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Study of the Bunsen–Roscoe Reciprocity Law in Solar Water Disinfection (Optical Effect) for *E. coli*, *E. faecalis* and *C. perfringens*

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Abstract: Water stress and water quality represent major environmental challenges in the 21st century. In response, wastewater management and its potential reuse emerge as strategies to mitigate these problems. This research aims to verify the law of reciprocity in the solar disinfection process of real secondary wastewater effluents for different faecal microorganisms. Flat disinfection reactors, subjected only to natural and continuous UV radiation, were used. The study focused on the optical effect of UV radiation, eliminating the significant influence of the thermal effect and its synergy in solar disinfection at temperatures above 45 °C, by controlling the temperatures of the water samples to levels below 20 °C. Three experimental tests were carried out on sunny days. Each test comprised two trials, under the following conditions: (a) low solar irradiance over a prolonged time (duration approximately: 2.6 h) and (b) high solar irradiance and a shorter period of time (approximately 2 h), with each receiving the same UV dose. Inactivation kinetics was analysed for *E. coli*, *E. faecalis*, and *C. perfringens* (including spores). The results validated the reciprocity law for *E. coli* in all tests for UV doses > 20 Wh/m^2, showing no significant deviations, with inactivation rates of 0.44 to 0.51 m^2/Wh for initial concentrations of 10^6–10^7 CFU/100 mL. In contrast, for *E. faecalis*, the reciprocity was only valid at intensities < 700 W/m^2, with rates of 0.04 and 0.035 m^2/Wh for 10^5–10^6 CFU/100 mL; above this irradiance value, the law varied significantly and was not valid. *C. perfringens* did not show significant disinfection results during the experiments to verify this law, mainly due to the resistance of its spores. Additional experimentation with *C. perfringens* is necessary, by extending the length of the experiments and/or conducting them at higher irradiance values, in order to reach bacterial inactivation to enable the analysis of the reciprocity law. In general, the main conclusion from these results is that the reciprocity law in solar disinfection would be difficult to use for the estimation of water solar disinfection based on the irradiance and exposure times, as there are deviations from it at least in one specie (*E. faecalis*). Mores studies should be carried out to fully understand and determine the validity of this law and its potential application for forecasting solar water disinfection.

Keywords: reciprocity; UV disinfection; solar energy; *E. coli*; *E. faecalis*; wastewater

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1. Introduction

Global water resources are increasingly limited, and water availability and quality are major challenges affecting both developed and developing countries. Approximately 40% of the world's population lives in areas with water scarcity, and it is projected that more than 50% will face this situation by 2050 [1,2]. In view of this situation, one of the measures to mitigate water stress and limited access to water is the management of wastewater and its possible reuse. Disinfection technologies using renewable energy sources, such as solar energy [3–5], are attracting increasing attention in current wastewater treatment research due to their potential to address challenges associated with conventional methods, such as high energy costs and implementation difficulties. Among these technologies, SODIS [6] is a

simple and low-cost method that uses solar energy to disinfect water and improve its quality. Sunlight disinfects water and kills microorganisms, thanks to the combination of the optical effect of UV radiation and the thermal effect of temperature. It is known from the scientific literature that when water temperatures above 45 °C are reached during solar disinfection, a strong synergistic effect between the optical and thermal inactivation processes is observed, which enhances and accelerates microbial inactivation [7–9]. However, when temperatures are in the range close to the optimal temperature for microbial growth, generally between 20–45 °C, an antagonistic effect seems to occur that impairs solar water disinfection [10,11]. Other studies indicate that temperatures below 20 °C do not significantly affect the SODIS process [11]. On the other hand, the law of reciprocity is fundamental to understanding how the intensity of solar radiation and the time of exposure to the sun interact to achieve effective water disinfection. This law is crucial, as solar radiation can vary considerably during the day due to clouds or other factors (exposure time, time of year, geographical location, atmospheric conditions, etc.). The validity of this application is essential for determining minimum sun exposure times in SODIS, as UV irradiance changes throughout the day, and the results may vary between exposure times beginning at 9 a.m. as opposed to 12 p.m. However, this issue has not been widely studied in the research literature.

