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Impact of *Methylobacterium* in the drinking water microbiome on removal of trihalomethanes

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ABSTRACT

A major class of chlorine disinfection by-products in water treatment and distribution systems is the trihalomethanes. When they occur at high concentration in drinking water they may cause serious problems to human health. Little is known about the capacity of bacterial species that are endemic to drinking water to affect the fate of those chlorination by-products. *Methylobacterium* species have been previously found to play an important role in the degradation of another major group of chlorine disinfection by-products: the haloacetic acids. Thus, the role that *Methylobacterium* might play in the concentration of trihalomethanes in drinking water was explored in this study. Concentrations of trihalomethanes were measured in drinking water for different concentrations of *Methylobacterium* and under different organic matter and chlorine concentrations. The results revealed that when the *Methylobacterium* DSM 18358 is present in drinking water, even at a low relative abundance of 1%, it plays a key role in decreasing the concentration of trihalomethanes up to 48% from the initial one after 24 h.

1. Introduction

Disinfectants that are routinely used in drinking water distribution systems (DWDSs) include chlorine, chloramines and chlorine dioxide. Where chlorine or chloramines are used as disinfectants, trihalomethanes (THMs) and haloacetic acids (HAAs) emerge as by-products (Borgmann-Strahsen, 2003 ; Herath et al., 2015 ; Debiec et al., 2017). Trihalomethanes are produced when the hydrogen atoms in methane are replaced by halogen atoms: chlorine, fluorine, bromine, iodine and astatine. Haloacetic acids are produced when the hydrogen atoms in acetic acids are replaced by halogen atoms. These two classes of disinfection by-products are the most common and have demonstrated carcinogenic activity in animals (Pereira, 2000 ; Hamid et al., 2014 ; Jaouadi et al., 2014). Acute effects of THMs in drinking water on humans are rare. However, there are two main long-term health outcomes of THMs in drinking water on humans, which are the cancer, such as rectal, colon and bladder cancer, and developmental or reproductive outcomes (WHO, 2008).

The THMs that are mostly present in drinking water are chloroform, bromoform, dibromochloromethane and bromodichloromethane (Rodriguez and Serodes, 2001). The regulated concentration for THMs is 100 μg/l (WHO, 2008 ; Brown et al., 2011). The guideline value for chloroform is 300 μg/l, for bromoform is 100 μg/l, for dibromochloromethane is 100 μg/l and for bromodichloromethane is 60 μg/l. The sum of the ratio of each of the four levels to their individual guideline value should not exceed 1. Also, the tolerable daily intake for chloroform, bromoform and dibromochloromethane is 15, 21.4 and 17.9 μg/kg/day, respectively. Public water supplies are monitored very frequently for THMs and thus, common concentrations do not exceed the guideline values (WHO, 2008). Chloroform is the most common of THMs and accounts for more than 90% of the total THMs (Krasner et al., 1989). It is found in a variety of environments, such as the outdoor air of urban areas, the indoor air by release from water, a variety of food and soft drinks, and drinking water (WHO, 2008). Examples of anthropogenic chloroform sources are pulp and paper mills, water treatment plants, chemical manufacturing plants and waste incinerators (Cappelletti et al., 2012). Acute health problems from exposure to chloroform are rare and associated with high concentrations. For instance, anaesthesia using chloroform can result in respiratory and cardiac arrhythmias. Occupational exposure to chloroform can cause renal tubular necrosis and liver toxicity (WHO, 2008). There is evidence that the chronic effects of long-term exposure to chloroform might cause cancer and reproductive problems (Waller et al., 1998 ; Gallagher et al., 1998 ; Melnick et al., 1994).

In general, high concentrations of THMs correlate with high concentrations of free and total chlorine, high concentrations of humic and non-humic substances, high temperature, high pH, and high concentrations of bromide ions (Sadiq and Rodriguez, 2004 ; Brown et al.,

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2011). In water treatment, chlorine is applied in one of three forms: as a compressed gas under pressure, which is dissolved in water at the point of application, as sodium hypochlorite solution or as calcium hypochlorite solution. The rate of decay of chlorine concentration in the bulk water is primarily a function of the concentration of organic matter in drinking water, the water temperature and the initial chlorine concentration. However, chlorine can also decay through its interactions with the materials of pipes, wall tanks and fittings, or with the adhering on them biofilms (Brown et al., 2011).

