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Ozone inactivation of resistant microorganisms: Laboratory analysis and evaluation of the efficiency of plants

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ABSTRACT

In this work, the ozone inactivation of resistant microorganisms is studied and a method to assess the efficiency of a drinking water plant to inactivate resistant microorganisms using ozone is proposed. This method aims at computing the fraction of resistant microorganisms that are not inactivated at the exit of an ozonation step by evaluating the duration of the lag phase of the ozone inactivation of these microorganisms and the contact time distribution of these microorganisms with the ozone in the step. To evaluate the duration of the lag phase of the ozone inactivation of resistant pathogenic microorganisms, an experimental procedure is proposed and applied to *Bacillus subtilis* spores. The procedure aims at characterizing the ozone inactivation kinetics of *B. subtilis* spores for different temperature and ozone concentration conditions. From experimental data, a model of the ozone inactivation of *B. subtilis* spores is built. One of the parameters of this model is called the lag time and it measures the duration of the lag phase of the ozone inactivation of *B. subtilis* spores. This lag time is identified for different temperature and ozone concentration conditions in order to establish a correlation between this lag time and the temperature and ozone concentration conditions. To evaluate the contact time distribution between microorganisms and the ozone in a disinfection step of a drinking water plant, a computational fluid dynamics tool is used. The proposed method is applied to the ozonation channel of an existing drinking water plant located in Belgium and operated by Vivaqua. Results show that lag times and contact times are both in the same order of magnitude of a few minutes. For a large range of temperatures and ozone concentrations in the Tailfer ozonation channel and for the highest hydraulic flow rate applied, a significant fraction of resistant microorganisms similar to *B. subtilis* spores is not inactivated.

1. Introduction

Until now, European standards for assessing the microbiological quality of drinking water are focused only on *E. Coli* and *Enterococcus* (European Union Council Directive 98/83/EC, 1998). New coming standards could probably also consider the inactivation of pathogenic resistant microorganisms such as *Cryptosporidium parvum* oocysts or the inactivation of their indicator species such as *Bacillus subtilis* spores. In order to anticipate these new standards and ensure consumer's health and safety, drinking water producers need new methods to assess the efficiency of their disinfection step. Hitherto, few

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Nomenclature

A_0	pre-exponential constant (min^{-1})
E	reaction activation energy ($J\ mol^{-1}$)
$E(t)$	contact time distribution (min^{-1})
f	fraction of resistant microorganisms similar to *Bacillus subtilis* spores that are still in their lag phase at the exit of an ozonation step (–)
IOD	instantaneous ozone demand of the water entering in the chamber 1 ($mg\ L^{-1}$)
k_a	initial inactivation constant (–)
k_b	kinetic constant for the ozone inactivation of *Bacillus subtilis* spores ($L\ mg^{-1}\ min^{-1}$)
k_{O_3}	kinetic constant of the ozone concentration decrease (min^{-1})
$L(t)$	latency function (–)
l	parameter of the lag time correlation (min)
m	parameter of the lag time correlation (min)
n	parameter of the lag time correlation (min)
Q_G	gas flow rate injected into the chamber 1 of the Tailfer ozonation channel ($L\ min^{-1}$)
Q_L	hydraulic flow rate in the Tailfer ozonation channel ($L\ min^{-1}$)
R	perfect gas constant ($J\ mol^{-1}\ K^{-1}$)
$S_{O_3,0}$	dissolved ozone concentration at the initial time in the batch reactor ($mg\ L^{-1}$)
$S_{O_3,in}$	instantaneous surrounding dissolved ozone concentration when the microorganisms arrive in contact with ozone in the continuous step ($mg\ L^{-1}$)
$S_{O_3}(t)$	dissolved ozone concentration at time t ($mg\ L^{-1}$)
$S_{O_3,G}$	ozone concentration into the injected gas in the Tailfer ozonation channel ($mg\ L^{-1}$)
$S_{O_3,ref}$	reference ozone concentration of 1 $mg\ L^{-1}$
t	time (min)
t_c	contact time distribution between microorganisms and the ozone in a Tailfer ozonation channel (min)
t_L	lag time (min)
t_{ref}	reference time of 1 min
T	temperature (K)
$T_{réf}$	reference temperature of 273.15 K
X_0	concentration in *Bacillus subtilis* spores at the initial time t_0 ($spores\ L^{-1}$)
$X(t)$	concentration in *Bacillus subtilis* spores at the time t ($spores\ L^{-1}$)

methods enabling the producers to fully assess the capacity of a drinking water plant to inactivate resistant pathogenic microorganisms have been proposed. Some previous studies have proposed Computational Fluid Dynamics (CFD) tools to assess an ozonation step in a drinking water plant (Audenaert et al., 2010; Cockx et al., 1999; Talvy et al., 2011). These tools provide information such as the dynamics of the liquid and gas phases, the ozone concentration field in the disinfection step and the contact time between microorganisms and the disinfectant in the process. Nevertheless, these tools do not allow an estimation of the concentration of pathogenic resistant microorganisms at the outlet of the ozonation step. Indeed, few investigations have been done to set up the kinetics of the ozone inactivation of resistant microorganisms. Hence, the inactivation kinetics of resistant microorganisms needs to be established to complete the CFD tools and to get information on the inactivation of pathogenic resistant microorganisms during an ozonation step.

Ozone resistant microorganisms present typical inactivation kinetics. When these microorganisms get in contact with the disinfectant, their resistance results in an initial lag phase during which they are not, or weakly, inactivated. The duration of this phase depends on several parameters upon which the most important is the temperature (Dow et al., 2006). The lag phase is followed by a decaying phase that can be modeled by a pseudo-first order reaction (Dow et al., 2006; Driedger et al., 2001; Larson and Mariñas, 2003).

The general purpose of this paper is to propose a method to assess the efficiency of a drinking water plant to inactivate resistant microorganisms.

The first goal of this work is to draw up and apply to *B. subtilis* spores an experimental protocol to characterize the ozone inactivation kinetics of ozone resistant microorganisms. From experimental data, a model of the ozone inactivation of these microorganisms is built. One of the parameter of this model is called the lag time and measures the duration of the lag phase of the ozone inactivation of *B. subtilis* spores. This lag time is identified for different temperature and ozone concentration conditions in order to establish a correlation between this lag time and these operating conditions, called the lag time correlation.

The second goal of this paper is to assess the efficiency of an existing ozonation step to inactivate resistant pathogenic microorganisms similar to *B. subtilis* spores using the CFD tool proposed by Talvy et al. (2011) and the lag time correlation established in this work. The studied process is the ozonation step of the Tailfer drinking water plant operated by Vivaqua (Belgium). From the CFD tool proposed and validated by Talvy et al. (2011), the contact time distribution between microorganisms and the ozone in this step is determined. Evaluation of the Tailfer plant disinfection step efficiency is obtained by computing the fraction of the resistant microorganisms that are still in their lag phase at the exit of the step, and therefore that are not inactivated, using the lag time correlation and the contact time distribution of these microorganisms with the disinfectant in the step.

The Tailfer drinking water plant, managed by Vivaqua (Belgium), is daily producing 120,000 m^3 and provides drinking water to about 700,000 inhabitants. The plant line is shown in Fig. 1. The water is pumped from the river Meuse and undergoes pretreatments and physical separation steps before entering into ozonation channels. After the ozonation step, the water goes through a post-filtration, a chlorination and a pH correction. Finally, the produced drinking water is transported to Brussels.

In this work, *B. subtilis* spores are used as a surrogate for *C. parvum* oocysts. *C. parvum* oocysts are known to be problematic in drinking water production because of their persistence