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Surrogate parameters for the rapid microbial monitoring in a civil protection module used for drinking water production

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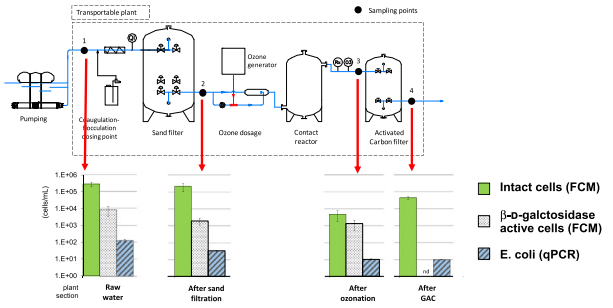
HIGHLIGHTS

- A mobile drinking water treatment plant used for civil protection was monitored.
- Intact cells, coliforms and *E. coli* are proposed as surrogate microbial parameters.
- Intact cells and coliforms were measured in less than 1 h using flow cytometry.
- *E. coli* cells were measured in less than 3 h using qPCR.
- High presence of viable-but-not-culturable cells in all the stages of the plant.

GRAPHICAL ABSTRACT



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ABSTRACT

Rapid analyses based on flow cytometry (FCM) and quantitative PCR (qPCR) were proposed and applied in a full-scale mobile water treatment plant (flow rate of 4.4 L/s) utilized as a civil protection module for drinking water production for quasi real-time monitoring. The rapid methods applied here are two cultivation-independent techniques (FCM and qPCR). The microbiological quality of water was monitored on the basis of alternative microbial parameters, detecting cells with an intact and permeabilised membrane (in 20 min), cells with β-D-galactosidase activity (in 40 min) and *Escherichia coli* (*E. coli*, in less than 3 h). These rapid techniques were compared with some conventional culturable bacteria groups (aerobic mesophilic bacteria, total coliforms and *E. coli*).

Although intact bacteria were two orders of magnitude higher than culturable aerobic mesophilic bacteria (due to a large fraction of viable-but-not-culturable cells, but also chemolithotrophic bacteria), they both showed not significant reduction in cells after filtration, 2–3 log of removal after ozonation and a regrowth of about 1 log after granular activated carbon.

Cells with β-D-galactosidase activity (belonging to the group of total coliforms) were higher than culturable total coliforms, due to a large presence of active-but-not-culturable cells, especially in ozone treated water.

E. coli quantified by qPCR decreased through filtration and they were under the quantification limit after ozonation, analogously to culturable *E. coli*. Despite a higher quantification limit for FCM and qPCR, they appear sufficiently accurate and suitable as surrogate microbial parameters, considering their

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rapidity (about an half hour with FCM). In the case of strong stress conditions such as ozonation, the surrogate microbial parameters, which include viable-but-not-culturable cells, might result more sensible in the evaluation of treatment efficiency.

1. Introduction

The World Health Organization has developed the Water Safety Plan approach [34,35] with the aim of providing safe drinking water. Quantitative Microbiological Risk Assessment (QMRA), a framework for evaluating infectious risks from human pathogens, has also been developed for managing waterborne microbial hazards, particularly where the fraction of diseases attributable to drinking water is low or difficult to be evaluated [35]. In QMRA, reference pathogenic microorganisms have to be directly quantified in the source water, whilst they can only be indirectly estimated in the supplied water on the basis of their reduction during the treatment.

In several legislations in the industrial sector, the performance in pathogen reduction can be easily estimated using: (1) surrogate parameters such as turbidity (for removal) or the $C \times T$ concept (for inactivation) where the $C \times T$ value is the disinfectant concentration C multiplied by the contact time T applied for water disinfection; (2) conventional cultivation-based methods such as heterotrophic plate count (HPC) and faecal indicators.

If turbidity and $C \times T$ can be monitored with intensive frequencies or on-line to reduce uncertainties in the abatement efficacy, the analyses of HPC and faecal indicators require relatively long periods of time to be completed (1–2 days). HPC indicates the load of bacterial water contamination [26] without information about bacterial group or species, and it was removed as parameter to indicate safety and quality of drinking water in the European Union legislation.