Levels of THMs vary seasonally; concentrations have been found to be higher in summer, when water temperature is higher than in winter (Dyck et al., 2015). In a DWDS it was shown that there were spatial changes for THMs, the concentrations of which were increased and finally stabilised in the distribution system extremities (Rodriquez et al., 2004). However, due to the complexity and uncertainty of reactions between chlorine and organic matter, no successful models of predicting THMs formation had been developed until 2011; most existing models were empirical and therefore they included a number of constants with no physical meaning. Since 2011, there have been many studies on modelling THMs and these models have also considered the effects of the organic matter on THMs formation (Kumari and Gupta, 2015 ; Mishra et al., 2016 ; Singh et al., 2012 ; Azeem et al., 2014 ; Li et al., 2016).

For most of the human population their greatest exposure to chloroform at low concentrations is as a disinfection by-product in drinking water. The primary source of chloroform in chlorinated drinking water has been found to be the reaction between chlorine and naturally occurring organic compounds (Pramanik et al., 2015 ; Venkatnarayanan et al., 2016). Examples of organic compounds that can be found in drinking water supplies come from pharmaceuticals, fragrances, flame retardants, plasticizers, components of personal care products, *etc*. Some of these compounds may not be completely degraded or removed during wastewater treatment (Stackelberg et al., 2007 ; Wang et al., 2017).

Microbial chloroform degradation occurs under both anaerobic and aerobic conditions and has been mostly studied as a cometabolic process with chloroform used by bacteria as carbon and energy source. Alkanes, hydrocarbons and ammonia have been used as growth substrates to support chloroform cometabolism via the activity of enzymes produced by microorganisms (Cappelletti et al., 2012). Methane is a common substrate used for chloroform cometabolism. By the oxidation of methane via the activity of methane monooxygenases, methanol is firstly produced and then methanol is oxidized in three consecutive steps to formaldehyde, formate, and carbon dioxide (Lieberman and Rosenzweig, 2004). Methylotrophs under aerobic conditions and methanogens under anaerobic conditions have been associated with the biodegradation of chloroform (Zamani et al., 2015 ; Cappelletti et al., 2012) as one-carbon organic compounds, such as methane and methanol, can be utilized as carbon sources by these bacteria.

Methanotrophic bacteria include several species such as the genera *Methylobacter* and they are able to form methanol. Methanotrophs are able to utilize only one-carbon compounds, whereas Methylotrophs can also utilize more complex organic compounds. Thus, methylotrophic bacteria such as *Methylobacterium* are facultative methylotrophs. *Methylobacteria* are pink-pigmented facultative methylotrophs and they are ubiquitous in nature (Green et al., 1988 ; Goldman and Green, 2008). There are efficient and inexpensive methods for the cultivation of the *Methylobacterium* genus of bacteria. These bacterial cultures can be used to improve plant agriculture as they play an essential role in plant physiology, including positive effects on nitrogen metabolism, seed germination, and stimulation of plant growth. There have been several methods of using fermentation broths, fermentation broth products, fermentation products and compositions to produce large quantities of *Methylobacterium* to treat plants or plant parts in the US9181541B2 patent (Bogosian, 2015). Also, *Methylobacterium* species have been found to be able to utilize chlorinated methanes as the sole carbon and energy source (Leisinger and Braus-Stromeyer, 1995 ; Kayser, 2001 ; Trotsenko and Doronina, 2003). Thus, based on the US9181541B2 patent these useful *Methylobacterium* species could be densely produced and stabilized by fermentation.

Drinking water bacteria including *Methylobacterium* isolated from DWDSs have been shown to be capable of degrading HAAs after they grew in enrichment cultures with high concentration of HAAs (Zhang et al., 2009). The *Methylobacterium* CRL-26 strain was found to be able to degrade chloroform with methane used as available substrate (Patel et al., 1982 ; Cappelletti et al., 2012). These findings motivated our research to study if the presence of *Methylobacterium* DSM 18358 in drinking water might have a significant effect on the concentration of THMs. Thus, THMs concentrations were conducted in raw drinking water and in drinking water with *Methylobacterium* at different relative abundances. In addition, the role of the organic compounds and chlorine concentrations in THMs concentration was studied as these are two key factors affecting THMs formation in drinking water.

