Assimilable organic carbon (AOC) detection: a step towards understanding microbiological stability of drinking water

Frederik Hammes and Thomas Egli

Microbial regrowth in drinking water can adversely affect the hygienic and aesthetic quality of the water. Eawag has developed a fast and accurate method for determining regrowth determining assimilable organic carbon (AOC) in water. In combination with flow cytometric assessments of the viable microbial population in a water sample, this has the potential to provide new insights into the microbial stability of water.

Limiting heterotrophic regrowth

From a microbial perspective, the aim of drinking water treatment is to eliminate all pathogenic bacteria and to minimise the presence and potential regrowth of heterotrophic bacteria in the distribution system. Such regrowth can give rise to biofilm formation in pipelines which in the long run causes operational problems such as biofouling and biocorrosion. Microbial regrowth can also adversely influence consumer preferences, such as the taste and odour of the water, or in the worst case, it can lead to potential health hazards caused by pathogenic proliferation. Assimilable organic carbon (AOC) is low molecular weight dissolved organic carbon that can easily be utilised by bacteria leading to growth. Previous studies have already related the concentration of AOC in water to heterotrophic regrowth (van der Kooij, 1992). Even though this is an important parameter it is not yet regularly measured as routine parameter in drinking water treatment. The main reason for this is that existing AOC assays are time and labour consuming, and few clear guidelines on the interpretation thereof are available.

A rapid method

AOC is usually measured by measuring the total concentration of a pure culture inoculum that has grown into stationary phase in a water sample. We have developed a new method for AOC determination using a natural microbial community as inoculum and fluorescent staining and flow cytometry for enumeration of the cells (Hammes and Egli, 2005). The advantage of this method is that the natural microbial community gives a realistic interpretation of the AOC content, because it covers a broader substrate range than single pure cultures do. In addition, flow cytometry is an extremely rapid
and accurate method for cell enumeration, which allows the processing of large numbers of samples as well as the collection of additional kinetic data (Figure 1). A large inoculum density and high incubation temperature (30 °C) speeds up the analysis time to about 24 – 48h.

**Figure 1.** Growth kinetics for a natural microbial community growing on natural assimilable organic carbon. Total cell counts were measured with SYBRgreen staining and flow cytometry, while ATP was measured with the luciferin-luciferase assay.

**Standardisation and application**
As part of the Techneau project (WA 3) the Eawag AOC assay is being standardised for common use. The optimised assay was tested during the last 6 months in a local drinking water treatment and distribution system (Lengg, Zürich; 70,000 m³/day). We have also used the assay to characterise specifically the mechanistic and kinetic aspects of AOC formation during the ozonation of surface water (Hammes et al., 2006). In similar fashion to ozone, AOC is also formed from conventional treatments such as chlorination, thus questioning the applicability of this to drinking water as a final treatment step.

**Interpretation: a niche for regrowth**
AOC as a key parameter for drinking water stability should, however, not be viewed in isolation. The degree of microbial regrowth is as dependant on the concentration of viable bacteria in the system as on AOC. Hence, we combine flow cytometric absolute cell counting with fluorescent viability markers to characterise accurately the total viable and active population in a water sample. This biomass
concentration, relative to the available nutrients, provides a more realistic interpretation of potential regrowth in.

References