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Solar disinfection – An appropriate water treatment method to inactivate faecal bacteria in cold climates

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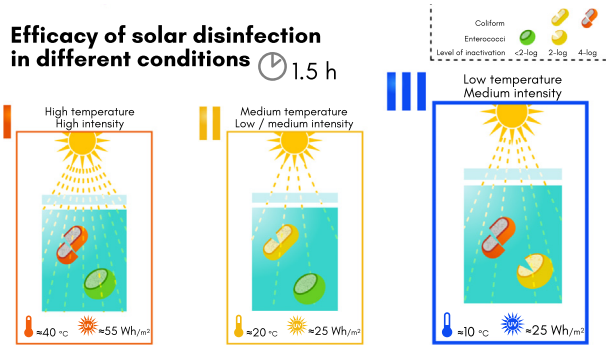
HIGHLIGHTS

- SODIS is underused in cool low-income areas as its efficacy is unknown in the cold.
- Here SODIS tested in Finland: low temperature (~9 °C) & low UV intensity (~19 W/m^2).
- As a result, 4-log coliform disinfection reached markedly with 25 Wh/m^2 in 1.5 h.
- Best performance in coldest weather due to low bacterial UV resistance in the cold?
- Results indicate SODIS is a potential water treatment method also for cold climates.

GRAPHICAL ABSTRACT



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ABSTRACT

Solar disinfection (SODIS) is an inexpensive drinking water treatment method applied in tropical and sub-tropical low-income countries. However, it has been unclear whether it functions adequately also in colder climates. To investigate this issue, SODIS experiments were performed in the humid continental climate of Finland by exposing faecally contaminated drinking water to natural solar radiation at different water temperatures (8–23 °C) and UV intensities (12–19 W/m^2) in polyethylene (PE) bags. To establish an adequate benchmark, SODIS experiments with the same experimental design were additionally conducted in the Mediterranean climate of Spain in typical conditions of SODIS application (~39 °C and 42 W/m^2).

Out of all experiments, the highest coliform and enterococci inactivation efficiencies in terms of lowest required doses for 4-log disinfection (25 Wh/m^2 and 60 Wh/m^2, respectively) were obtained in humid continental climate at the lowest studied mean water temperature (8–11 °C). Despite the low mean UV irradiance (~19 Wh/m^2), 4-log disinfection of coliforms and enterococci were also reached fast in these conditions (1 h 27 min and 3 h 18 min, respectively). Overall, the doses required for disinfection increased as the water temperatures and UV intensities of the experiments rose. Disinfection of 4-logs (> 99.99%) of both bacteria was reached in all SODIS experiments within 6 h, suggesting SODIS could be a sufficient household water treatment method also in colder climates, unlike previously thought.

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The effects of different water temperatures on bacterial inactivation were also tested in the absence of sunlight. Together the obtained results indicate that while water temperatures below or close to the optima of coliforms and enterococci (~10 °C) alone do not cause inactivation, these temperatures may enhance SODIS performance. This phenomenon is attributed to slower bacterial metabolism and hence slower photorepair induced by the low water temperature.

1. Introduction

According to WHO and UNICEF (2017), about 2.1 billion people have no access to safe drinking water at home, and 844 million of them lack basic drinking water service altogether. Solar water disinfection (SODIS) is one of the household water treatment methods that help to alleviate these issues in tropical and sub-tropical low-income countries especially in rural and emergency settings. SODIS is based on exposing microbially contaminated water to disinfecting UVA and UVB radiation of natural sunlight in transparent containers for at least 6 h. It has been proven to be an effective method in disinfecting water by extensive laboratory and field studies (Wegelin et al., 1994; Sommer et al., 1997; McGuigan et al., 1998; Oates et al., 2003; Dejung et al., 2007; Graf et al., 2010; Figueredo-Fernández et al., 2017). Furthermore, SODIS is one of the recommended methods for emergency point-of-use drinking water treatment (WHO, 2005).

SODIS makes drinking water safer because it inactivates microbes such as pathogenic bacteria. Bacteria causing disease in the human body (~37 °C) are mostly mesophilic, meaning they can grow in a mid-temperature range, i.e. temperatures of approximately 10–45 °C (Madigan, 2017). Thereby, SODIS of mesophilic bacteria, such as coliforms, is accelerated, when solar heating raises the temperature of treated water beyond this range to above 45 °C (McGuigan et al., 1998; Vivar et al., 2017a) or 50 °C (Wegelin et al., 1994; Joyce et al., 1996; Sommer et al., 1997). This is because when the temperature maximum of a bacterium is surpassed, denaturation of proteins starts and other essential cell structures ultimately become damaged, which may lead to cell death (Madigan, 2017). More specifically, Wegelin et al. (1994) noticed that if water temperatures stay above 50 °C during SODIS of coliforms, the UV dose required for a degree of disinfection becomes three times lower compared to SODIS in temperatures from 20 to 40 °C. Moreover, enterococci studied by Wegelin et al. were not affected by water temperatures below 55 °C, above which the required UV dose for a certain level of disinfection was reduced by one-half, compared to lower temperatures examined in their study. Similarly, Vivar et al. (2017a) found that the disinfection kinetics of SODIS of enterococci and *E. coli* were unaffected when water temperatures remained between 15 °C and 40 °C. They also noted that temperatures between 40 °C and 45 °C, close to the optima of these bacteria, may actually hamper SODIS effectiveness, i.e. have an antagonistic effect. Giannakis et al. (2014) similarly found that simulated SODIS of *E. coli* decelerated when temperatures rose from 20 °C to 40 °C, the inactivation being the least efficient at 40 °C among studied temperatures (20–60 °C). This causation is likely due to the rate of bacterial metabolism and thus bacterial UV damage repair (photorepair), being the fastest when temperatures are optimal (Giannakis et al., 2014).

It is plausible that temperatures below or close to the growth-allowing minima of bacteria might similarly produce a synergistic disinfecting effect together with radiation. As far as the authors know, the combined effect of cold temperature (< 15 °C) and radiation on microbial damage has not been studied extensively. Nevertheless, Rincón and Pulgarin (2004) remarked that the susceptibility of *E. coli* against photocatalytic treatment increased in winter conditions (6–10 °C water). García-Fernández et al. (2015) and Vivar et al. (2017b) additionally studied SODIS at ~15 °C water temperature. Moreover, in simulated aquaculture streams, hydrogen peroxide enhanced SODIS of *Pseudomonas*, *Aeromonas* and *Enterobacter* species was improved in the Finnish conditions of cold water and low radiation intensity (5–7 °C, 13 W/m^2) compared with Spanish conditions (31–32 °C, 44 W/m^2) (Villar-Navarro et al., 2021). However, to the best of the authors' knowledge, the effects of simple SODIS in water temperatures below 15 °C have not been studied closely.

If point-of-use SODIS was found to be effective and relatively fast in cooler climates, it would make the method potentially available to millions of new low-income households in climatic zones with cold or temperate seasons. Potential places for SODIS application could be found for example in the Himalaya region and in the cooler southern parts of South America. Currently, SODIS is thought to be inefficient in temperate, subpolar and polar climates: for example Moreno-SanSegundo et al. (2021) estimate that applicability of SODIS is questionable in areas with comparatively cool temperatures and low UV radiation. Borde et al. (2016) also state that SODIS, especially in a larger container holding several litres, is a promising low-cost drinking water treatment solution in low-income and disaster settings, but they list cold weather as one of the possible challenges for its efficacy.