2. Materials and methods

Drinking water was sampled from a domestic tap in Glasgow and used for the experimental analysis of this study. Also, the *Methylobacterium* DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Leibniz Institute, Germany) 18358 was used in these experiments. This strain was previously isolated from a drinking water network in Seville (Gallego et al., 2005b). After it was reactivated (WHO, 2004), it was cultured in R2A agar plates at 28 °C in the incubator for 72 h (Gallego et al., 2005c). A colony, which was created by streaking on the agar plates (Thiel, 1999), was inoculated into 10 ml R2A medium (Simões et al., 2007), and then it was incubated at 28 °C at 150 rpm speed for 72 h (Gallego et al., 2005c). The R2A is a low-nutrient medium, which has been used for viable bacterial count and isolation of bacteria from drinking water (Reasoner and Geldreich, 1985 ; Sandle, 2004 ; Kalmbach et al., 1997) like *Methylobacterium* (Gallego et al., 2005a ; Hiraishi et al., 1995). *Methylobacterium* cells were harvested from the pure culture at the exponential phase of growth by centrifugation for 20 min at 13,000 × g speed, washed 3 times in 0.1 M of phosphate-buffered saline, and re-suspended in drinking water (Simões et al., 2007) in order to inoculate the sampled drinking water with different relative abundances of *Methylobacterium* at 1% and 10%.

The USEPA DPD Method 8167 (Chamberlain and Adams, 2006 ; Greenberg et al., 1992) was followed to measure the total chlorine concentration in drinking water using the DR 900 Hach colorimeter (Manchester, UK). A total chlorine reagent powder pillow (Hach) was used for each measurement. During this measurement, combined chlorine, which is part of the total chlorine, oxidizes iodide. Then, iodine and free chlorine, which is the other part of total chlorine, react with DPD (N,N-diethyl-p-phenylenediamine) to form a pink colour, which is proportional to the total chlorine concentration. Results were recorded as total chlorine (Cl_2) in mg l^{-1}. The measurements were performed in triplicates in 10 ml samples and the wavelength of the measurements was at 520 nm.

The concentration of THMs was measured following the THMs Plus™ Method 10132 (Khan et al., 2014 ; Fujiwara, 1916) using the DR 2800 Hach spectrophotometer (Manchester, UK). Four chemical solutions from Hach were used for each measurement; the 27539-29 THMs Plus reagent, the 27540-48 THMs Plus reagent, the 27541-42 THMs Plus reagent and the 2756559 pH storage solution. During this measurement, trihalomethanes present in the sample react firstly with N, N,-diethylnicotinamide under heated alkaline conditions to form a dialdehyde intermediate using the first 2 reagents. The sample is then cooled and acidified using the third reagent. Then, the dialdehyde intermediate reacts with 7-amino-1,3 napthalene disulfonic acid to form a coloured Schiff base using the last Hach solution. The colour formed is proportional to the total amount of THMs present in the sample. The

measurements were performed in triplicates in 10 ml samples and the wavelength of the measurements was at 515 nm. Results were recorded as THMs in mg l^{-1}. Additional disinfection by-products, apart from THMs, included in the result from this test are: 1,1,1-trichloro-2-propanone, 1,1,1-tricholoacetonitrile, chloral hydrate and the HAAs: dichlorobromoacetic acid, dibromochloroacetic acid, tribromoacetic acid and trichloroacetic acid.

2.1. Chlorine and trihalomethanes measurements at different times of water sampling

Chlorine and THMs concentrations were measured in raw drinking water, sampled from the tap, from 8.00 h to 18.00 h every 2 h in order to study if there were differences in their concentrations depending on the time of water sampling. There was no control of the temperature in those measurements because the samples were processed immediately after water sampling. Therefore, the temperature of the samples at the time of measurement was the same as that of drinking water that was sampled from the tap.

2.2. Chlorine and trihalomethanes concentrations under different Methylobacterium concentrations

Chlorine and THMs concentrations were measured in raw drinking water and in drinking water with the *Methylobacterium* strain DSM 18358 inoculated at relative abundances of 1% and 10%, to study the effect that *Methylobacterium* might have on their concentrations. The role of *Methylobacterium* in THMs concentration was also studied in a solution, in which there was no chlorine or any organic matter other than *Methylobacterium* cells. The goal of this measurement was to exclude the effect of chlorine and organic matter on THMs concentration, and only focus on the potential effect of *Methylobacterium* on the removal of THMs. This solution was a standard THMs solution of 100 μg/l. *Methylobacterium* cells were injected into that standard solution at 10^5 cells ml^{-1} concentration and THMs concentrations were then measured after 0, 1, 3, 6 and 24 h at 4 °C. The temperature of the samples was maintained at 4 °C as THMs are volatile chlorination by-products (Nikolaou et al., 2002).