The reciprocity law, or the Bunsen–Roscoe law for photochemical processes, suggests that the inactivation efficiency is proportional to the UV radiation dose applied (Wh/m^2), which is defined as the product of the radiation intensity times the exposure time, independent of the UV irradiance used (W/m^2). The first experiments regarding this law are attributed to Bunsen and Roscoe [12], who concluded that the photochemical reaction mechanisms depended only on the total energy absorbed, independent of the radiant intensity and exposure time that determine this energy. Assuming that this law is valid, to achieve equal effects, the radiant intensities and the times that determine the total irradiated energy (UV dose) could be varied. The photoresponse of organisms receiving the same UV dose should then be equivalent, irrespective of whether the effect is realised (a) with a low radiant flux for a prolonged period of time or (b) with a high flux for a shorter period of time. However, although the reciprocity law has been verified in the vast majority of biological applications (including the inactivation of viruses, bacteria, etc.), it has also been shown that this law suffers from experimental deviations or is not fulfilled in several applications [13]. Its validity has been studied for UV inactivation in water under various UV irradiation methods and with different microorganisms. (See Section 1.1). The choice of water disinfection method and the characteristics of the sample, including its composition, can critically influence reciprocity. Solar radiation, with its daily and climatic variability, poses challenges that may contribute to deviations from this law, especially when compared to controlled technologies such as the use of UV lamps or LEDs. Furthermore, the microbial disinfection of water depends not only on photochemical reactions, but also on biological processes, which means that variations in radiation intensity, temperature, exposure time, microbiological load, and species sensitivity can also cause such deviations. However, deviations from the reciprocity law in solar disinfection have not been analyzed in real wastewater samples, which include wild strains of microorganisms, turbidity and large amounts of organic matter and nutrients; or without temperature significantly affecting the disinfection process (synergistic or antagonistic effect of temperature)".

The aim of this research is to verify the reciprocity law in regards to the solar disinfection process (SODIS) of real wastewater, using the secondary effluent of a wastewater treatment plant (WWTP). The study is based exclusively on the optical effect of solar radiation (UV radiation), excluding the significant influence of the thermal effect and its synergy on the experimental disinfection results when the water temperature is controlled below 20 °C. Moreover, it analyses the effectiveness of reciprocity on different microorganisms present in the water samples, to be treated to check whether this law is uniformly enforced, as well as in different seasons of the year. Future results will be relevant for tertiary treatment applications driven by solar disinfection in wastewater treatment plants, with particular interest in the feasibility of treatment at moderate temperatures (<20 °C).

1.1. Reciprocity Law in Microbial Water Disinfection

Pousty et al. [14] used a UV-LED system (PearlBeam, AquiSense) to expose *E. coli* MG1655 strains (laboratory prepared) to different wavelengths, using their respective average power densities (0.11–0.55 mW/cm^2). They observed that at the shortest wavelength (265 nm), the inactivation rate of *E. coli* depended solely on the UV dose, whereas at longer wavelengths (275, 285 and 295 nm), the inactivation rate did not follow the law of reciprocity, as it depended on both irradiance and exposure time. On the other hand, Kamel et al. [15] argued that this law was valid when they tested the disinfection of *E. coli*, *E. faecalis*, and *C. perfringens* in natural wastewater samples, using 265 nm LEDs with optical powers of 2.5 mW and 50 mW, and 275 nm LEDs with powers of 1.6 mW and 50 mW.