Some related accounts likewise confirm the potential of PE (polyethylene) bag SODIS as an easy-to-distribute, low-cost, short-term drinking water treatment solution in disaster situations (McGuigan et al., 2012; Gutiérrez-Alfaro et al., 2017). PE bags are also better at disinfection than commonly used PET (polyethylene terephthalate) bottles because PE allows penetration of more efficiently disinfecting UVB rays into the treated water (Lawrie et al., 2015; Gutiérrez-Alfaro et al., 2017). Finally, PE bags have been found to be safe for SODIS due to the low chemical reactivity of the material. For instance, after twelve weeks of daily SODIS use in PE bags, Danwittayakul et al. (2017) found the levels of leaching organic compounds to be well below official safety limits.

For the above-described reasons, the aim of this study was to explore the effectiveness of SODIS in a cool climate. Whilst potential areas of household application are located in low-income countries, we chose to test SODIS efficacy in the cold humid continental (temperate) climate of southern Finland. In the experiments of this study, spring water contaminated with wild faecal bacteria was exposed to natural sunlight in PE bags in different natural temperatures. Possible survival and repair of bacteria exposed to SODIS were subsequently studied. A set of experiments with the same experimental design was also conducted in the Mediterranean (subtropical) climate of Spain. These experiments were carried out to be able to contrast the feasibility of SODIS application in cold climate to SODIS in the conditions it is usually applied in through the comparison of time and dose required for disinfection. The effect of water temperature on disinfection in the absence of solar radiation was also examined. This was conducted by exposing samples to temperatures similar to those of the SODIS experiments in the dark (dark tests). Furthermore, the costs of using PE bag SODIS as a household water treatment method were estimated.

2. Materials and methods

2.1. Test water

The test water used in all experiments was store-bought spring water inoculated with urban wastewater influent according to household water treatment evaluation recommendations of WHO (2011). The wastewater influent, used as a source of wild faecal bacteria, was acquired from either the Viikinmäki or Puerto Real wastewater treatment plants in Finland and Spain, respectively. Wild local bacteria were chosen in order to perform SODIS experiments under more realistic conditions. To obtain test water for SODIS experiments, a mix of spring water and filtered (25 μm, VWRI 5160063) wastewater influent (0.1–1% v/v) was prepared so that the initial concentrations of total coliforms and enterococci were approximately 10^5 CFU in 100 ml of ready sample. Transmittance of test waters (≥ 95.5% for a wavelength interval of 280–400 nm) was measured with UV-

1800 Shimadzu spectrophotometer and Jenway 7315 spectrophotometer in Finland and Spain, respectively.

Concentrations of cations in used spring waters (99–99.9% v/v in test water) were measured by means of an 882-Compact IC Plus (Metrohm; C4 250/4.0 column) ion chromatograph (IC) equipped with a conductivity detector. Additionally, concentrations of anions were analysed using an 881-Compact IC Pro (Metrohm; ASupp5 250/4.0 column) equipped with a conductivity detector. A mixture of CO_3^{2-} (3.2 mM) and HCO_3^- was used as a mobile phase for anion analysis at a flow rate of 0.7 ml/min. A mixture of HNO_3 (1.7 mM) and $C_7H_5NO_4$ (0.7 mM) was used as a mobile phase for the cation analysis, and the applied flow rate was 0.9 ml/min.

2.2. SODIS experiments

Humid continental (temperate) climate experiments were conducted at Aalto University (Otaniemi, southern Finland: 60.184° N, 24.830° E) during March – April of 2019 and March 2021. Mediterranean (subtropical) climate experiments were conducted at Cadíz University (Puerto Real, southern Spain: 36.532° N, 6.183° W) in April 2019.

PET bottles are the most commonly used vessels for SODIS (McGuigan et al., 2012). However, commercial PE bags (capacity 1 l, plastic wall thickness 0.05 mm) were chosen for all experiments of this study, as they are also used in household SODIS. This choice was due to their high transmittance of UVB wavelengths as opposed to PET bottles with low UVB transmittance (measured with a UV-1800 Shimadzu spectrophotometer) (Fig. 1).

In all SODIS experiments, water-filled PE bags were placed in rows in direct sunlight (Fig. 2). The total time spans of these experiments were 6 h in Finland and 4 h in Spain. At each sampling time, one PE bag was removed from sunlight exposure for bacterial cultivation and the air temperature, water temperature and radiation intensity were recorded. The air temperature was measured with a thermometer placed next to the bags. A thermometer placed inside a water-filled PE bag that was identical to the samples was used to measure the water temperature. The volume of test water in each PE bag was 500 ml, which resulted in 16 mm ± 2 mm water layer thickness in the bags in the lying down position of the experiments.

The SODIS experiments were performed on two different roofs (Fig. 2). Roof materials were non-reflective composition shingles (Finland) and stone pebbles (Spain). Possibly differing heat transfer properties of roof materials were accounted for by tracking the water temperature. Snow was removed from the experiment area before the March 2021 experiment conducted in Finland so that it would not change the heat transfer and reflective properties of the roof.

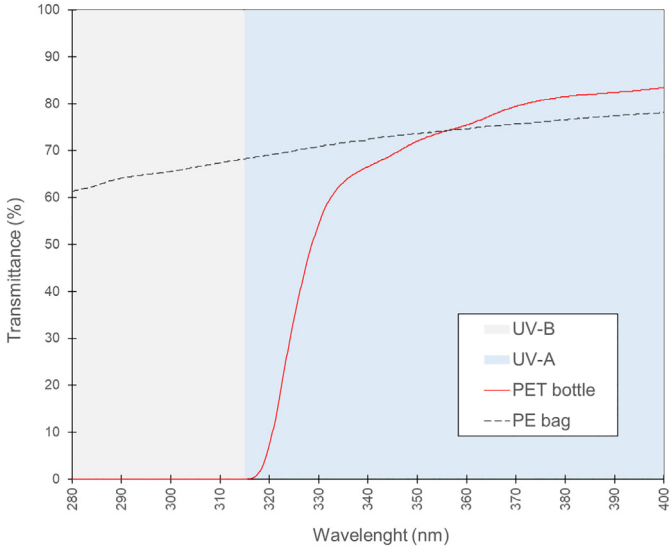

Fig. 1. Transmittance spectra of PE bags and PET bottles.

Solar radiation intensity was measured with a General Tools radiometer (UV513AB) in Finland and additionally a Kipp & Zonen Meteon Irradiance meter (CUV 5) in Spain. The spectral range in both was 280–400 nm. An adjustment coefficient calculated from the measurement differences between the radiometers was applied to measurements of the General Tools radiometer. The solar dose was calculated based on the formula presented by Gutiérrez-Alfaro et al. (2017):

$$Q_{UV} = Q_{UV-1} + UV_n \cdot (t_n - t_{n-1}) \quad (1)$$

where Q_{UV} is the cumulated dose at sampling point n, Q_{UV-1} is the cumulated dose at sampling point n − 1, UV_n is the UV radiation intensity at sampling point n, t_n is time in hours at sampling point n, and t_{n-1} is time in hours at sampling point n-1.

Bacteria from sample bags from each time point were cultivated using the membrane filtration method in quadruplicate. Additionally, one bag that had been exposed to sunlight for the full experiment duration was stored in the absence of light at room temperature (20 °C ± 1 °C) for 24 h after each experiment. Membrane filtration was subsequently used to record possible bacterial post-SODIS reactivation. The reactivation percentage was calculated with a formula presented by Lindenauer and Darby (1994):

$$\text{reactivation}\% = 100\% \cdot \frac{N_t - N}{N_0 - N} \quad (2)$$

where N_t is the concentration of bacteria after the 24-h reactivation time (CFU/100 ml), N is the concentration at the end of experiment (CFU/100 ml) and N_0 is the initial concentration before the experiment (CFU/100 ml).

