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**Paper 8.2**

# Biofilm formation at non chlorinated swimming pool conditions

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## Abstract
This study is part of the DIPool project. In the DIPool Project, an alternative pool water treatment concept is developed, based on UV technology. Control and reduction of biofilm formation is an important step in this project. The objective of this study is to develop a concept that limits biofilm formation by controlling nutrient concentrations in the pool water with different treatment techniques. Furthermore the biofilm will be reduced by using mechanical and/or chemical cleaning methods. The concept is optimized by using special materials and coatings.

This paper focuses on the key parameters influencing the biofilm formation. The results on biofilm formation can also be used to reduce biofilms in chlorinated pools. These biofilms may provide a habitat and shelter to pathogens like *Legionella* and *Cryptosporidium*. At Delft University of Technology, in a lab scale installation, water is recirculated through an open flow-lane system, at swimming pool conditions and exposed to daylight. Different nutrients of a bathing load cocktail are added to the recirculating water. The effect on the biofilm formation is determined by analyzing parameters like dry weight, ATP, DOC and microbial numbers by qPCR. The experiment is started with dosage of citric acid and urea and later with dosage of a more realistic body fluid analog.

## 1. Introduction
This study is part of the DIPool project (Dutch Innovative Pool). Within this project a new pool water treatment concept is developed based on advanced UV treatment for disinfection. The project is based on a multi disciplinary approach in several sub projects. All the sub projects are closely related to each other (figure 1).

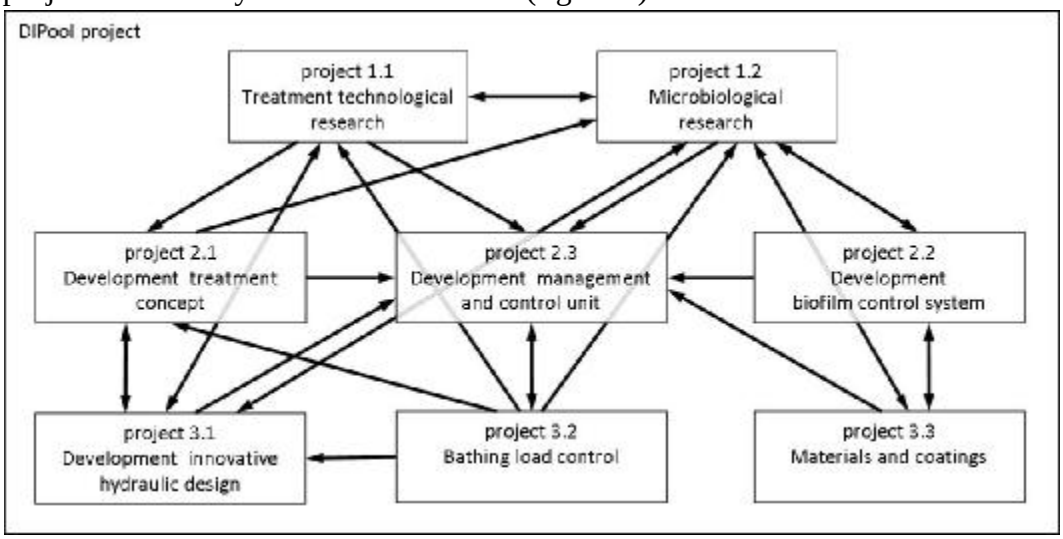


Figure 1 Projects within DIPool project

Treatment technological research on different techniques in the absence of a residual disinfectant is investigated in project 1.1. The result is a new treatment concept that will be tested in project 2.1. Microbiological research is done in project 1.2. The result of this research is a biofilm control system which is developed in project 2.2. The pool water quality within the DIPool project can not easily be managed and controlled by measuring the concentration of a residual disinfectant. Therefore a new technique to manage and control the pool water quality within the DIPool concept is developed in project 2.3. Project 3.1 focuses on the hydraulic design of swimming pools with the DIPool concept. A mixed hydraulic system, desirable in a chlorinated pool, is not desirable in the DIPool concept because contaminations and pollutants are diluted in the pool and not quickly removed from the pool basin to the pool water treatment. Project 3.2 focuses on the management and control of human bathing load. Project 3.3 finally focuses on the effect of special materials and coatings on biofilm formation and the cleanability of these biofilms.

