

# Analysis of microbiological stability in different water samples

## Introduction

Microbial water quality is an important analysis in drinking water as past research has determined that uncontrolled and excessive microbial regrowth can lead to a deterioration of aesthetic water quality, leading to undesirable tastes or odors. It can also promote the growth of opportunistic pathogens, and treatment process malfunctions such as clogging of filters, biofouling, and biocorrosion (Gatza, Hammes, & Prest, 2013). One way to control the microbial regrowth in drinking water distribution systems (DWDS) is by the addition of disinfectants such as chlorine, chlorine dioxide, or monochloramine after the treatment train (F. Hammes, Berger, Köster, & Egli, 2010). This method has been effective for over a century, but there are some consequences. Some bacteria are resistant to chlorine, there are some health risk associated with disinfection by-products, and there is a negative consumer perception associated to the chlorinous taste (F. Hammes et al., 2010). Looking for new alternatives that can help control and stabilize the microbial population in DWDSs is a new challenge.

Scientists are studying biological stability as an option to confront the regrowth of microorganism in DWDSs. Biological stability (biostability) is defined as the inability of drinking water to support microbial proliferation (F. Hammes et al., 2010). The two key parameters for biostability are biologically available substrate and microbial biomass (F. Hammes et al., 2010). Removing the nutrients (e.g. carbon, nitrogen, and phosphorus) in water that support the proliferation of microorganism is a present trend in drinking water treatment (F. A. Hammes & Egli, 2005). Carbon, specifically assimilable organic carbon (AOC), is the limiting nutrient in most drinking waters (F. A. Hammes & Egli, 2005). Past research has determined some guidelines that indicate the ideal concentrations of AOC in DWDSs to achieve biostable water; for water in the absence of disinfectants <10 mg/l, and for water in the presence of disinfectants <100 mg/l (Lautenschlager et al., 2013).

## Objectives

In order to prepare a much larger experiment, it is better to first understand how a smaller system works. The major task was to develop a system to simulate the conditions of a drinking water storage tank and DWDS. Analyses pertinent to DWDS, like microbiological measurements, nutrient, and chlorine concentrations are essential for understanding the quality of water. The water was collected from Strawberry Creek, a stream passing through UC-Berkeley (UCB) campus, from the tap at the 303 laboratory in the O' Brian Hall at UCB and from the Chevron Refinery's Richmond Advanced Recycling Expansion (RARE). Although such a system was not implemented, microbiological measurements were carried out, as detailed below.

## Methods and Results

### *Flow Cytometry (FCM)*

FCM was used to determine the amount of microorganisms in the water samples. The Eawag, Switzerland staining protocol and the BD Accuri™ C6 software analysis template were used (Berney, 2006; für Gesundheit, 2012; F. Hammes et al., 2008). Two fluorescent, nucleic-acid staining dyes were utilized for counting cells. SYBR Green I (SG) was used for differentiating between cells and debris to obtain the total cell counts in the water sample, and the SYBR Green I mix with propidium iodide (SGPI) was used to differentiate between intact cells and damaged cells. All samples were filtered through a 70-micron filter to avoid clogging the flow cytometer. The samples were done in triplicate.

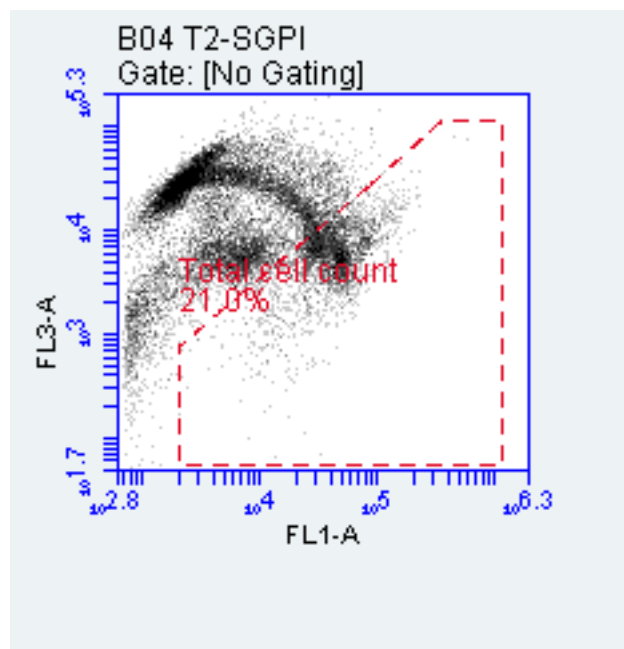


Figure 1: Intact cell count of tap water using SGPI staining

### *Assimilable Organic Carbon (AOC)*

The AOC measurements represent small organic molecules readily available for uptake into the microbial cells. The sampled water was filtered through a 0.1-micron filter to remove all the cells in water. The filtered water was then inoculated using bottled Evian water to yield approximately  $1 \times 10^4$  cells/mL in the sample. These samples were incubated in a water bath for 3 days at 30°C. The microbial growth was measured using the total cell count protocol of flow cytometry. The concentration of carbon was obtained by measuring the cell growth after the incubation, using the equation of  $\text{AOC } \mu\text{g/L} = \text{net growth cells/L} / \text{conversion factor } 1 \times 10^7 \text{ cells}/\mu\text{g}$  (F. Hammes et al., 2010). All samples were done in triplicate.

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