Rincón and Pulgarín [16] conducted experiments with direct solar radiation using a compound parabolic collector (CPC). Using natural water with *E. coli* K12 (ATCC 23716) strains, they concluded that the UV dose was not an adequate standard to achieve the desired bacterial disinfection, unless the intensity of irradiation was taken into account to ensure effective disinfection results. In contrast, Giannakis et al. [17] concluded that, in addition to irradiation intensity and light dose received by the sample, temperature conditions are also crucial. Their study, which simulated solar disinfection in synthetic secondary wastewater effluents with *E. coli* strains, evaluated the effects of UV doses according to the reciprocity law. It was observed that temperature could generate significant deviations in this law, with different inactivation rates for the same UV dose applied, highlighting that the rates were higher at lower intensities and lower temperatures, and lower at higher intensities and higher temperatures.

Berney et al. [18] conducted experiments with laboratory-grown strains, including *E. coli* K-12 MG1655 (ATCC 700926), under exposure to sunlight. Natural sunlight exposures were carried out at 37 °C, with UV doses calculated between 350 and 450 nm. The results showed that the inactivation curves did not change significantly according to the dose applied, and that the law of reciprocity was fulfilled for natural sunlight. Bosshard et al. [19], under similar conditions, but with higher dose rates (163–1315 W/m^2), studied the disinfection of laboratory-grown *Shigella flexneri* and *Salmonella typhimurium*, which were compared with the previously studied *E. coli* strain. The results showed some deviations from the reciprocity law with exposures to very high irradiance intensities (>700 W/m^2), while reciprocity was fairly well fulfilled under sunlight intensity (<400 W/m^2). Interestingly, the validity of this law for *E. coli* [18] and *S. typhimurium* was maintained over a wider range (50 to 700 W/m^2) compared to that for *Sh. flexneri*, which was generally validated whenever intensities lower than 400 W/m^2 were applied.

2. Materials and Methods

2.1. Experimental Setup

Three experimental tests were conducted. Each experiment was divided into two trials on the same day: (a) before solar noon and (b) during solar noon. The first trial was performed in the early morning, when light has less favourable spectral characteristics for bacterial inactivation than during solar noon. In the second trial, the sun was at its highest elevation above the horizon, compared to its positions during the rest of the day, presenting the highest amount of solar irradiance compared to those emitted in the morning or in the afternoon. The experimental days were chosen randomly in the autumn (tests #1 and #2) and spring (test #3) seasons. The experimental tests took place on the rooftop facilities of the Higher Polytechnic School (E.P.S.) of Linares, at the University of Jaén in Linares (Spain). Linares is located at 38°5′3.487″ north latitude and 3°38′46.006″ west longitude, corresponding to a temperate climate. The tests were carried out outdoors on a horizontally levelled table under sunny climatic conditions.

The Linares wastewater treatment plant (WWTP) provided the effluent samples, obtained directly after secondary treatment, with varying microbiological loads. This WWTP consisted of a pretreatment, a primary treatment, and a secondary treatment for wastewater, using a sludge line and a gas line [20]. The wastewater samples contained

wild bacterial strains and organic matter. Representative samples were always collected between 9:00 and 10:00 a.m. on the day of each experiment. In this way, the pollutant loads are studied under the same temporal variability. The total exposure of the water samples was conducted under real sunlight.

In order to carry out the experiment, first of all, and taking into account that the weather forecast was stable and with very similar characteristics to the day of the experiment, a few days before each experimental test, prior analyses were carried out (data not shown) of the climatological parameters (see Section 2.2). Based on the exposure time and the collected data of solar radiation intensity (recorded in units of W/m^2 every 60 s), the UV radiation doses throughout the day were calculated and the necessary estimates were made for the sampling time points, which have been used for each test. These calculations were adjusted to the hours of highest solar radiation between 10:00 a.m. and 16:00 p.m. For the first trial (a), a specific time interval of 2.58 h was set to allow adequate solar disinfection of the waste effluent in order to verify the reciprocity law, without the intention of obtaining high quality. Thus, from the data obtained in the trial performed before solar noon, the time required to reach the same dose of UV radiation for the test that took place during solar noon (second trial (b): approx. 2 h) was calculated. The ultraviolet (UV) radiation dose was calculated as follows:

$$\text{UV Dose } (Wh/m^2) = \sum_{i=1}^{n} [((I_{final} - I_{initial})/2) * 60\ s]/3600\ s \quad (1)$$