2.3. Chlorine and trihalomethanes measurements in drinking water under different organic matter concentrations

In order to understand the role of organic matter in chlorine and THMs concentrations, the raw drinking water and the one with *Methylobacterium* at 1% and 10% relative abundances were autoclaved so that there are no active bacteria that could affect these measurements. They autoclaved at 121 °C for approximately 60 min and then, chlorine and THMs concentrations were measured after 0, 1, 3, 6 and 24 h at 4 °C.

Extracellular polymeric substances (EPS) are important polymers of high molecular weight secreted by microorganisms (Staudt et al., 2004). The potential change of the amount of EPS in drinking water might explain the change in the concentration of THMs in drinking water. The surface area of EPS was measured for 3 samples (the raw drinking water and drinking water with *Methylobacterium* at 1% and 10% relative abundances) of 10 ml each after 0, 1, 3, 6 and 24 h at 4 °C. The liquid samples were filtered through 47 mm Whatman® 0.2 μm membrane filters (Sigma-Aldrich, Gillingham, UK). The samples were covered with 1 ml of 10 μg ml^{-1} Fluorescein Aleuria aurantia lectin (Vector laboratories, Peterborough, UK) for 10 min in the dark to stain the EPS (Garny et al., 2008 ; Flemming and Wingender, 2010). The EPS was visualised directly on the membrane filters using fluorescence microscopy (Olympus IX71 Tokyo, Japan) with the oil immersion UPlanFLN objective lens (UPlanFLN, Tokyo, Japan) with 100× magnification/1.30 numerical aperture. The filter used was the FITC with excitation at 495 nm and emission at 525 nm. The surface area of EPS was calculated in Matlab by processing more than 30 images obtained from microscopy. The original images were firstly converted to gray-scale images using the Matlab command called “rgb2gray” and then to binary images using the Matlab command called “im2bw” in order to separate the EPS from the background of the image. After these surface areas were calculated, they were divided to the total surface area of the image in order to finally calculate the percentages of these surface areas (%).

Finally, the effect of glucose on the concentrations of chlorine and THMs in drinking water was studied in order to further explore the role of organic matter in THMs formation. That allowed us to test if chlorine would react with this additional organic matter added from the glucose to drinking water so that THMs be formed. Chlorine and THMs concentrations were measured in raw drinking water after 0, 1 and 3 h, and after the 3-h measurement 1% glucose was added to drinking water. Measurements of chlorine and THMs concentrations were conducted after 3 and 21 h from the glucose addition. The temperature was maintained at 4 °C.

2.4. Trihalomethanes measurements in drinking water under different chlorine concentrations

The effect of chlorine concentration on THMs concentration was studied for the raw drinking water and for drinking water with *Methylobacterium* inoculated at 1% and 10% relative abundances. Specifically, sodium thiosuphate was added to drinking water in order to reduce chlorine concentration (Barbera et al., 2012 ; Jarroll et al., 1981). The effect of that chlorine decay on THMs concentration in drinking water was studied. A crystal of sodium thiosuphate at 0.1 N (248.18 g mol^{-1}) (WaterCommittee, 1953) was added to the samples and then, chlorine and THMs concentrations were measured after 5 and 10 min at 4 °C.

3. Results

3.1. Chlorine and trihalomethanes concentrations in raw drinking water

The time of sampling of drinking water from the tap was found to affect the concentration of chlorine. The lowest chlorine concentration was found at 8.00 h, then it was increased until 14.00 h and finally, it was decreased until 18.00 h. Also, the time of sampling affected the concentration of THMs, which was generally decreased from 8.00 h to 18.00 h (Fig. 1).

