MULTI ReUse

Flow cytometry for quick determination of microbiology and growth potential in the water

Technology

The majority of waterborne bacteria are non-cultivable and don't form colonies on available standard microbiological culture media. As a consequence, these bacteria are not picked up by cultivation-based detection methods. Among the cultivation-independent methods, flow cytometry is prominent due to its speed and reproducibility of results with different types of water (Hammes et al. 2008; Van Nevel et al. 2017). The diagnostic procedure, which already has a long tradition in the medical field when it comes to blood tests, has evolved into an efficient and practicable analytical method for microbiological water testing over the course of the last few years. The determination of the bacterial concentrations can be performed within 15 minutes and is possible in an online format. Apart from pure quantification the technology allows the differentiation between intact and membrane-compromised bacteria. The integrity of the membrane serves as an indirect viability criterion: live bacteria have intact cell membranes while dead bacteria are often damaged. In addition to the quantification of the total and intact cell numbers in water, characteristic groups of bacteria can be distinguished.

In practice, small volumes of water samples (200 µL to 1 mL) are typically stained with fluorescence dyes that bind to the genetic material of the bacteria. The differentiation between intact and damaged bacteria is based on the fact that some dyes (e. g. propidium iodide, red) only enter bacteria with damaged cell membranes, while others (e. g. SYBR Green) penetrate bacteria independent of their integrity status. The combined use of those dyes is very useful for determining the efficiency of biocide treatments that inflict membrane damage (example in figure 1).

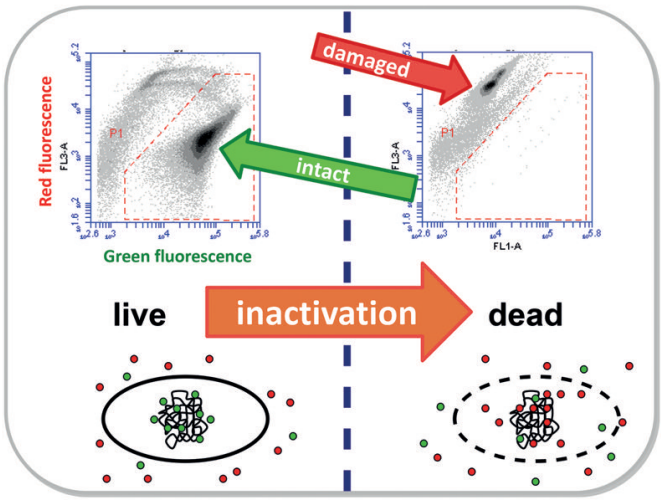

Figure 1: Exemplary flow cytometry density diagram of a bacterial suspension with living/ intact membrane cells (in red-dotted area), resp. with dead damaged membrane cells (outside of the dotted area) after colouration with two dyes; SYBR green and propidium iodide. Every dot stands for a bacterium. Under the fluorescence microscope living/intact bacteria appear in green and dead/damaged bacteria in red.

Flow cytometry for the determination of growth potential

Next to the determination of the number of total and intact cells that represent the actual microbiological state of a water sample, flow cytometry also allows the deter-

mination of the growth potential (Whitton et al. 2018). For this purpose, water samples are collected in sampling vessels devoid of nutrients. Bacterial concentrations are measured on day 0 (= actual state) and after seven days of incubation at a temperature similar to the ambient temperature the water is typically exposed to (for MULTI-ReUse a temperature of about 22 ± 1 °C was chosen). This procedure and an example of a result are shown in figure 2.

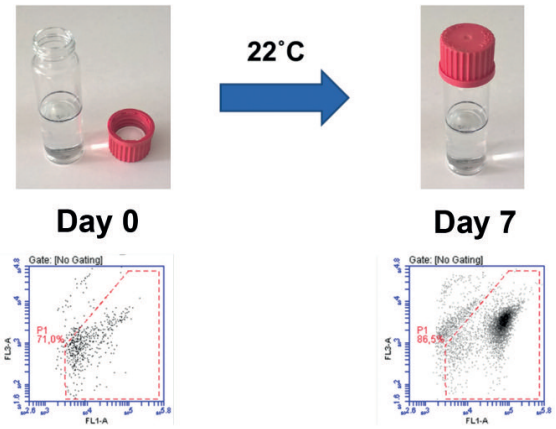

Figure 2: Exemplary presentation of determination of growth potential. The growth is measured by the difference in bacteria numbers on day 0 and day 7. The multiplying factor shows the growth potential that can occur under defined temperatures in stagnant water in the worst case.

In case that assimilable organic nutrients are available in the water sample, the bacteria multiply within the given time period. The difference between bacterial concentrations on day 0 and day 7 is expressed as a factor that is referred to as growth potential. The more nutrients are present in the water sample on day 0, the higher the growth potential.

Change in bacteria concentration during water treatment

The change in bacterial concentration during the treatment of a wastewater treatment plant effluent can be visualized by flow cytometry. Figure 3 shows the concentration of intact bacteria in the effluent as well as after ultrafiltration (UF) and reverse osmosis (RO).

While the concentration of intact bacteria in the effluent is almost 107 intact cells per mL, the UF causes a log-reduction of at least three log-units. Since flow cytometry also captures bacteria growing on the permeate side of the membrane (which is not a sterile environment), the performance of ultrafiltration can be considered to be substantially higher. The number of bacteria after RO is under the detection limit of this method.

