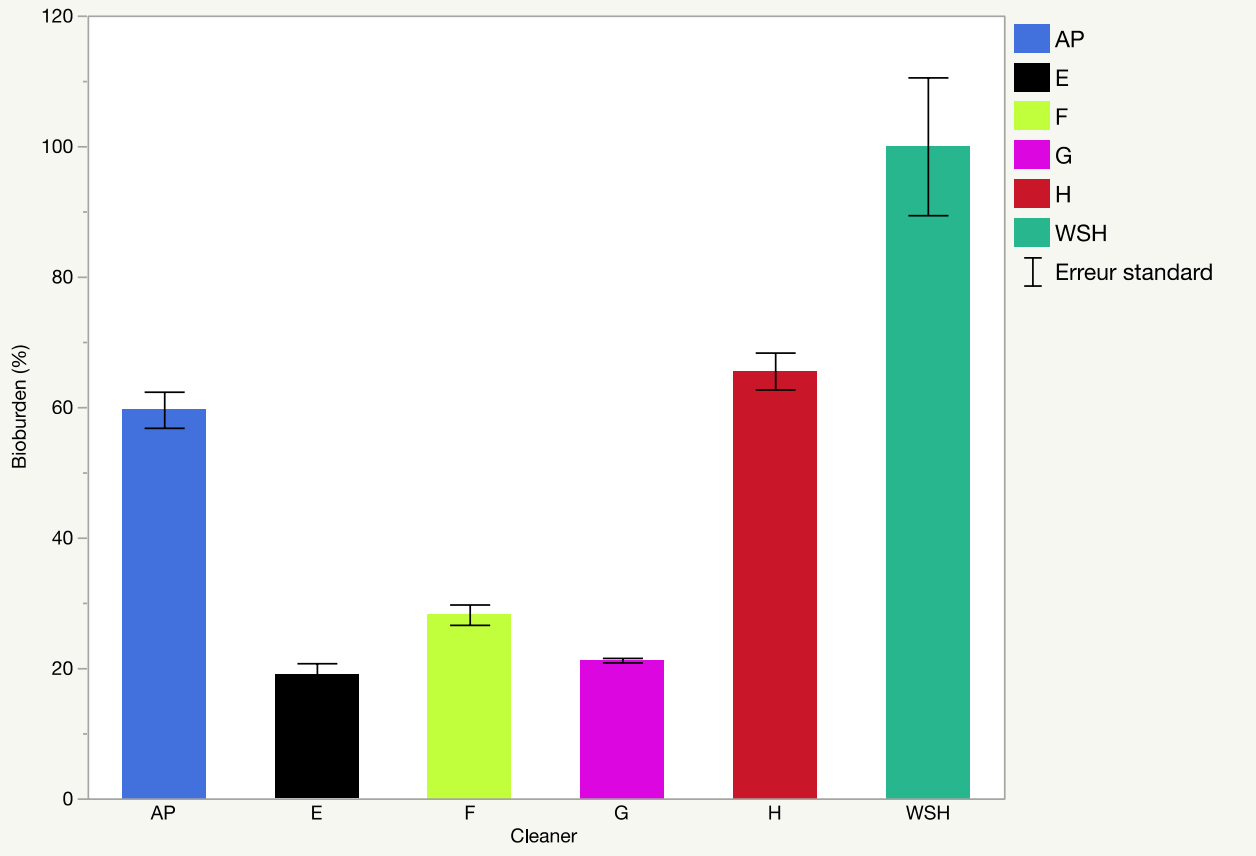
1 cycle or use-reprocessing with 24 hours incubation in ATS (3 min cleaning time at 20°C)

E = mild-alkaline, enzymatic cleaner

F = neutral, multi-enzymatic cleaner

G = neutral, multi-enzymatic cleaner

H = neutral, multi-enzymatic cleaner with biocidal activity



Cleaner				Mean Bioburden (%)
WSH	A			102.3
AP		B		59.6
E			C	18.9
F			C	28.7
G			C	21.2
H		B		65.5

Cleaner H has a cleaning efficacy comparable to water