2.3. Control experiments in absence of solar radiation

To study the sole effect of water temperature on enterococci and total coliforms, test water identical to the one used in the SODIS experiments was exposed to different temperatures without exposure to sunlight for the same or slightly longer time periods compared the SODIS experiments. First, spring water was preheated or cooled to 0 °C, 10 °C, 20 °C or 43 °C in PE bags in ovens at these temperatures or in a fridge at 4 °C. Temperatures were measured at the start before adding wastewater influent and at each sampling time point with a thermometer inside a PE bag identical to the sample bags. At the start of the experiments, wastewater was added to preheated/cooled spring water bags to reach a concentration of 1%, and the samples in the bags were thoroughly mixed. Subsequently, bags were immediately put back into ovens or the fridge at the assigned temperatures. The 0 °C sample bag was kept on ice in the fridge and the 20 °C sample bag was kept in room temperature (20 °C ± 1 °C). Bacterial cultivation was conducted identically to SODIS experiments.

2.4. Bacterial cultivation and enumeration

The following media were used for bacterial cultivation in experiments performed in Finland and Spain: Slanetz Bartley agar (Merck, 1.05262 and Pronadisa, 1109) to cultivate enterococci, *m*-Endo LES agar (Sigma, 85,766) as well as *E. coli*–coliforms chromogenic medium (Pronadisa, 1340) to cultivate total coliforms and finally TCBS media (Sigma, 86,348 and Pronadisa, 1074) in an attempt to cultivate *Vibrio* species. The membrane filtration method (ISO 9308-1:2014, modified as described here) was used in analysing samples of all experiments. Samples were plated in quadruplicate with the exception that samples where reaching the detection limit was close and those from dark tests were plated in duplicate. Quantification of colonies was performed after incubation of 24 h (total coliforms and bacteria growing on TCBS medium) or 48 h (enterococci) at 37 °C. Bacterial colonies were enumerated according to the colony-count instructions of the respective media manufacturers. Bacteria growing on TCBS media were identified by sequencing as described in supplementary

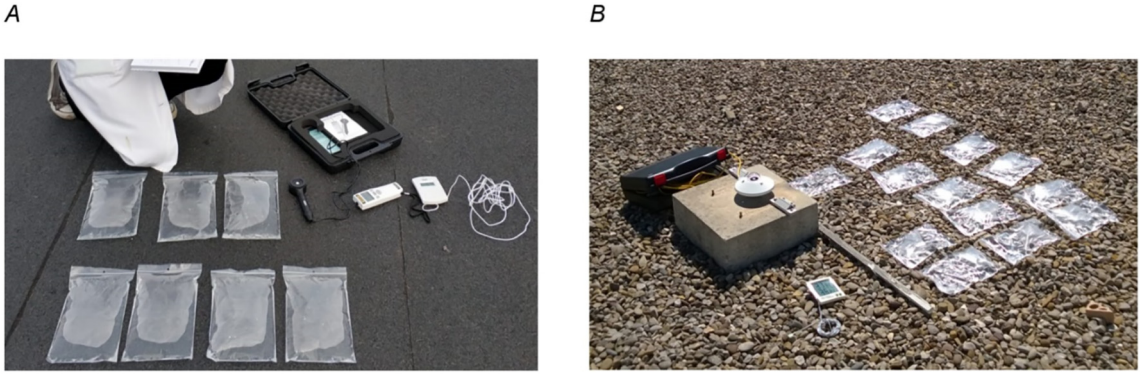

Fig. 2. Images of SODIS experiment setups. Finland (A) and Spain (B).

materials. SODIS results on these bacteria are also only described in supplementary materials due to the identification revealing that they consisted of a mix of enterococci and coliform species. Additionally, identification was also performed on reactivation colonies from the experiment with the highest total dose (165 Wh/m^2, Spain). This analysis is similarly described in supplementary materials.

The SODIS and dark test results are presented as a logarithmic change in concentration (log [N/N$_0$]) as a function of time (min) and/or dose (Wh/m^2). The initial bacterial concentration is depicted with N$_0$, and the concentration at time t is depicted with N. The results were fitted to linear microbial survival models using GInaFiT software developed by Geeraerd et al. (2005). Using only linear models was chosen to avoid over-fitting data to more complex models and to be able to compare disinfection curves to each other. The three best-fitting linear models available in the software were log-linear + shoulder (Eq. (3)), log-linear + tail (Eq. (4)) and log-linear + shoulder and tail (Eq. (5)). Out of these options, the model with the smallest RMSE and highest R^2 values was selected for each inactivation curve. Additionally, doses required for 2-log and 4-log inactivation as well as time to reach 4-log inactivation were calculated with the GInaFiT software.

$$N_d = N_0 \cdot e^{(-k_{max} \cdot Q_{UV})} \cdot \frac{e^{(k_{max} \cdot SL)}}{1 + (e^{(k_{max} \cdot SL)} - 1) \cdot e^{(k_{max} \cdot Q_{UV})}} \quad (3)$$

$$N_d = (N_0 - TL) \cdot e^{(-k_{max} \cdot Q_{UV})} + TL \quad (4)$$

$$N_d = (N_0 - TL) \cdot e^{(-k_{max} \cdot Q_{UV})} \cdot \frac{e^{(k_{max} \cdot SL)}}{1 + (e^{(k_{max} \cdot SL)} - 1) \cdot e^{(k_{max} \cdot Q_{UV})}} + TL \quad (5)$$

where N$_d$ is the bacterial concentration (CFU/100 ml) at a certain dose, N$_0$ is the initial bacterial concentration (CFU/100 ml), Q$_{UV}$ is the radiation dose (Wh/m^2), k$_{max}$ is the specific maximum inactivation rate (m^2/Wh), SL is the shoulder length (Wh/m^2) and TL is the tail length (Wh/m^2).

3. Results and discussion

3.1. Conditions of SODIS experiments and spring water compositions

The weather was mostly sunny throughout experiments. No freezing in sample bags was observed at any time. The conditions of the experiments are summarized in Table 1.

Spring water (99% v/v in test water) used in Spain was slightly harder than the water used in Finland (Table 2). A recent article by Rommozzi et al. (2020) describes how SODIS is affected by different ions in their typical concentrations for natural waters, such as lakes and rivers. They report that out of the ions found in the test waters in our study, NO_3^- (≥ 30 mg/l), NO_2^- (≥ 0.1 mg/l) and Cl^- (≥ 10 mg/l) may enhance SODIS of *E. coli* in the respective concentrations, whereas SO_4^{2-} had no effect even at the highest studied concentration (500 mg/l). Consequently, in our experiments, the spring water used in Spain may have accelerated SODIS to a small extent in comparison to the water used in Finland. This is due to the Spanish spring water having higher concentrations of NO_2^- and Cl^- than the respective thresholds that Rommozzi et al. found.

3.2. SODIS performance in humid continental and Mediterranean climatic zones

Fig. 3 shows all the results of the SODIS experiments conducted in the humid continental (Finland, Espoo) and Mediterranean (Spain, Cádiz) climatic zones. These experiments were carried out under the local experimental conditions that occur naturally in both locations including local temperature, solar irradiance as well as physical, chemical and microbiological characteristics of the waters. Therefore, the results obtained in each location are not directly comparable with each other. However, they do constitute evidence of the feasibility of applying SODIS in both locations since the experiments have been carried out under real conditions.

The detection limit (LOD) of <1 CFU/100 ml was reached in all experiments for coliforms and half of the experiments for enterococci. Inactivation of 4-logs of both bacteria was reached in all experiments, meaning

Table 1
Overview of conditions of each SODIS experiment. The mean temperatures and intensities for coliforms and enterococci referred to are weighted means recorded during the experiment before possibly reaching the detection limit of <1 CFU/100 ml. Temperature and intensity ranges before reaching the detection limits are indicated in brackets.

