Small reactor for fast and simple studies of biofilms

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What are biofilms?

- Biofilms are structures formed by bacteria on almost every wet surface
- Biofilms provide highly efficient protection to bacteria against external adversary factors
- The are no „gentle“ chemical procedures to remove biofilms
- The only efficient removal of biofilms is by mechanical scrubbing

Why should biofilms be a problem?

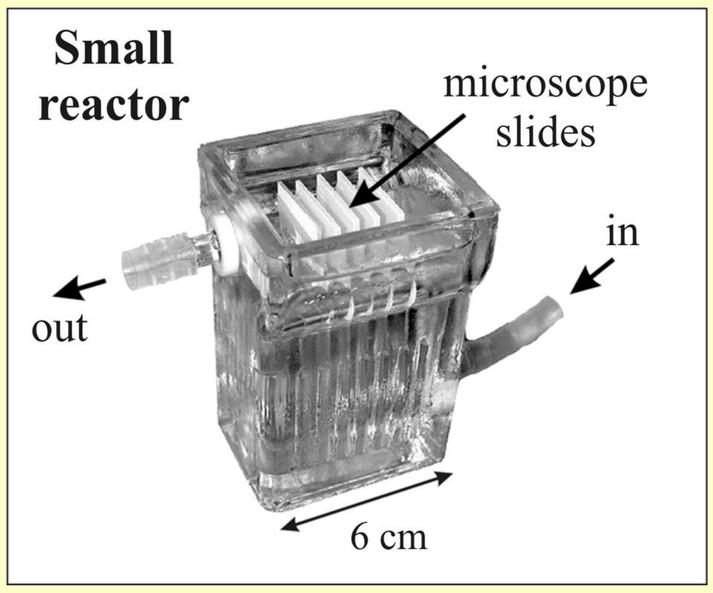
- Biofilms as a source of pathogens pose a health risk
- Biofilms are extremely difficult to eliminate
- Disinfection of biofilms might eliminate the bacteria, the biofilm structures, however, remain and are readily available for a rapid re-colonization

Design of the Model

Container:
glas staining device with silicone-tubes at the sides for in- and outflow of water.

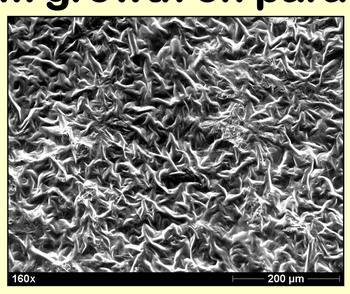
Water source:
tap water

Flow rate:
constant of 50 ml / minute.

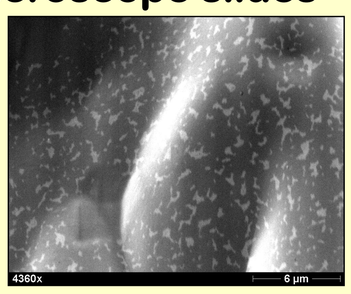


Across the flow direction of the water coupons (e.g. microscope slides) can be placed.
The coupons can be taken out single without disturbance of the others.
By injection of fluid into the input tube it is very easy to simulate contaminations of the system.
Not shown in the picture is the glass lid covering the whole reactor.

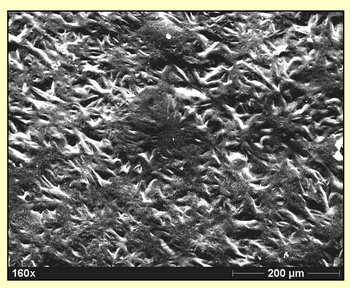
Morphological studies : Biofilm growth on paraffinated microscope slides



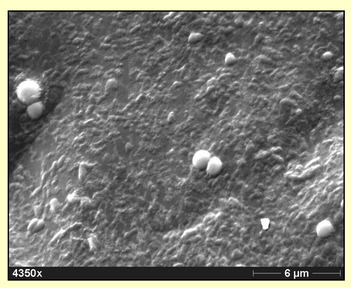
0 days



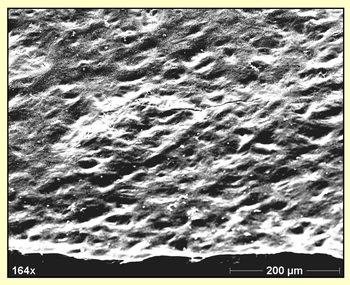
Fresh Paraffin has a highly structured surface with fossettes and hightenings



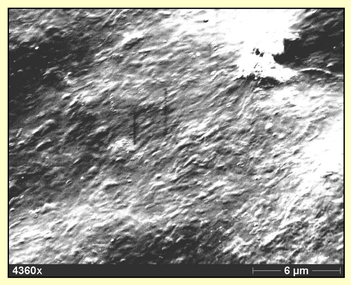
14 days



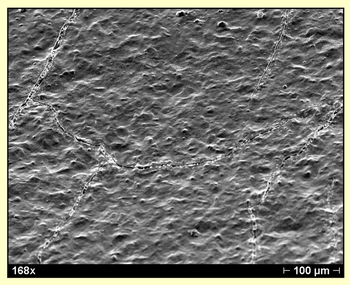
After 14 days Biofilm covers most of the surface.
The structure of the paraffine, however, is still recognizable.