While the flow cytometric measurements of the actual state of the treated water clearly show a reduction of the bacteria in the water, bacterial concentrations after seven days of incubation indicate that the microbiological growth potential in the water was reduced remarkably less (red bar in figure 3). This regrowth is caused by the nutrients contained in the system. Bacterial growth during water reuse can possibly cause multiple technical complications, e. g. the blocking of nozzles in agricultural irrigation systems or excessive formation of deposits at the walls of industrial pipe systems.

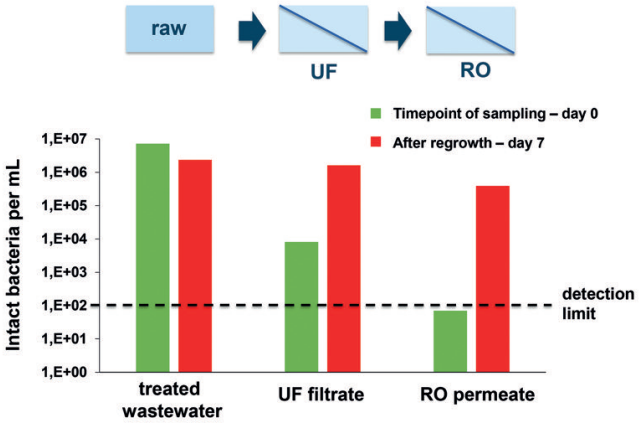

Figure 3: Concentrations of inactive bacteria in the effluent (raw water), resp. after undergoing the ultrafiltration (UF) and the reverse osmosis (RO) processes on the day of the water sampling (Day 0, green bar), resp. after bacterial growth (Day 7, red bar). The detection limit of flow cytometry is at about 100 bacteria per mL.

Microbiological regrowth is therefore usually suppressed by adding a biocide. Flow cytometry can help with the decision about the biocide concentrations necessary to sustainably suppress bacterial growth. Figure 4 shows the effect of different concentrations of hypochlorite on bacterial regrowth in an ultrafiltrate. It could be shown that a disinfectant concentration of ≥ 2 mg/L efficiently suppressed microbiological regrowth in the specific water that was studied over the course of two weeks.

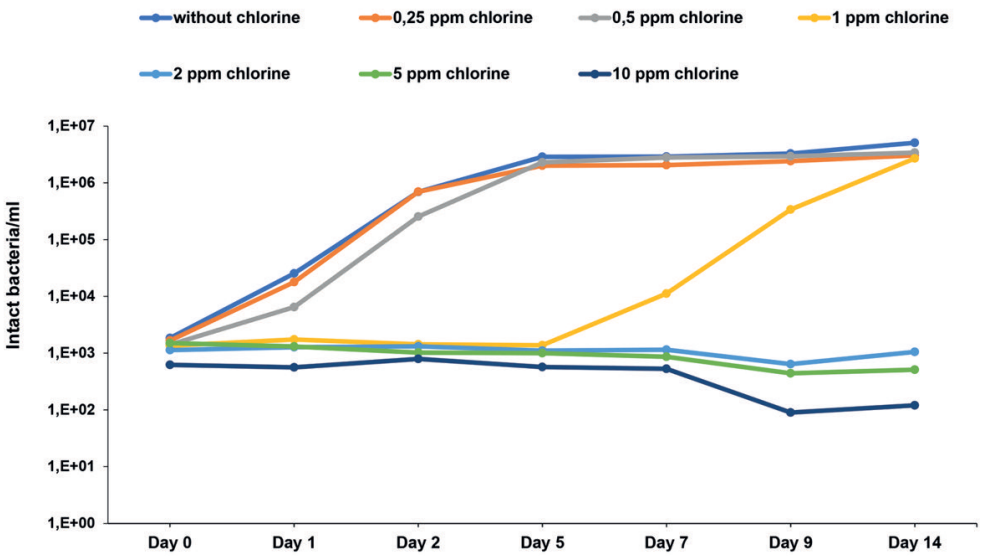

Figure 4: Determination of the sustainability of different chloride concentrations (hypochlorite) on the bacterial growth in UF permeate via flow cytometry.

Conclusion

As for other water types, flow cytometry offers fast and reliable determination of bacterial cell numbers in the field of monitoring water reuse processes. Based on the fact that the detection isn't based on the cultivation of the bacteria, the entire bacterial population in the water is measured independent of their growth requirements. While traditional hygienic indicator bacteria such as coliforms, intestinal enterococci or clostridium perfringens are typically not detectable after membrane filtration and total colony counts are only available after 2–3 days, flow cytometry offers a sound data base for the microbiological assessment of the efficiency of different water treatment steps. Information about the actual microbiological status of a water sample, i.e. about the total and intact cell concentration, is supplemented by information about the microbiological growth potential and therefore indirectly about the assimilable organic nutrients contained in the water. The method is compatible with the 'Hazard analysis and critical control points' (HACCP) concept as the rapid detection of changes in the microbiology provides a good basis for process control decisions.

Literature

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Short description of the MULTI-ReUse project

Treated wastewater is an important part of the water cycle. It usually is fed into rivers, something that is acceptable from an environmental point of view but for the use in agriculture or industry the water often is unsuitable. MULTI-ReUse closes this gap by developing and implementing of new procedures for the reuse of service water. The aim of MULTI-ReUse therefore is the development, demonstration and evaluation of a modular water treatment system, in order to offer service water in different qualities and quantities for the different purposes and to competitive prices.

Imprint

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