Date and location of experiment	Total duration of experiment (h)	Air temperature, whole experiment (°C)	Maximum dose received in experiment (Wh/m^2)	Intensity, coliforms (W/m^2)	Intensity, enterococci (W/m^2)	Water temperature, coliforms (°C)	Water temperature, enterococci (°C)	Experiment label: I = intensity, T = temperature
19.3.2021, Finland	6	5 (0–8)	86	19 (13 – 21)	18 (7–21)	8 (6–13)	11 (6–14)	Low T & Med I
22.3.2019, Finland	6	11 (5–14)	81	16 (12 – 20)	12 (5–20)	17 (15–17)	15 (8–17)	Med T & Low I
2.4.2019, Finland	6	15 (10–19)	109	18 (15–20)	19 (15–23)	18 (11 – 22)	23 (11–28)	Med T & I
11.4.2019, Spain	4	38 (30–40)	165	42 (38–43)	42 (38–44)	38 (19–41)	39 (19–43)	High T & I

Table 2
Compositions of spring waters used for experiments in Finland and Spain.

	Cl^-, mg/l	NO_3^-, mg/l	SO_4^{2-}, mg/l	Na^+, mg/l	K^+, mg/l	Ca^{2+}, mg/l	Mg^{+2}, mg/l	F^-, mg/l	NO_2^- mg/l
Spring water, Finland	4.7	2.3	19.2	**5.5**	2.7	19.6	4.8	–	–
Spring water, Spain	12.1	7.7	44.0	8.7	1.1	75.3	15.1	0.4	0.7

SODIS as a household water treatment method had a highly protective effect against total coliforms and enterococci in all experiment conditions (WHO, 2011). The SODIS results of total coliforms and enterococci are discussed in detail in their respective sections.

3.2.1. Total coliform inactivation by SODIS

The results of total coliform inactivation by SODIS performed in Finland and Spain at different natural water temperatures and solar radiation intensities were compiled into Fig. 4. All total coliform inactivation results fitted best to a model with a shoulder. This might indicate that the populations exhibit a level of resistance towards the low dose received at the beginning of the experiments. The tailing in the curves points to some more UV resistant subpopulations being present in the experiments.

As Fig. 4a-b and Table 3 show, the efficiency of total coliform inactivation by SODIS was clearly the highest (k_{max} = 0.53 m^2/Wh) in the Low T & Med I experiment (mean water temperature 8 °C, mean intensity 19 W/m^2) among all experiments. Although experimental results obtained in Finland and Spain are not readily comparable, it can be noticed that High T & I experiment (k_{max} = 0.21 m^2/Wh, mean water temperature 38 °C, mean intensity 42 W/m^2) conducted in Spain shows much lower efficiency of total coliform inactivation with respect to the required dose. Almost identically low disinfection efficiency (k_{max} = 0.20 m^2/Wh) was also obtained in the Med T & Low I experiment (mean water temperature 17 °C, mean intensity 16 W/m^2). 4-log inactivation in the Low T & Med I experiment performed in Finland was reached with a dose (25 Wh/m^2) that is less than a half of the dose (53 Wh/m^2) needed for High T & I experiment conducted in Spain.

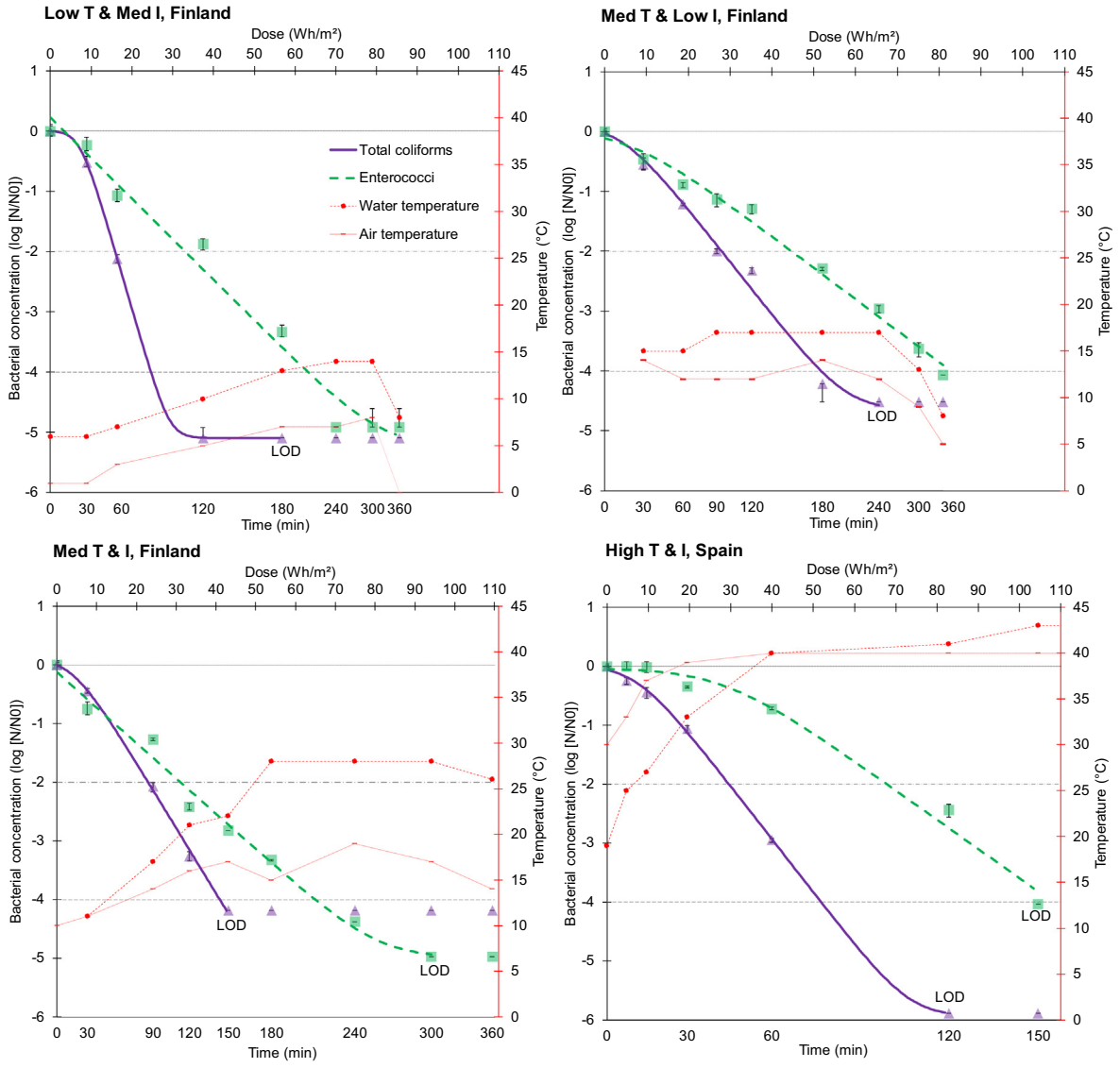

Fig. 3. Coliform & enterococci inactivation in SODIS experiments conducted in two different climatic zones at different water temperatures and solar radiation intensities. I = intensity, T = temperature, Med = medium, LOD = detection limit. Error bars represent the range of included bacterial concentration measurements, i.e. maximums and minimums. Experimental data is represented by symbols: ■ - observed enterococci, ▲ - observed total coliforms. Data fitted to kinetic models is represented by lines. All plots are scaled based on the UV dose (top horizontal axes), which is why the time scale is non-linear in the bottom horizontal axes.