3.2. The role of Methylobacterium in chlorine and trihalomethanes concentrations

It was found that the chlorine concentration in raw drinking water was decreased over the 24-h period (Fig. 2A). The THMs concentration in raw drinking water was initially decreased sharply, but after the first

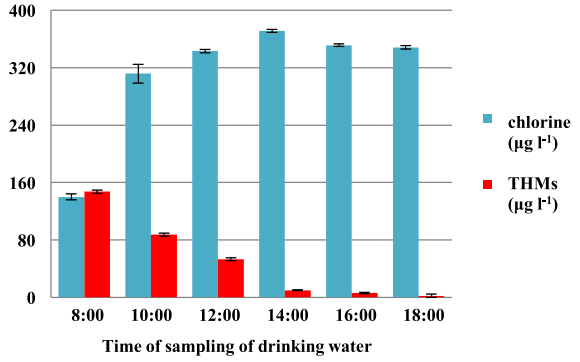

Fig. 1. Chlorine and THMs concentrations in raw drinking water at different times of water sampling from 8.00 h to 18.00 h every 2 h.

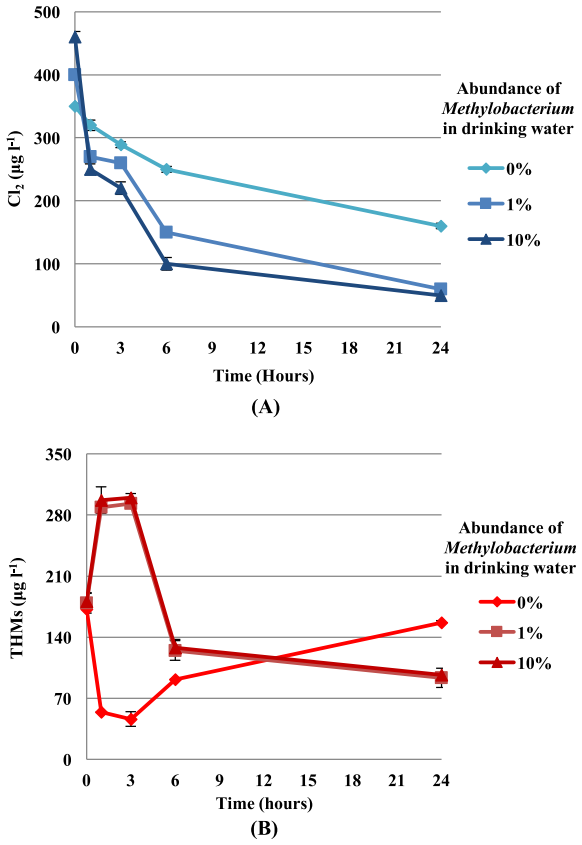

Fig. 2. Chlorine **(A)** and THMs **(B)** concentrations in raw drinking water and in drinking water with *Methylobacterium* at 1% and 10% relative abundances after 0, 1, 3, 6 and 24 h at 4 °C.

3 h there was a steady and slow increase for the remaining 21 h for which measurements were taken (Fig. 2B).

Chlorine concentration was found to be decreased with time in drinking water with the presence of the *Methylobacterium*. The rate of decline was marginally affected by the relative abundance at which the *Methylobacterium* was inoculated into drinking water (Fig. 2A). Also, when *Methylobacterium* was present in drinking water, it was shown that the change in the concentration of THMs over time displayed the opposite behaviour from that in raw drinking water; an initial sharp increase up to the first 3 h followed by a steady decline. The THMs concentration at the last measurement was almost the half from the initial one (Fig. 2B).

Also, the presence of *Methylobacterium* in the THMs standard solution was found to have a profound effect on the concentration of THMs. Specifically, the concentration of THMs was decreased over the 24-h period. The concentration of THMs at the last measurement was less than the half from the initial one (Fig. 3).

3.3. The role of organic matter in chlorine and trihalomethanes concentrations

The chlorine concentration from the first measurement at 0 h was found to be very low in the autoclaved samples and its concentration was equal to zero after the 6-h measurement. In addition, it was shown that the presence of the dead *Methylobacterium* cells in the samples accelerated slightly the decrease of chlorine concentration. The rate of decline was marginally affected by the relative abundance at which the *Methylobacterium* was inoculated into drinking water (Fig. 4A). Also, THMs were found to be formed only up to the first 6 h that chlorine was still present in the sample. The higher was the amount of organic matter, depending on the number of dead cells in each of the 3 samples,