The results in this paper are part of project 1.2, microbiological research. The success rate of the DIPool concept strongly depends on the microbiological control of swimming pool water at non-chlorinated conditions.

Biofilms can be defined as a community of microorganisms with extracellular products attached to a surface [1]. The formation of biofilms can be viewed as a succession process which occurs in three major steps [2]:

1) attachment of single cells; initial diversity of microbes is high;
2) clonal growth; diversity decreases as competition on the surface increases;
3) biofilm maturation; diversity increases because the biofilm community facilitates a variety of microhabitats.

Formation of biofilms in swimming pools reduces in the presence of a residual disinfectant.

Biofilms are unwanted in swimming pools for hygienic, aesthetic and safety reasons. From a hygienic point of view, biofilms are unwanted because they provide a shelter for pathogens like *Pseudomonas aeruginosa*, *Legionella spp*. and *Cryptosporidium* [3]. *Pseudomonas* and *Legionella* can easily multiply in a biofilm under swimming pool conditions. These pathogens can be harmful to visitors and need to be removed or inactivated. From an aesthetical point of view, biofilms are unwanted, especially when they result in a contrasting colour in a swimming pool, like brown or green. Next to the visual aspect, a slimy biofilm surface is also unwanted. Surfaces with a slimy surface are also slippery and can cause accidents and are therefore also unwanted from a safety point of view.

The formation of biofilms in chlorinated swimming pools is controlled by the level of chlorine. Although biofilms seem to have no occurrence in chlorinated pools, they most certainly are present, but they will not form large amounts of slimy surfaces in chlorinated pools [4]. In storage tanks, sand filters and activated carbon filters, biofilms are present and even play an important role in pool water treatment by removing Urea from the pool water. Urea is a parameter in Dutch legislation [5] for swimming pools and each swimming pool is monthly measured by a laboratory on this parameter. Most Dutch pools therefore have a biological filtration to remove urea. Biological activity will mainly occur in the absence of chlorine. Activated carbon is therefore used in chlorinated swimming pools as filter medium to remove the chlorine from the pool water and provide a shelter for bacteria. Since a Dutch Legionella outbreak in 1999 [6], Dutch swimming pools are regularly checked for Legionella.

These bacteria can be found in biological filters and need to be monitored closely to prohibit (re)contamination of the pool water.

Several parameters influence biofilm formation. Growth accelerating parameters in swimming pools are: water temperature, presence of nutrients and presence of daylight. Growth reducing parameters in swimming pools are: efficiency of mechanical or chemical cleaning and use of growth reducing chemicals. Biofilm morphology influencing parameters in swimming pools are: flow velocity of pool water at pool basin surfaces, level of mixing in the pool basin and pool water system, the use of chemicals and the use of special materials and coatings. This research focuses on the determination of the level of influence of these different parameters. The different parameters are varied between realistic boundaries. A special biofilm incubator was used for this study.

## 2. Materials and methods

### *2.1 Biofilm incubator*

Biofilms are cultivated in a temperature controlled flow-lane incubator which was used in previous research [7]. This flow-lane incubator system contained four separate flow channels, 1.5 m long, 10 cm wide (figure 2).

Figure 2 Biofilm incubator

Through which water was circulated over a surface covered with glass or polycarbonate slides (76 x 25 x 1 mm). The slides were used as a substratum for biofilm adhesion (figure 3). The incubator had a variable depth from 1-5 cm and was covered with a transparent lid which enabled air circulation, but disabled contaminations with external solids like dust. The circulation speed of the water could be regulated precisely.