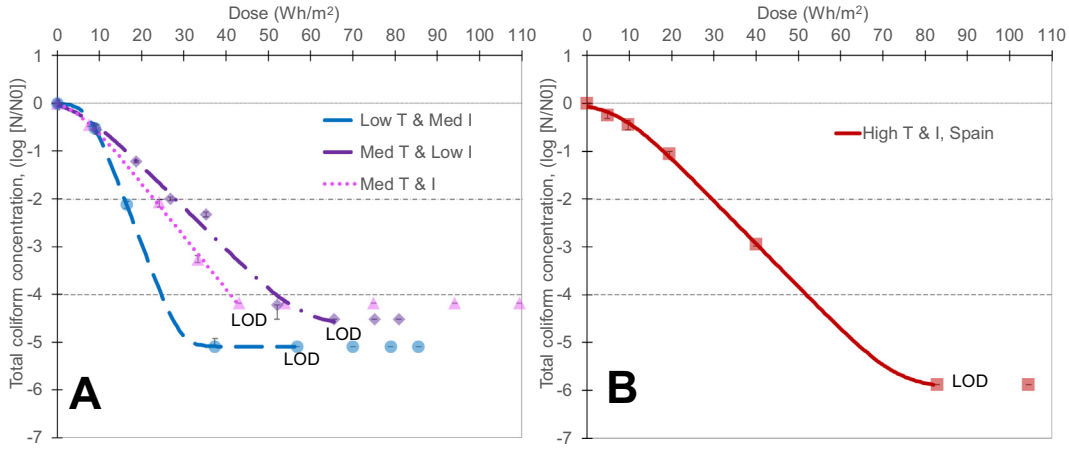

Fig. 4. Total coliform disinfection at different water temperatures and solar radiation intensities in two climates: Finland (4a, left) and Spain (4b, right). I = intensity, T = temperature, Med = medium, LOD = detection limit. Error bars represent the range of included measurements, i.e. maximums and minimums. Experimental data is represented by symbols: ● - Low T & Med I, ♦ - Med T & Low I, ▲ - Med T & I, ■ - High T & I. Data fitted to kinetic models is represented by lines.

Based on the results of Gutiérrez-Alfaro et al. (2016), the required SODIS dose for a 4-log reduction in *E. coli* in spring water is ~35 Wh/m^2 in PE bags (water temperature 18–28 °C, intensity 20–35 W/m^2). To the best of our knowledge, PE bag SODIS of other coliforms besides *E. coli* has not been reported.

A key parameter, which should be taken into account when assessing SODIS efficacy, is experimental time required to reach 4-log disinfection (Table 3). Experimental time should be examined especially when considering SODIS tests performed in Finland and Spain, as the wastewater source and experimental conditions (solar intensity, water and air temperature) were intrinsically different. Interestingly, the time required for 4-log disinfection is very similar in the Low T & Med I (1 h 27 min) and High T & I (1 h 24 min) experiments. This is surprising, because the mean intensity of the High T & I experiment (42 W/m^2, Spain) was much higher in contrast to the Low T & Med I experiment (19 W/m^2, Finland). Additionally, these two experiments are much faster in reaching 4-log total coliform disinfection compared to the two other experiments with medium temperatures and low to medium intensities.

As mentioned before, the approximate minimum, optimal and maximum temperatures of *E. coli* and other faecal coliforms are 8 °C, 39 °C and 48 °C, respectively (Madigan, 2017). The water temperature of the High T & I experiment (max. 41 °C, mean 38 °C) is hence very close to the optimum of faecal coliforms. This might explain why the k_{max} value of total coliform inactivation in the High T & I experiment is relatively low, and the 2-log and 4-log inactivation required the highest doses out of all the experiments. Similarly, Vivar et al. (2017a) discovered that SODIS (mean intensity 45 W/m^2) of *E. coli* in wastewater influent (filtered influent passed through an Imhoff tank) decelerated when the experiment temperatures remained between 40 °C and 45 °C. Giannakis et al. (2014) also reported similar results when studying the effect of temperatures from 20 °C to 60 °C on SODIS (global solar intensities of 800 W/m^2 and 1200 W/m^2) of *E. coli* in synthetic secondary effluents: disinfection was the weakest at 40 °C. Naturally, compared to our test water, the nutrient content was significantly different in the synthetic effluent matrices used by both Giannakis et al. (2014) and Vivar et al. (2017a). Thus, growth might explain some of the antagonistic effects in their experiments as temperatures remained close to the coliforms' optimum.

Overall, the rates of inactivation increased slightly as the temperatures of the experiments dropped (except for Med T & Low I experiment), but the doses were dissimilar in each experiment as well. Therefore, the sole effect of water temperature on experiments is difficult to single out precisely. Villar-Navarro et al. (2021) recently obtained similar results when studying hydrogen peroxide enhanced SODIS of *Pseudomonas*, *Aeromonas* and *Enterobacter* species of simulated aquaculture streams in Finnish (5–7 °C, 13 W/m^2) and Spanish (31–32 °C, 44 W/m^2) conditions. They observed that in Finland, 4-log disinfection was reached with a lower solar radiation dose of 25 Wh/m^2 (32 Wh/m^2 in Spain) and the k_{max} value was correspondingly almost double compared to that observed in the Spanish conditions. The same researchers also noticed a similar trend with UVA and photocatalysis tests enhanced with hydrogen peroxide as well as simple photocatalysis tests: disinfection was reached with a smaller dose in 6 °C compared with 22 °C.

The lower required doses in colder experiments conducted in Finland could be caused by cold-induced slower metabolism rates because of membrane stiffening (Madigan, 2017) and decreased production of housekeeping proteins (Jones et al., 1987). Both of these mechanisms might decelerate cellular photorepair in colder temperatures. The best performance of the Low T & Med I experiment could be explained by the fact that it is the only experiment with a temperature mostly below the cardinal temperature minimum of coliforms (Fig. 3), and hence photorepair processes might have been close to non-existent. It can be argued that the Med T & Low I experiment provided the least efficient conditions for

Table 3
Statistical parameters of SODIS experiments' results, total coliforms. Weighted mean temperature refers to the temperatures recorded during the experiment before possibly reaching the detection limit of <1 CFU/100 ml.

Experiment label based on water temperature & intensity	Weighted mean water temperature (°C)	Best-fitting linear model	k_{max} (m^2/Wh)	k_{max}, standard error	Dose to reach 2-log inactivation (Wh/m^2)	Dose to reach 4-log inactivation (Wh/m^2)	Time to reach 4-log inactivation	RMSE	R^2
Low T & Med I	8	shoulder + tail	0.53	0.00	16	25	1 h 27 min	0.00	1.00
Med T & Low I	17	shoulder + tail	0.20	0.02	28	52	3 h 2 min	0.23	0.99
Med T & I	18	shoulder	0.26	0.01	24	41	2 h 26 min	0.10	1.00
High T & I, Spain	38	shoulder + tail	0.21	0.01	31	53	1 h 24 min	0.08	0.99

SODIS in terms of time because the intensity was low, and the water temperature was not high or low enough to hamper bacterial functions.

3.2.2. Enterococci inactivation by SODIS

Fig. 5a-b show the compiled results of enterococci inactivation by SODIS performed in Finland and Spain at different water temperatures and solar radiation intensities. Overall, SODIS was less efficient in inactivating enterococci compared to more UV sensitive total coliforms in all studied temperatures, both in humid continental and Mediterranean climatic zones (Figs. 4a-b and 5a-b & Tables 4 and 5), which is in agreement with other studies (Wegelin et al., 1994; Gutiérrez-Alfaro et al., 2016; Levchuk et al., 2019). This dissimilarity between disinfecting UV doses is likely due to gram-positive bacteria, such as enterococci, having a thicker, more durable cell wall compared to gram-negative bacteria such as coliforms (Rincón and Pulgarin, 2004; Gomes et al., 2009; Figueredo-Fernández et al., 2017).