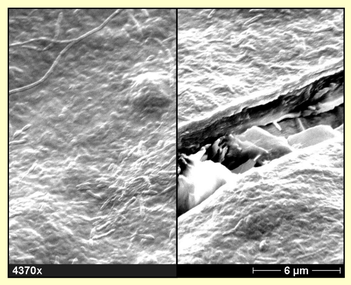
21 days



After 21 days in the reactor the paraffin surface is complete overgrown with biofilm. One only can guess the structure underneath the bacteria and the EPS-material they have produced.



42 days



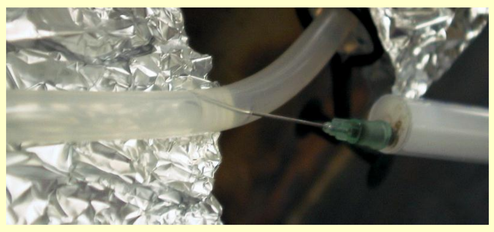
Another three weeks later the surface of the biofilm is almost completely smooth.
It is not possible to determine, what structure lies underneath it.
On the surface fungi can be seen.
The film has grown so thick, that it crackes during the preparation for the SEM. At theese crackes it is possible to measure the thickness of the (highly dried) film: It is about 3 µm.

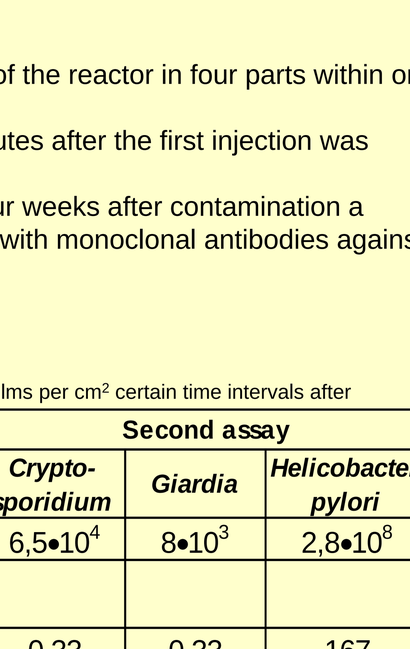
The aim of this Model

- construct a facility that allows to generate biofilms on any materials used in distribution systems under flow conditions
- easy retrieval of the biofilms for various studies, such as cultivation and microscopy observations
- simulate the accidential contamination of drinking water biofilms with pathogens or microorganisms of fecal origin
- make statements about their survival in these biofilms

Injection of china ink as a test for the distribution of contaminants in the system

By injecting china ink we could show that contaminants were spread equally all over the whole volume in the reactor within 40 seconds.





Physiological Studies:

- Contamination with potential pathogens (*Helicobacter pylori* or cysts/oocysts of *Giardia lamblia* and *Cryptosporidium sp.*)
- Observation of the traceability of the organisms in the biofilm and outflow

Method

- The suspension was injected into the input tube of the reactor in four parts within one hour
- the outwash of pathogens during the first 15 minutes after the first injection was examined.
- One hour, one day, one week, two weeks and four weeks after contamination a coupon was taken out of the reactor and stained with monoclonal antibodies against the pathogens

Results

Table 1: Findings of parasite cysts/oocysts and *Helicobacter pylori* in biofilms per cm^2 certain time intervals after contamination

	First assay			Second assay		
Number of organisms injected	***Crypto-sporidium***	***Giardia***	***Helicobacter pylori***	***Crypto-sporidium***	***Giardia***	***Helicobacter pylori***
	$4 \bullet 10^5$	$4 \bullet 10^4$	$2{,}5 \bullet 10^6$	$6{,}5 \bullet 10^4$	$8 \bullet 10^3$	$2{,}8 \bullet 10^8$
Retrieval on Coupons after						
1 hour [1/cm²]	2,3	-	-	0,33	0,33	167
1 day [1/cm²]	1,7	-	-	0,33	-	43
8 days [1/cm²]	1	-	48	-	-	-
16 days [1/cm²]	-	-	24	-	-	-

Table 2: Contamination and outwash of parasites and *Helicobacter pylori* at certain time intervals

	Injection (Total counts)			Outwash [organisms/100mL]		
time [min]	***Crypto-sporidium***	***Giardia***	***Helicobacter pylori***	***Crypto-sporidium***	***Giardia***	***Helicobacter pylori***
0	$1{,}6 \bullet 10^4$	$2{,}0 \bullet 10^3$	$7{,}2 \bullet 10^7$			
1				20	10	670
5				0	0	
10				0	0	
15	$1{,}6 \bullet 10^4$	$2{,}0 \bullet 10^3$	$7{,}2 \bullet 10^7$			
30	$1{,}6 \bullet 10^4$	$2{,}0 \bullet 10^3$	$7{,}2 \bullet 10^7$			
45	$1{,}6 \bullet 10^4$	$2{,}0 \bullet 10^3$	$7{,}2 \bullet 10^7$			
60						1

Conclusions

Characteristics of the model

- rapid growth of biofilms on paraffin-coated microscope slides
- biofilm-coated slides are easy to take out of the dish without disturbing other slides
- biofilms grown on slides can be easily prepared for different types of microbiological and/or microscopy examination
- the model is suitable for studying the growth and the behaviour of biofilms on different materials

Studies performed with the model

- biofilms growing in the reactor can be contaminated with pathogens injected at the dish input
- permanent forms of parasites (cysts and oocysts) do not have a high affinity to the biofilm
- *H. pylori* is capable of persisting in biofilms for more than 2 weeks, during which time the number of viable organisms decreases by six log units

Acknowledgements

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