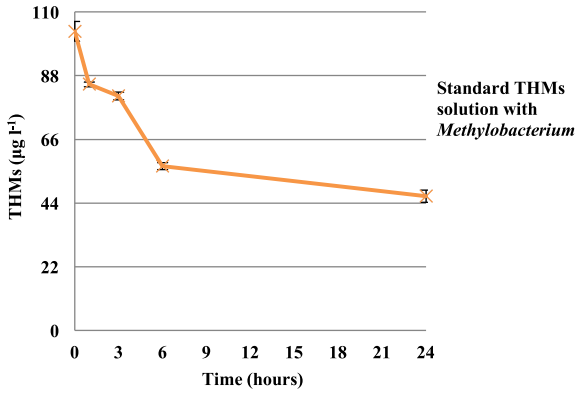

Fig. 3. Concentration of THMs in a THMs standard solution after 0, 1, 3, 6 and 24 h at 4 °C; *Methylobacterium* was inoculated to the solution with a concentration of 10^5 cells ml^{-1} at the 0-h measurement.

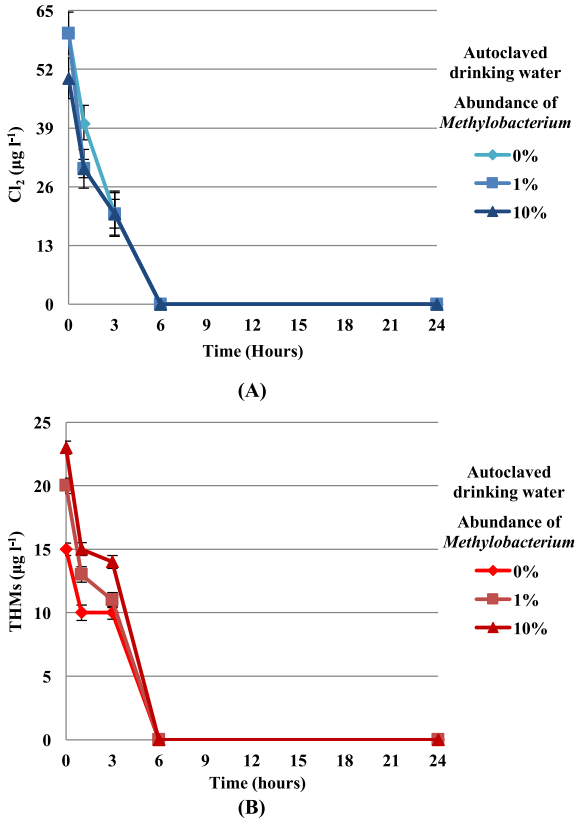

Fig. 4. Chlorine (A) and THMs (B) concentrations in autoclaved samples of raw drinking water and drinking water with *Methylobacterium* at 1% and 10% relative abundances after 0, 1, 3, 6 and 24 h at 4 °C.

the higher was the THMs formation. The rate of this increase was marginally affected by the relative abundance at which the *Methylobacterium* was inoculated into drinking water (Fig. 4B).

In raw drinking water only modest differences were found in the surface area of EPS during the 24-h period. However, in the drinking water inoculated with *Methylobacterium* a shift increase in the surface area of EPS was found that was most evident up to the first 3 h. The rate of this increase in the concentration of EPS was marginally affected by the relative abundance at which the *Methylobacterium* was inoculated into drinking water (Fig. 5).

The addition of 1% glucose to drinking water caused a sharp decrease in the concentration of chlorine with time; the concentration of chlorine was equal to zero at the 24-h measurement. The concentration

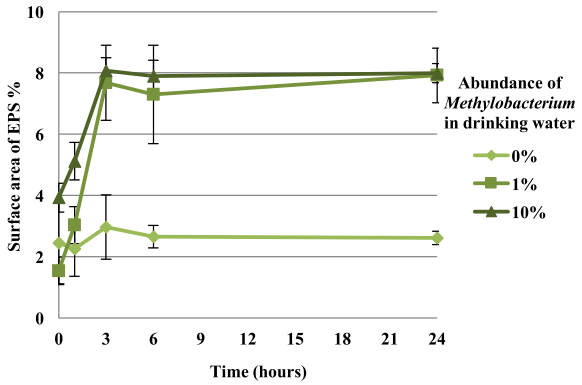

Fig. 5. Percentage of surface area of EPS for raw drinking water and drinking water with *Methylobacterium* at 1% and 10% relative abundances after 0, 1, 3, 6 and 24 h at 4 °C.