The longer shoulder length of the enterococci curve in the High T & I experiment compared to that of coliforms in the same experiment is one of the other factors indicating that enterococci are more UV resistant compared to total coliforms (Figs. 4b and 5b). Similarly to total coliforms, the lowest dose needed for 4-log disinfection (60 Wh/m^2) was obtained in the Low T & Med I experiment (Finland, mean water temperature 11 °C, mean intensity 18 W/m^2), whereas the corresponding much higher, largest dose (108 Wh/m^2) was required in the High T & I experiment (Spain, mean water temperature 39 °C, mean intensity 42 W/m^2) (Table 4). Here it must be noted that the time to reach 4-log inactivation was the shortest (2 h 40 min) in the High T & I experiment conducted in Spain, which had clearly the highest mean UV intensity. However, 4-log disinfection in the Low T & Med I experiment was only 38 min slower than that, further indicating SODIS can be very effective also in cold conditions.

Gutiérrez-Alfaro et al. (2017) and Figueredo-Fernández et al. (2017) reported that a dose of approximately 90 Wh/m^2 was required for 2-log enterococci inactivation by SODIS in PE bags. The respective water temperatures and solar intensities in these studies, also conducted in Cadíz (Spain), were 18–28 °C & 20–35 W/m^2 (Gutiérrez-Alfaro et al., 2017) and 41 °C & 30–50 W/m^2 (Figueredo-Fernández et al., 2017). Similarly to our study, the aforementioned studies used nutrient-poor water matrices (bottled spring water, well water, river water, tap water), which were inoculated with wastewater influent. In our study, the most similar dose required for 2-log enterococci inactivation (68 Wh/m^2) was obtained in the High T & I experiment (Spain). It is slightly lower compared to the previously mentioned studies, but it falls within the standard deviation obtained from the three experiments in the Figueredo-Fernández et al. study. Conversely, the corresponding 2-log inactivating doses of our other experiments, all of which were conducted in Finland, were only a third or a half of what Gutiérrez-Alfaro et al. and Figueredo-Fernández et al. observed.

The respective minimum, optimum and maximum temperatures of enterococci calculated with the Rosso model are circa 7 °C, 43 °C and 48 °C, respectively (Van den Berghe et al., 2006). These temperatures could explain why the highest doses for 2-log and 4-log enterococci inactivation were obtained in the experiment with the highest water temperature (mean 39 °C), i.e. the High T & I experiment (Spain). This outcome is conceivably due to enterococci attaining a high rate of metabolism protecting them from UV damage in the water temperature of the experiment, as it is very close to their temperature optimum. Lower doses required for 4-log disinfection (60 Wh/m^2, 68 Wh/m^2 and 83 Wh/m^2) were obtained in all experiments conducted in Finland. The reason the Med T & Low I experiment performed the poorest out of the SODIS experiments conducted in Finland both in terms of required dose and time, is probably due to it having the lowest mean intensity out of the three (12 W/m^2). The intensity of the Low T & Med I experiment is not that much higher (18 W/m^2), but its temperature is under or very close to the cardinal minimum (< 7 °C) for most of the experiment (Fig. 3). As we suspect also in the case of total coliforms (previous section), the low temperature close to the temperature minimum of enterococci must have slowed down their metabolism enough to make them less able to resist UV damage in that experiment.

A few characteristics could further explain the differences in enterococci survival in different SODIS experiments. Firstly, some accounts point out that the optimal temperature for bacterial growth may not always be optimal for their survival (Rozen and Belkin, 2001). Lessard and Sieburth (1983), for instance, noticed that *E. coli* and enterococci in a sewage – seawater mixture in Plexiglas diffusion chambers placed outdoors from February to August (0–20 °C), survived the best in cold temperatures. Additionally, according to some studies, cold tolerance is developed in enterococci if they are incubated at low positive temperatures (8–16 °C), and this tolerance is increased if the length of this incubation period is extended (Thammavongs et al., 1996).

3.3. Reactivation of bacteria after SODIS experiments

The magnitude and rate of bacterial reactivation after SODIS determines how long treated water can be stored before it may become undrinkable again. Reactivation in 24 h after all SODIS experiments in our study remained under a very low value of 0.3%. Unsurprisingly, the lowest reactivation of total coliforms and enterococci occurred in the experiments with the highest received total doses. After the High T & I (total dose 165 Wh/m^2), Med T & I (total dose 109 Wh/m^2) and Low T & Med I (total dose

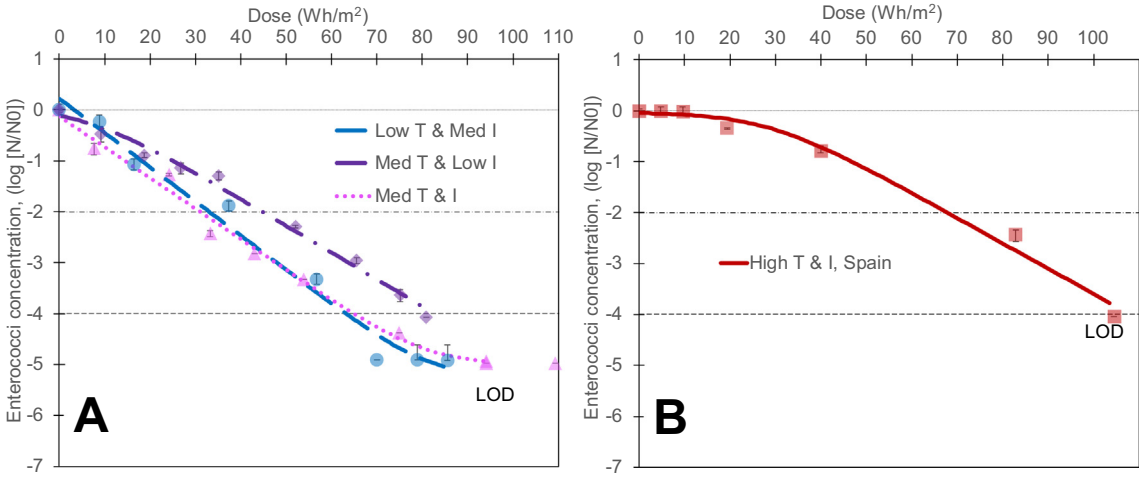

Fig. 5. Enterococci disinfection at different water temperatures and solar radiation intensities in two climates: Finland (5a, left) and Spain (5b, right). I = intensity, T = temperature, Med = medium, LOD = detection limit. Error bars represent the range of included measurements, i.e. maximums and minimums. Experimental data is represented by symbols: ● - Low T & Med I, ♦ - Med T & Low I, ▲ - Med T & I, ■ - High T & I. Data fitted to kinetic models is represented by lines.

Table 4
Statistical parameters of SODIS experiments' results, enterococci. Weighted mean temperature refers to the temperatures recorded during the experiment before possibly reaching the detection limit of <1 CFU/100 ml.

