



How to B-TOX

An instruction on how to use our biofilm in wastewater treatment plants

Safety protocol:

- Every employee needs to have an **instruction** into genetically modified organisms (GMOs) and biosafety prior to working - with our biofilm to make sure they are aware of all risks
- Always wear the following **protective gear** when working with the biofilm:
 - Protective clothing (e.g. lab coat)
 - Protective goggles
 - Gloves for single-use (made from nitrile or latex)
- When the skin comes into contact with the biofilm, immediately disinfect and wash with soap:
 - Rub the disinfectant over the skin until it is dry
 - Scrub your hands (or other parts of the skin) at least 20 seconds with soap before rinsing them with water
- Anything that has come or might have come into **contact** with the biofilm is either **to be thrown away or needs to be sterilized**
- Any **trash** that occurs from working with the biofilm needs to be **sterilized**
- **Do not touch** the biofilm or the carrier, unless it cannot be avoided
- After working with the biofilm always **wash and disinfect** your hands
- Only open the container with the biofilm in immediate vicinity of the clarifier

The risks:

The biofilm contains **GMOs**. These are not allowed to leave an S1-labelled area under any circumstances (biosafety level 1: lowest biosafety level necessary when working with GMOs). **Bacteria of the biofilm escaping** this area could have **severe consequences** like the emergence of pathogenic multi-resistant organisms. Microorganisms are small and hence are usually invisible to the human eye. On that account, the measures as described above should be carried out meticulously. This is the only way to ensure that no GMO is able to escape the plant.

The application:

We will send you glycostocks with our biofilm forming bacteria. The inoculation and biofilm growth should only be performed by experienced employees. We strongly advise you to obey the safety protocol during every step. Since the biofilm will not survive eternally in the clarifier, the carriers need to be exchanged every 2-3 months. In the following, there is a brief instruction on how this is to be executed:

- Once arrived, store the glycostocks at **-80°C** in a designated room (biosafety level 1)
- The designated room has following requirements:
 - Only accessible for trained employees
 - Filters for extract and supply air
 - Separated from other rooms
 - A sterile workbench
- Open the glycostock and inoculate a small amount of LB-medium
- Transfer cell culture into biofilm growth medium containing the biofilm carriers
- Transfer the carriers covered with biofilm into the designated clarifier
- **Do not spill** the content of the container next to the clarifier otherwise it must be decontaminated
- Close and store the container in the designated room
- **Sterilize everything**
- For removal first open a container
- Remove carriers with a net
- directly transfer them to the container
- Once all carriers are removed, **autoclave** and clean them, they can be **reused** afterwards.
- **Sterilize everything**