Equation (1) represents the calculation of the UV radiation dose as the summation over a time interval "*n*" for the average ultraviolet irradiance (I) = ($I_{final} - I_{initial}$) every 60 s, divided by 3600 s (from seconds to hours). The absorbance of the borosilicate glass and the water sample were not taken into account for the shown UV dose results during the experimental tests.

Consequently, for each experimental test, in trial (a), the solar exposure was carried out under long time conditions and low UV irradiances, while in trial (b), the solar exposure was carried out under shorter time conditions and high UV irradiances. Both trials exposed the water samples to the same UV dose. In addition, five sampling points were used in each test to carry out a kinetic study of microbiological inactivation, which included the analysis of the initial sample obtained directly from the WWTP and four other samples collected during the experiment. The previous estimations made for the sampling were very useful in determining the sampling time points (M0, M1, M2, etc.) of each of the trials carried out in each experimental test (see Table 1), as they showed a great similarity and presented minimum variations of 1–2 min. To verify this during the trials, the UV doses were recalculated every 60 s to control the variables and ensure the accuracy of the results.

For the experimental sampling conducted throughout the trials, the experimental sample was distributed in four Petri dishes (90 mm diameter × 20 mm high × 2 mm thick). These dishes were completely filled with the wastewater sample, and then each Petri dish was covered with a borosilicate glass (120 mm × 120 mm × 2 mm) to avoid contamination and the formation of bubbles (no air spaces) between the water and the glass. The borosilicate glass allowed a high transmittance for the UV spectrum (UVA-UVB), reaching 90% of the visible and infrared spectrum on the water sample (see Figure A1 in Appendix A). At each sampling point, the total volume of one dish was obtained. The initial volume of residual water in each Petri dish was 116 mL; however, the final volume of the sample obtained decreased to 114 mL (approx.) due to minor losses, primarily caused by removing the borosilicate glass for sampling. The final thickness of the water film in the Petri dish was 18 mm (22 mm is the total height of the dish with the borosilicate glass, 18 mm is the inner height of the Petri dish).

Table 1. Instantaneous global solar irradiance ($G_{irradiance}$) and UV irradiance ($UV_{irradiance}$) in the horizontal plane, accumulated UV dose, microbial inactivation expressed as Log_{10} reduction (N/N_0) and physicochemical parameters (pH, turbidity and conductivity) obtained during tests #1, #2 and #3, in experimental sampling.