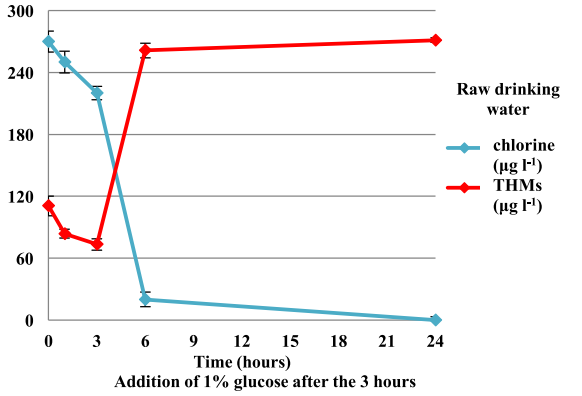

Fig. 6. Chlorine and THMs concentrations in drinking water after 0, 1, 3, 6 and 24 h at 4 °C; 1% glucose was added to drinking water after the 3-h measurement.

of THMs displayed the opposite behaviour of that of chlorine; THMs concentration was increased with time up to a final concentration which was more than 3 times higher than the initial one (Fig. 6). Also, the behaviour of chlorine and THMs in raw drinking water up to the first 3 h, before the addition of glucose to drinking water, was the same as it was previously shown (Fig. 2).

3.4. The role of chlorine in trihalomethanes concentration

After the addition of sodium thiosuphate to drinking water the concentration of chlorine was sharply decreased in all 3 samples regardless the presence or absence of *Methylobacterium* (Fig. 7A). This decrease of chlorine concentration was found to affect the concentration of THMs only in raw drinking water, in which the concentration of THMs was decreased. In the inoculated drinking water, the concentration of THMs was slightly increased. Again, the rate of this increase of the THMs concentration was slightly affected by the relative abundance at which the *Methylobacterium* was inoculated into drinking water (Fig. 7B).

4. Discussion

During the hours in which people do not use the tap often, biofilms may be formed at the inner surfaces of the pipes in stagnant waters (Simões and Simões, 2013 ; Beech and Sunner, 2004 ; Momba and Kaleni, 2002). This can cause decrease in the concentration of chlorine in drinking water through its interaction with the bacteria in the bulk water or with the adhering on them biofilms (Brown et al., 2011). This was shown in our experiments, where chlorine concentration was low

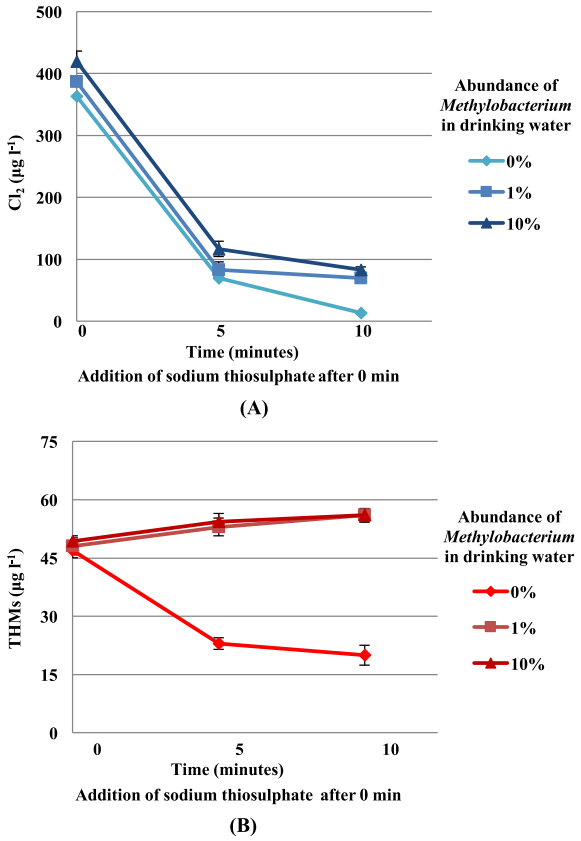

Fig. 7. Chlorine (A) and THMs (B) concentrations in raw drinking water and in drinking water with *Methylobacterium* at 1% and 10% relative abundances after 0, 5 and 10 min at 4 °C; sodium thiosulphate was added to drinking water after the 0-min measurement.

early in the morning and later in the afternoon. It was also shown that early in the morning the concentration of THMs was high. This might happen because even this low amount of chlorine that was still present in water had enough time during the night in order to react with the organics and form THMs. This should not be a surprising result since it has been previously shown that the longer time the chlorine has available to react with the organic compounds in drinking water, the higher is the formation of THMs (Sadiq and Rodriguez, 2004 ; Rodriguez and Serodes, 2001).