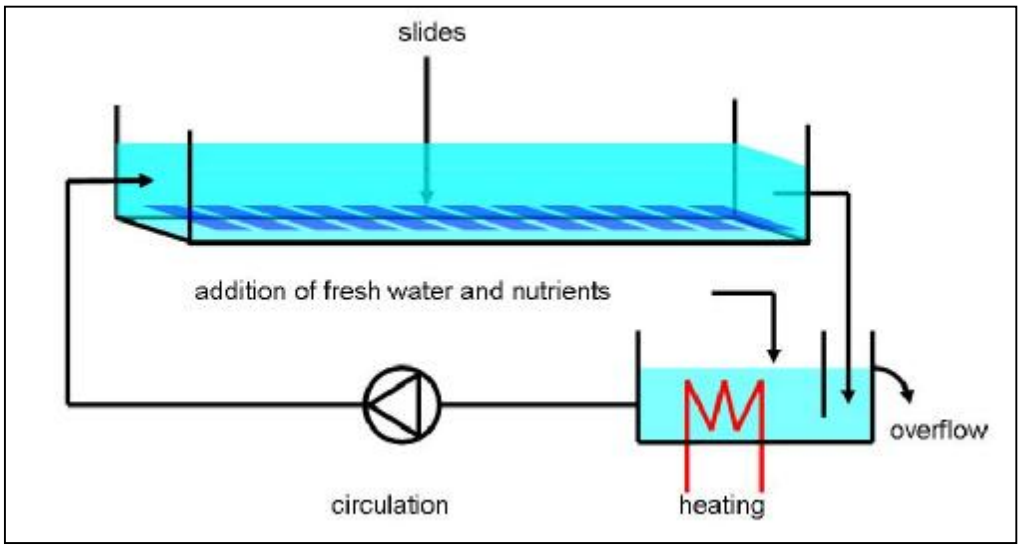

Figure 3 Schematic of biofilm monitor

The biofilm formation on the slides was monitored in time, by removing them and quantify bacterial growth. The flow lane was placed in a laboratory at room temperature. Delft municipal tapwater was used to fill and refresh the flow lane. Four flow lanes could be operated simultaneously with different settings for each flow lane.

Refreshment of the content of the biofilm incubator was comparable with the turnover in a realistic pool basin in order to keep nutrient concentrations constant in time. The flow velocity on surfaces in the incubator will be realistic compared to the real velocities on surfaces in pool basins. Water velocities induce shear stress which affects the biofilm formation. High shear stress leads to the formation of a smooth and dense biofilm [8].

Maximum shear stresses are found near a skimmer outlet or fresh water inlet, were velocities can go up to 2 m/s, but will reduce quickly at greater distances from the outlet/inlet. At an overflow edge, the surface flow velocity can be calculated from the circulation flow and the length of the overflow edge. This velocity at the overflow edge was calculated for a small competition pool at 0,27 m/s. The maximum velocity tested in the incubator is twice the calculated flow on the overflow edge. Low surface velocities can be down to zero at poor circulated areas. Minimum flow velocities can be estimated in a pool basin from pool floor to pool water surface, which is 0,5 m/h in a small competition pool. Single experimental setups will run 2-8 weeks to determine the effect of the chosen variables.

The influence of nutrients, which are pollutants from swimmers, is tested first. Starting with Citric Acid as a Carbon source at different concentrations followed by Citric Acid combined with Urea for more realistic C/N distribution. At the end of the research a realistic synthetic dynamic bathing load is dosed to simulate swimmers and their behaviour. Results from the bathing load experiments are used to determine minimum and maximum concentrations and a realistic C/N distribution.

The influence of specific chemicals, materials and coatings on the biofilm formation is also included in the study. Chemicals with different characteristics were selected and the effect of continuous or shock treatment and different concentrations on biofilm formation are

investigated. Continuous used chemicals are selected carefully because they must be used in the presence of swimmers in the pool. Chemicals for chock treatment are used in the absence of swimmers and therefore have to meet less criteria. The influence of special materials and coatings on biofilm formation is tested in combination with the effect of mechanical and chemical cleaning.