Experiment label based on water temperature & intensity	Weighted mean temperature (°C)	Best-fitting linear model	k_{max} (m^2/Wh)	k_{max}, standard error	Dose to reach 2-log inactivation (Wh/m^2)	Dose to reach 4-log inactivation (Wh/m^2)	Time to reach 4-log inactivation	RMSE	R^2
Low T & Med I	11	tail	0.15	0.01	33	60	3 h 18 min	0.35	0.98
Med T & Low I	15	shoulder + tail	0.10	0.02	45	83	5 h 57 min	0.25	0.99
Med T & I	23	tail	0.14	0.01	31	68	3 h 39 min	0.23	0.99
High T & I, Spain	39	shoulder	0.11	0.01	68	108	2 h 40 min	0.22	0.99

86 Wh/m^2) SODIS experiments, respective 0.001%, 0% and 0% reactivations were observed, as shown in Table 5. These doses have apparently been sufficient to irreversibly damage bacterial structures beyond repair, and very few if any cultivable bacteria survived. Conversely, the detection limit of enterococci was not reached in the Med T & Low I experiment (total dose 81 Wh/m^2). Thereby, some enterococci reactivation was likely to occur in the reactivation test. We also suspect that the low intensity and harmless water temperature of the experiment additionally enabled the observed minor photorepair of enterococci and total coliforms.

Levchuk et al. (2018) reported much higher (< 5%) reactivation of total coliforms and enterococci after a quite similar SODIS dose (76 Wh/m^2) compared to the Low T & Med I and Med T & Low I experiments. As in our own study, they used bottled drinking water inoculated with wastewater influent as their water matrix, and their experiments were also carried out in Cádiz. In our study, reactivation of total coliforms occurred in the Med T & Low I experiment (81 Wh/m^2) but not in the Low T & Med I experiment with a very similar dose (86 Wh/m^2). We presume that photorepair did not occur because the water temperature was close to or below the coliform (~8–48 °C, Madigan, 2017) and enterococci (~7–48 °C, Van den Berghe et al., 2006) cardinal ranges in the Low T & Med I experiment (6–14 °C). Like described in the previous sections, bacterial metabolic functions could have been hindered enough by the cold (Jones et al., 1987; Madigan, 2017) to make bacteria unable to resist UV damage during the SODIS treatment. This mechanism probably contributed to no bacteria surviving the Low T & Med I experiment (mean water temperature 11 °C for enterococci), whereas conversely, the more comfortable mean temperature (15 °C for enterococci) of the Med T & Low I experiment could have allowed a higher degree of cell repair (photorepair), and hence a few bacteria survived. Another possible reason for the reactivation of total coliforms in the Med T & Low I experiment is that the initial respective concentrations of total coliforms and enterococci were ~ 0.8 logs and ~ 1.7 logs larger than those in the Low T & Med I experiment. Nonetheless, Giannakis et al. (2014) found initial concentrations to be insignificant in terms of efficacy of SODIS in similar experiments.

The observed reactivation colonies from the High T & I SODIS experiment (Spain), which received the highest dose (165 Wh/m^2), were sequenced as described in supplementary materials. These colonies turned out to include *Acinetobacter* spp. with a genetic coincidence of 96%. This result suggests that the genus is relatively resilient against solar radiation. The solar radiation resistance of gram-negative *Acinetobacter* spp. have not been explored in SODIS studies, but this genus has been found fairly resistant to UVB compared to other bacteria tested by Zenoff et al. (2006). The authors also pointed out that a species of this genus may be able to develop and perform effective photorepair mechanisms after exposure to UV. In comparison, Santos et al. (2013) found the genus to be UV sensitive, but they proposed that *Acinetobacter* spp. may be able to develop UV resistance depending on the UV exposure levels of their habitat.

Table 5
Mean bacterial reactivation in irradiated samples after each SODIS experiment after 24 h dark storage in room temperature.

Experiment label based on water temperature	High T & I (Spain)	Med T & I	Med T & Low I	Low T & Med I
Reactivation, total coliforms (%)	0.001%	0.0%	0.24%	0.0%
Reactivation, enterococci (%)	0.0%	0.0%	[a]0.18%	[a]0.0%
Received total UV dose (Wh/m^2)	165	109	81	86
Water temperature range of experiment (°C)	19–43	11–28	8–17	6–14

[a] Detection limit was not reached during SODIS.

3.3.1. Thermal bacterial inactivation control: 0 °C, 10 °C and 20 °C dark tests

The experimental times and temperatures for these dark tests were selected to cover the temperature ranges and time spans of the SODIS experiments conducted in Finland. No significant inactivation of either enterococci or total coliforms was observed in 0 °C, 10 °C or 20 °C dark tests over the experiment period of 6 h 30 min (390 min) (Fig. 6). All bacterial concentrations stayed within ±0.5 logs of initial concentrations.

The temperature of the 20 °C dark test is well within the cardinal ranges of coliforms and enterococci, whereas the 10 °C dark test is close to the temperature minima of both bacteria. However, as there is no additional source of stress present for the bacteria in the conditions of the experiments, no significant change in their concentrations occur in either dark test. In the 0 °C dark test, both bacteria were apparently able to upkeep a level of basal metabolism for the duration of the dark test, and hence they did not become non-cultivable or die even in this experiment. Thereby, disinfection in the SODIS experiment with the lowest temperature range of this study (6–14 °C, Low T & Med I experiment) is due to UV damage, not cooling bacteria to death as could be suspected. Nevertheless, as mentioned before, cool temperatures may accelerate disinfection indirectly by slowing down metabolic functions (Jones et al., 1987; Madigan, 2017), making bacteria less able to repair UV damage. Dropping temperatures initially cause enzymatic reactions to slow down and thus metabolism rates decrease. This is due to stiffening of the cytoplasmic membrane of an organism, which hampers the transport of nutrients and protons to and from the cell. Many cell functions are increasingly compromised as temperatures decrease and membrane stiffening proceeds. This process prevents growth but does not kill the organism. (Madigan, 2017).

3.3.2. Thermal bacterial inactivation control: 43 °C dark test

The experimental time and temperature of this dark test was selected to match the maximum temperature (43 °C) and time span (4 h) of the High T & I experiment, which was conducted in Spain with local wastewater influent and spring water.

In this dark test, substantial inactivation in total coliforms (~2.6 logs) was observed after 4 h (240 min) (Fig. 7). This is somewhat unexpected, as the respective minimum, optimum and maximum temperatures of *E. coli* and other faecal (i.e. thermotolerant) coliforms are approximately 8 °C, 39 °C and 48 °C, respectively (Madigan, 2017). Their optimum temperature is surpassed in this experiment; nevertheless, their maximum temperature is not. Thereby low damage to structures could be expected (Madigan, 2017). However, already a temperature of 45 °C is known to have a synergistic effect with UV radiation on some coliforms when applying SODIS (McGuigan et al., 1998; Vivar et al., 2017a). Our results suggest that this synergistic effect could start at a slightly lower temperature than observed before. Similarly, Ubomba-Jaswa et al. (2009) also obtained somewhat comparable results to our study. They observed a ~ 1.5-log decrease in *E. coli* concentrations in natural well water in 1.5 h in a 45 °C water dark

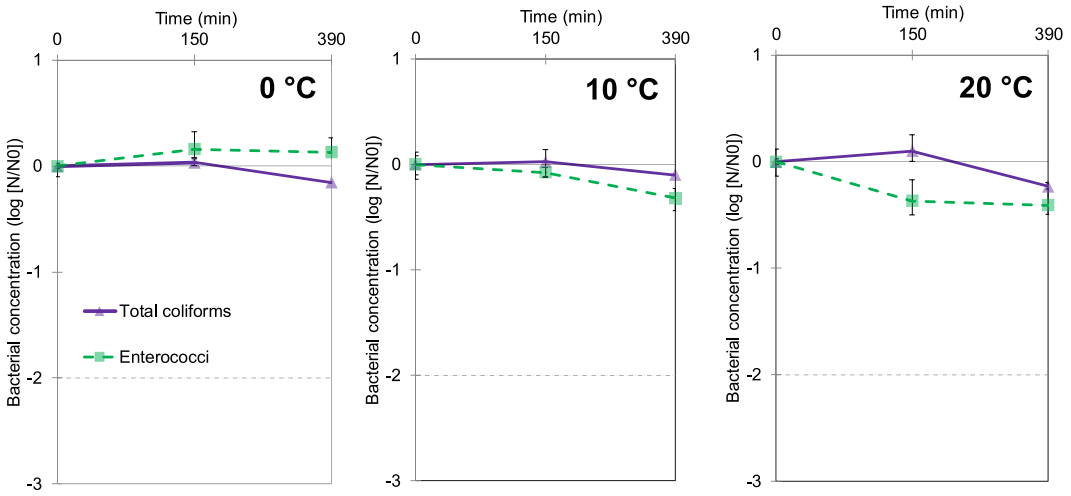

Fig. 6. Bacterial inactivation in 0 °C, 10 °C and 20 °C water in respective dark tests (Finland).