When chlorine concentration was monitored over time in raw drinking water, it was found that there was a stable decrease in its concentration for the first 3 h, and the same was found for the concentration of THMs. This happened because as chlorine concentration was decreased there was less available chlorine to react with the organic compounds and form THMs. After the first 3 h, even though chlorine concentration was again decreased with time, it was found that THMs concentration was increased. Over the last 21 h of measurements the remaining chlorine in drinking water reacted with the organics that were available in drinking water and that caused the formation of THMs.

When *Methylobacterium* was present in drinking water, the decrease in the concentration of chlorine with time was enhanced. Regarding the concentration of THMs, it was found that during the first 3 h, the *Methylobacterium* caused the increase in the concentration of THMs. The EPS measurements showed that the first 3 h were critical as the *Methylobacterium* produced EPS, which suggests the formation of aggregates in drinking water (Sheng et al., 2010). Aggregates are the coming-together of bacteria in clumps in the bulk water prior to adhesion onto the surfaces (Saur et al., 2017). *Methylobacterium* species have been previously reported to have an enhanced ability to form

aggregates when they are present with other drinking water bacteria (Ramalingam, 2012 ; Simões et al., 2007). The formation of these aggregates caused the formation of EPS, which caused the increase in the formation of organic matter and that subsequently caused the increase in the concentration of THMs in drinking water.

After the first 3 h, there was significant decrease in the concentration of THMs with a final concentration equal to almost the half of the initial one. Specifically, the degradation rate was found to be 47.9% for the relevant abundance of *Methylobacterium* in drinking water at 1%, and at 46.6% for the relevant abundance of *Methylobacterium* in drinking water at 10%. This may suggest that the formation of aggregates by the *Methylobacterium* accelerated the decrease of THMs concentration. This ability of the *Methylobacterium* to decrease the concentration of THMs was also proved by the measurements in the THMs standard solution, in which the *Methylobacterium* was found to be able to decrease the concentration of THMs with a final concentration equal to less than the half of the initial one. The reason why the *Methylobacterium* was found to be able to decrease THMs concentration might be that it utilised them as an energy and carbon source in order to grow and form aggregates in drinking water.

The addition of glucose to drinking water was proved to be important as chlorine concentration was rapidly decreased with time to a final concentration equal to zero. This happened because chlorine reacted with glucose and this caused THMs formation. The formation of THMs was shown by the rapid increase of their concentration with a final concentration equal to 2 times higher than the initial one. Similarly, the higher organic matter, derived from the dead *Methylobacterium* cells in the autoclaved inoculated drinking water, resulted to higher concentration of THMs than that in the autoclaved raw drinking water. The decrease of the concentration of chlorine here resulted in the decrease of the concentration of THMs.

This positive correlation in the concentrations of chlorine and THMs was also proved in the measurements with the sodium thiosulphate, in which the rapid decrease of the concentration of chlorine resulted in the decrease of the concentration of THMs in raw drinking water. On the other hand, in the inoculated with *Methylobacterium* drinking water, this did not happen as again the *Methylobacterium* proved to increase the concentration of THMs in the initial phase. Finally, the results from this work proved that the behaviour of *Methylobacterium* DSM 18358 was qualitatively the same when it was inoculated into drinking water at either low (1%) or high (10%) relative abundance.

Overall, it was shown that *Methylobacterium* played an important role in the concentration of THMs in drinking water. When the *Methylobacterium* DSM 18358 was present in drinking water, even at relative abundance of only 1%, it was found to be able to decrease the concentration of THMs after 24 h to almost the half of their initial concentration. This is an important knowledge for drinking water industries which continuously search for cheap and effective ways to improve drinking water quality because specific species like *Methylobacterium* might be key species in drinking water because even when they are present at low concentrations they might lead to safer drinking water.

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