

First successful application of developed specific gene probes for monitoring early biofilm forming bacteria in paper mill samples

The first process relevant tools developed in the AquaFIT4Use project are successfully applied and will be validated in the next months.

Water samples originating from the same spots along the water process flow of paper mill SAPPI Maastricht (SAP) sampled during different years (autumn 2008 and 2009) were analysed with a set of gene probes using the VIT-Profilig approach by Vermicon (VER). The cultivation-independent population profiles were established and compared. Different sample types showed different population profiles but each sample compared to the corresponding sample from the other year showed similar population patterns (see Fig. 1).

Thereby, most interesting population profiles regarding present diversity were retrieved for samples from production process. These samples were dominated by bacteria of the *Betaproteobacteria* and *Cytophaga-Flexibacter* subphylum. Both groups together represented more than 90% of all viable bacteria cells within these samples. All other analysed bacterial groups were underrepresented in these samples. More specific analysis with newly developed gene probes revealed that most bacteria of these two groups were related to *Tepidimonas arfidensis* (*Betaproteobacteria*) and *Cloacibacterium* spp. (*Cytophaga-Flexibacter* subphylum), respectively.

The obtained results are consistent with results of an earlier EU-project (“ODOUR CONTROL”) and confirm the findings of recent studies considering these two bacterial groups together with *Rhodobacter* species (*Alphaproteobacteria*) as the early biofilm forming bacteria in paper mills with neutral to alkaline pH process conditions (Tiirola et al., 2009). Our current findings within AquaFIT4Use project support these studies by the detection of at least two (*Tepidimonas*, *Cloacibacterium*) of the three bacterial groups in large quantities directly within samples of two different paper mills with neutral to alkaline process conditions. *Rhodobacter* was not detected in the analysed samples. Moreover, using specific probes the absence of *Deinococcus geothermalis* and *Meiothermus* was analysed. Both bacteria are supposed to be relevant as early biofilm forming bacteria in paper mills with acidic conditions. The findings of vermicon support strongly the theory that the biofilm formation in paper mills is pH-dependent.

Ongoing analysis with the developed specific gene probes in paper mill samples will deliver valuable insights and will help to elucidate the process of biofilm formation and succession *in situ*. Whereas, *Tepidimonas arfidensis* and *Cloacibacterium* seem to have a high potential to act as biofilm indicator organisms in paper mills with neutral to alkaline process conditions, the role of *Rhodobacter* is currently under investigation.

Possible effects of strategies for inhibiting biofilm formation by these organisms can easily be monitored by application of the new specific gene probes. If the relevance of the bacteria can be confirmed in further studies first easy-to-use testkits targeting *Tepidimonas* and *Cloacibacterium* for monitoring biofilm formation and success of biofilm inhibition strategies in paper mills will be developed for general use.

References:

Tiirola, M., Lahtinen, T., Vuento, M., Oker-Blom, C. 2009. Early succession of bacterial biofilms in paper machines. J. Ind. Microbiol. Biotechnol. 36:929-937.