The level of mixing can not be tested on this lab scale installation. This is tested on a more realistic scaled pilot plant in project 1.1 of the DIPool project. On this lab scale installation, the nutrients are well mixed, in contrast to a real swimming pool situation where pollution from swimmers are more concentrated in the region where the swimmers are; the top layer of the pool basin [9].

### *2.2 Experimental procedures*

#### 2.2.1 Cleaning procedures

The biofilm incubator was cleaned before each experiment. A warm soda solution was prepared with Delft municipal tapwater and a concentration of 10 g $NaHCO_3$ /l with a temperature of 50°C. The warm soda solution was recirculated in the biofilm incubator for 5 minutes. The surfaces of the biofilm incubator were mechanically cleaned with a brush during this recirculation. After 5 minutes recirculation and mechanical cleaning, the biofilm incubator was drained and rinsed with Delft municipal tapwater.

#### 2.2.2 Dosing chemicals

Citric acid and urea were used as nutrients. Stock solutions were prepared using demineralised water and chemicals. The concentration of the stock solutions was calculated from the desired C and N concentrations in the biofilm incubator. Dosed stock solution was verified by determining difference between weight of the stock solution at the begin and end of each specific experiment.

### *2.3 Analytical methods*

Quantification of the bacterial growth within the biofilm was done with Q-PCR. Within this technique, a specific sequence in a DNA sample is amplified and simultaneously quantified as absolute number of copies or relative amount when normalized to DNA input or additional normalizing genes [10].

## 3. Results and discussion

The experiments are currently running and the data on the key parameters influencing the BFP will be finished in 2009. The first results are presented during the conference presentation.

## 4. Conclusions

First conclusions will be in the presentation during the conference.

## Acknowledgements

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**References**


1. Davey, M.E., O'Toole, George A., *Microbial Biofilms: from Ecology to Molecular Genetics.* Microbiol. Mol. Biol. Rev., 2000. 64(4): p. 847-867.
2. Martiny, A.C., Jorgensen, Thomas M., Albrechtsen, Hans-Jorgen, Arvin, Erik, Molin, Soren, *Long-Term Succession of Structure and Diversity of a Biofilm Formed in a Model Drinking Water Distribution System.* Appl. Environ. Microbiol., 2003. 69(11): p. 6899-6907.
3. Leoni, E., Legnani, P., Guberti, E., Masotti, A., *Risk of infection associated with microbiological quality of public swimming pools in Bologna, Italy.* Public Health, 1999. 113(5): p. 227-232.
4. Goeres, D.M., Palys, T., Sandel, B. B., Geiger, J., *Evaluation of disinfectant efficacy against biofilm and suspended bacteria in a laboratory swimming pool model.* Water Research, 2004. 38(13): p. 3103-3109.
5. VROM, *Besluit Hygiene en Veiligheid Badinrichtingen en Zwemgelegenheden*, M.v. VROM, Editor. 1984.
6. Den Boer, J.W., Friesema, I. H. M., Hooi, J. D., *Reported cases of legionnaire's disease in the Netherlands, 1987-2000.* Gemelde legionella-pneumonie in Nederland, 1987-2000, 2002. 146(7): p. 315-320.
7. Roeselers, G., *Microbial Ecology of Phototrophic Biofilms.* 2007, Delft.
8. Rochex, A., Godon, J.J., Bernet, N., Escudié, R., *Role of shear stress on composition, diversity and dynamics of biofilm bacterial communities.* Water Research, 2008. 42(20): p. 4915-4922.
9. Ayar, K., *Zwembaden*. 1992, TU Delft. p. 270.
10. Higuchi, R., Fockler, C., Dollinger, G., Watson, R., *Kinetic PCR analysis: Real-time monitoring of DNA amplification reactions.* Bio/Technology, 1993. 11(9): p. 1026-1030.