Total coliforms, faecal coliforms and/or *Escherichia coli* are indicators of a possible contamination with faecal material (*inter alia* [30,37]. Their quantification is not suitable for automation or on-line monitoring, not allowing the making of immediate decisions about water quality. Therefore, surrogate microbial parameters able to offer rapid results (from a few minutes to a couple of hours) appear very interesting to the monitoring of water quality. The availability of a fast and sensitive indication of microbial contamination might support field monitoring of raw water sources, management and optimization of disinfectants dosages or the "early warning" of accidental events.

Two rapid, cultivation-independent tools to evaluate the microbiological parameters are flow cytometry (FCM) and quantitative PCR (qPCR), which appear very attractive and promising to quantify surrogate microbial parameters in water samples.

FCM, a cultivation-independent analysis at single-cell level for high-throughput and real-time cell quantification, was introduced about two decades ago but its routine application to investigate and quantify microbial concentration in the water sector is just in its infancy and few applications are available (*inter alia* [6,10,27]. FCM, coupled with fluorescent dyes, permits the identification of bacterial groups on the basis of their cellular properties [5,16,28,39].

qPCR is an indirect rapid method to detect and enumerate unique genetic sequences within target bacteria in few hours. qPCR has been proposed in the literature as a good tool to quantify faecal-indicator bacteria such as enterococci or *E. coli* in environmental waters [13,19,22,24,25] but up to now there is a need for further investigation and understanding in this field.

This paper is a contribution in the field of water treatment with the aim of applying FCM and qPCR to obtain a very rapid measurement of some microbiological parameters proposed as surrogates of conventional microbiological parameters based on cultivation methods.

Total bacterial cells, intact or permeabilised bacterial cells and cells with β-D-galactosidase activity (belonging to the group of total coliforms) were quantified by FCM whilst *E. coli* cells by qPCR. All these bacterial groups were monitored during drinking water production in a full-scale mobile water treatment plant used as a civil protection module. To evaluate the feasibility of doing rapid analyses with FCM and qPCR the results were compared with the data obtained by standard conventional cultivation-based methods.

This research, which cannot be exhaustive on the use of FCM and qPCR in monitoring drinking water microbial quality, has the scope of adding some new results, comparing new fast approaches with conventional cultivation-based methods and supporting a critical discussion in an area which requires further investigations in the near future (*inter alia* [8].

2. Materials and methods

2.1. Full-scale drinking water treatment plant

Raw water was pumped from the Adige river (North-East of Italy) and treated in a mobile full-scale plant for drinking water production. The mobile unit was used by the local protection agency in emergency cases. The stages of the drinking water treatment plant are indicated in Fig. 1: (A) rapid sand filtration; (B) ozone dosage and contact reactor; (C) granular activated carbon (GAC).

The raw water was introduced in the treatment plant at a flow rate of 4.4 L/s on average, with a temperature of around 12 °C and pH in the range of 6.3–6.6. The raw water pump was used for generating the necessary operating pressure at the inlet of the sand filter (1.3–1.7 bar), with a pressure drop across the filter of about 0.5 bar. The two-layer sand filter bed (diameter 1.2 m, height 2.1 m) was filled with quartz sand and hydro anthracite. Solid separation in the sand filtration stage could be enhanced by dosing a poly-diallyldimethylammonium chloride solution at a dosage of 5 g/m^3, depending on the quality of the entering raw water which might vary in wet or dry periods.

The ozone generator (Ozonfilt, ProMinent, Germany) was fed with air with an average ozone production of about 33 g O_3/h. The ozone gas was injected into the water stream through a hydrokinetic unit followed by a contact reactor with a volume of 0.6 m^3. The contact time t_{10}, that is the length of time during which the first 10% of the water passes through the ozone contact reactor, was 0.6 min as calculated with tracer studies using pulses of sodium chloride. Ozone residual concentration in water was measured with an amperometric sensor (OZE, ProMinent, Germany) and values of 1.2–1.3 mg/L were measured in all the runs. As a consequence, the $C \times T$ value was 0.74 mg L^{-1} min on average.

Residual ozone in water was removed in the subsequent activated carbon filter containing 400 kg of granular activated carbon.

Neither the effects of chlorine or UV disinfection, which could be applied at the end of the water treatment to improve drinking water quality, nor the water quality in the distribution were considered in this research.