test. Giannakis et al. (2014) likewise found that there was no drastic change in *E. coli* concentrations in synthetic secondary effluent when temperatures remained between 20 °C and 40 °C for 4 h. Nevertheless, at 50 °C, 3-log inactivation of *E. coli* occurred in 2.5–3 h. Another complementary explanation for the notable disinfection of total coliforms in our study could be that the inactivated subpopulation of coliforms did not consist of thermotolerant ones.

Conversely, the 43 °C temperature dark test caused less than 0.25-log inactivation in enterococci. This temperature matches their optimum, ~43 °C (Van den Berghe et al., 2006), so they thrive in the conditions of the experiment. Enterococci are regarded as somewhat heat resistant and the strong synergistic effect of radiation and heat is likely only significant at higher temperatures surpassing 55 °C (e.g. Wegelin et al., 1994).

3.4. Preliminary cost estimation of SODIS performed in PE bags

The annual household costs of using PE bags as SODIS vessels to treat water were estimated in this study to determine how economically viable they are in everyday use. These costs include the purchasing and annual operation costs for the drinking water needs of a family of four people. The PE bags used in the present study, as described earlier, had a one-litre capacity and were 50% filled, i.e. carried 500 ml of water. These bags were used for the purpose of this study, but families could use completely filled larger bags to make SODIS more convenient. Larger containers, even with a much thicker water layer, have been proven to have a similar disinfection efficacy compared to smaller ones (Keogh et al., 2015). Fast disinfection can still be reached with PE bags after five months of use (Gutiérrez-Alfaro et al., 2017). The bags used in this study were commercial multipurpose PE bags, which were available in a Finnish supermarket for EUR 0.08 / piece (Foodie, 2017).

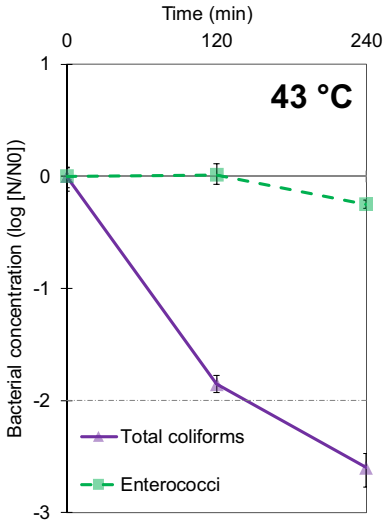

Fig. 7. Bacterial inactivation in 43 °C water in a dark test (Spain).

As can be seen from Table 6, compared to Finland, PE bags are cheaper in India, Ghana and online. Their everyday use is also cheaper compared to PET bottles, which are widely available in low-income countries (EAWAG, 2016). On the other hand, whilst conducting the experiments of this study, PE bags would sometimes break down from their seams. The fragility of PE bags in SODIS application has also been noticed by Gutiérrez-Alfaro et al. (2017) and it could hence pose an issue in household use, even if they can be very inexpensive (~0.3 EUR/year/family). Additionally, as mentioned before, PE bags are more efficient in SODIS compared to PET bottles, because they do not absorb UVB rays (Lawrie et al., 2015; Gutiérrez-Alfaro et al., 2017).

4. Conclusion

In this study, the effectiveness of point-of-use SODIS on total coliforms and enterococci was examined in the humid continental (temperate) climate of southern Finland. The Finnish climate acted as a substitute for climates of cool low-income areas where SODIS could potentially be utilized. SODIS experiments (6 h) were performed in PE bags under natural conditions (mean UV intensity 12–19 W/m^2, mean water temperature 8–23 °C). To compare the feasibility of SODIS application in this climate, an additional SODIS experiment (4 h) was also conducted in the Mediterranean (subtropical) climate of southern Spain (42 W/m^2, 38–39 °C). Reactivation after all SODIS experiments was additionally recorded, and the effect of temperature alone was studied in dark tests. The costs of using PE bag SODIS as a household water treatment method were additionally estimated.

- At least 4-log inactivation (> 99.99%) of enterococci and total coliforms was reached in all SODIS experiments in both the humid continental and the Mediterranean climatic zones. Additionally, reactivation of bacteria in the dark after each SODIS experiment was miniscule (> 0.3%) throughout experiments. The good performance of SODIS in the coldest conditions of this study (8–11 °C mean water temperature) was demonstrated here for the first time, to the best of the authors' knowledge.

Table 6
Cost estimation of using PE bag SODIS as a daily household drinking water treatment method.

Constituents of annual costs (in 2019)	Finland (€)	Online retailer (€)	India (€)	Ghana (€)	PET bottles (€)
Cost of one bag (one-litre volume)	0.08	0.01	0.02	0.05	0.09
Cost of eight bags (daily need per family)	0.64	0.08	0.16	0.4	0.72
Total annual costs incl. replacing bags every four months	2.56	0.32	0.64	1.6	2.88
Annual average cost per litre of disinfected water	0.0009	0.0001	0.0002	0.0005	0.0010

Based on these results, SODIS as a drinking water treatment method has a highly protective effect (as defined by WHO, 2011) against total coliforms and enterococci also in humid continental climate, unlike previously thought. SODIS performance in these conditions against viruses and protozoa still needs to be examined in future research.

- Even if lower doses were required for any level of disinfection in experiments conducted in humid continental climate, the same level of disinfection was mostly reached faster in the conditions of the experiment conducted in the Mediterranean climate. This was likely due to the significantly higher UV intensity in the latter. However, 4-log coliform disinfection was reached equally fast (in ~1.5 h) in the experiment with the coldest mean water temperature conducted in humid continental climate, as in the one conducted in the Mediterranean climate. Enterococci disinfection was also almost as fast in the coldest conditions as in Spain. The great speed of disinfection in the experiment with the coldest mean water temperature further highlights the good applicability of SODIS in cold conditions.
- The results suggest that a synergistic effect between cold temperatures and SODIS may exist because in terms of required dose, coliform and enterococci disinfection largely accelerated as temperatures of experiments dropped. This might be explained by slower metabolism and thus slower UV repair mechanisms in mesophilic bacteria in low temperatures, especially if temperatures drop below or close to their specific growth-allowing minimum temperatures.

CRediT authorship contribution statement

Anni Juvakoski: Writing – original draft, Methodology, Formal analysis, Investigation, Writing – review & editing, Data curation, Visualization, Funding acquisition, Project administration, Software. **Gaurav Singhal:** Investigation, Writing – review & editing. **Manuel A. Manzano:** Validation, Resources, Writing – review & editing. **Miguel Ángel Moriñigo:** Formal analysis, Writing – review & editing. **Riku Vahala:** Resources, Writing – review & editing, Supervision, Funding acquisition. **Irina Levchuk:** Conceptualization, Methodology, Software, Validation, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154086.

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