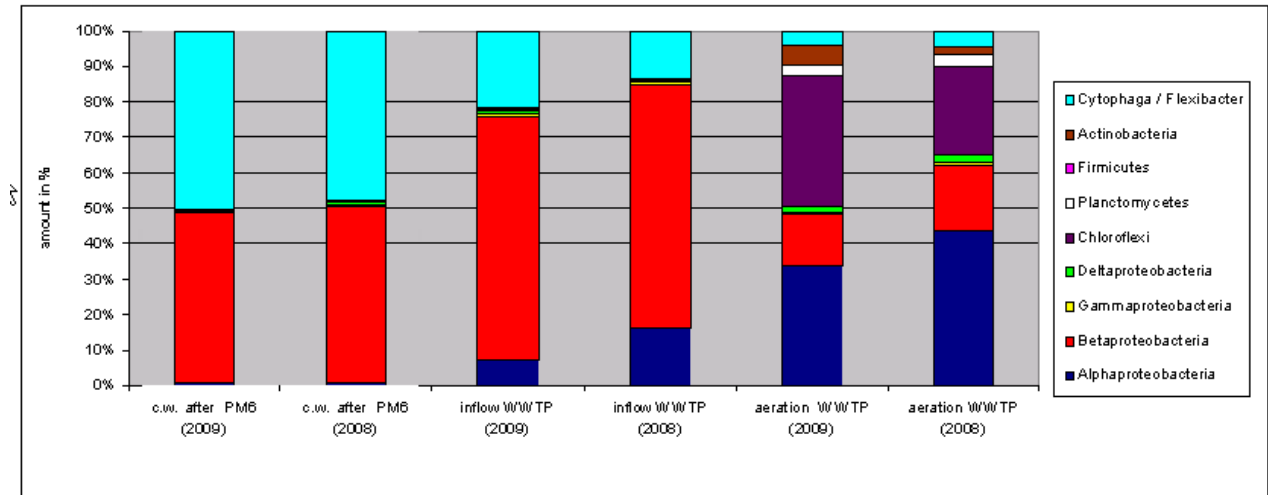


Figure 1: Diagram of obtained gene probe results. (Graphical) Comparison of population profile data obtained by VIT-Profiling analysis on corresponding sampling spots in paper mill SAPPI taken during different years (2008 and 2009).

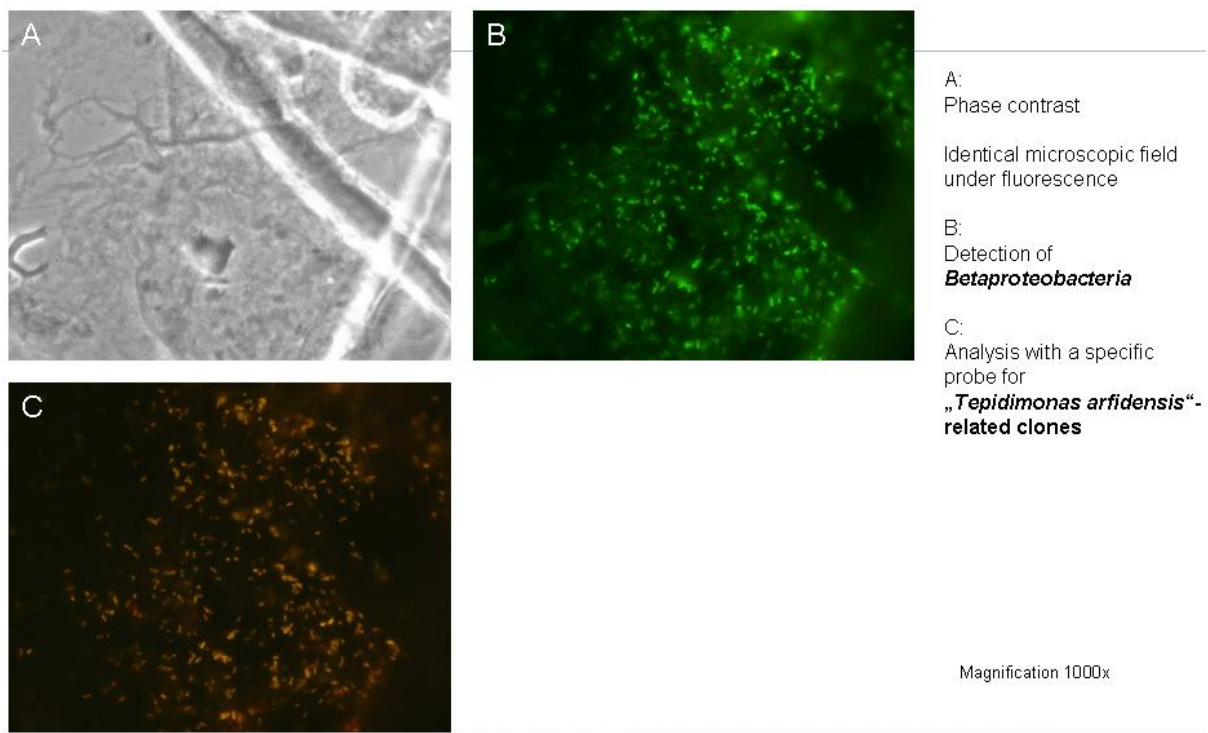


Figure 2: Detection of bacteria related to *Tepadimonas arfidensis* in sample “clear water after filtration at PM6 (2009).