Test	Sample	Time (h)		$G_{irradiance}$ (W/m^2) (280–3000 nm)	$UV_{irradiance}$ (W/m^2) (280–400 nm)	UV Dose		Log_{10} Reduction (N/N_0)			pH	Turbidity (NTU)	Conductivity (µS/cm)	COD (mg/L)	BOD_5 (mg/L)
		Real	Exp.			(Wh/m^2)	(KJ/m^2)	*E. coli*	*E. faecalis*	*C. perfringens*					
Exp. #1a	* M0	10:30:19	0.00	419.35	18.07	0.00	0.00	0.00	-	0.00	7.77	8.70	955	44	19
	M1	11:30:19	1.00	600.52	27.00	11.49	41.36	0.29	-	0.08	7.87	8.20	957	-	-
	M2	12:00:19	1.50	672.76	30.58	18.73	67.42	0.58	-	0.09	7.95	7.61	958	-	-
	M3	12:30:19	2.00	732.60	33.77	26.79	96.43	0.94	-	0.15	7.73	9.34	961	-	-
	M4	13:05:19	2.58	780.63	36.19	37.02	133.26	1.99	-	0.13	7.92	8.90	955	-	-
Exp. #1b	* M5	13:30:19	0.00	797.17	36.98	0.00	0.00	0.00	-	0.00	7.77	8.70	955	44	19
	M6	14:05:19	0.58	813.92	37.63	11.22	40.37	0.60	-	0.00	8.19	7.82	555	-	-
	M7	14:29:19	0.97	809.40	37.21	18.67	67.20	0.84	-	0.03	8.81	9.98	942	-	-
	M8	14:55:19	1.40	785.57	35.52	26.60	95.74	1.11	-	0.05	8.26	9.58	949	-	-
	M9	15:31:19	2.02	747.74	33.41	37.06	133.40	2.06	-	0.11	8.24	8.85	835	-	-
Exp. #2a	* M10	10:30:44	0.00	319.16	12.94	0.00	0.00	0.00	0.00	-	7.70	8.61	951	54	23
	M11	11:30:44	1.00	482.48	21.24	8.72	31.39	0.25	0.45	-	7.91	8.09	945	-	-
	M12	12:00:44	1.50	551.28	24.84	14.51	52.23	0.63	0.50	-	7.84	8.30	955	-	-
	M13	12:30:44	2.00	606.58	27.58	21.11	76.00	1.03	0.88	-	7.74	8.64	942	-	-
	M14	13:05:44	2.58	653.13	29.87	29.54	106.33	1.65	2.16	-	7.74	8.75	947	-	-
Exp. #2b	* M15	13:30:44	0.00	669.83	30.88	0.00	0.00	0.00	0.00	-	7.70	8.61	951	54	23
	M16	14:03:44	0.55	678.54	31.07	8.80	31.67	0.20	0.34	-	7.93	8.20	928	-	-
	M17	14:25:44	0.92	676.42	31.13	14.50	52.21	0.58	0.47	-	7.94	8.38	566	-	-
	M18	14:51:44	1.35	654.73	29.82	21.08	75.87	0.90	0.67	-	7.99	8.52	942	-	-
	M19	15:26:44	1.93	614.52	27.69	29.45	106.01	1.53	1.09	-	8.01	8.47	907	-	-
Exp. #3a	* M20	10:30:59	0.00	569.78	25.41	0.00	0.00	0.00	0.00	0.00	7.64	4.77	921	50	22.5
	M21	11:30:59	1.00	754.35	34.87	15.14	55.53	0.48	0.00	0.07	7.92	3.98	919	-	-
	M22	12:00:59	1.50	828.41	39.00	24.36	88.86	0.74	0.00	0.00	7.95	3.79	916	-	-
	M23	12:30:59	2.00	893.16	42.09	34.48	125.40	1.31	0.31	0.12	7.96	4.28	918	-	-
	M24	13:05:59	2.58	950.09	44.75	47.15	171.10	2.55	0.55	0.24	8.09	4.88	911	-	-
Exp. #3b	* M25	13:30:59	0.00	973.07	45.96	0.00	0.00	0.00	0.00	0.00	7.64	4.77	921	50	22.5
	M26	14:08:59	0.63	996.81	46.88	14.73	54.43	0.36	0.00	0.06	7.90	5.23	918	-	-
	M27	14:31:59	1.02	991.40	46.47	23.70	86.72	0.65	0.03	0.06	7.91	4.37	917	-	-
	M28	14:59:59	1.48	975.28	45.44	34.47	125.46	1.33	0.40	0.11	7.90	4.34	910	-	-
	M29	15:33:59	2.05	935.55	43.10	47.08	170.78	2.68	0.95	0.13	7.98	4.60	1026	-	-

(*) Raw water samples; (-) analysis not conducted.

On the other hand, the Petri dishes were modified with a small notch on the edge to incorporate NTC sensors to measure the temperature of the water. In addition, the dishes were placed on (white and flat) containers inside white plastic trays (two trays with two Petri dishes each) containing a water bath and crushed ice to lower the temperature of the water samples. The white colour of the trays allows practically all incoming radiation to be reflected and scarcely absorbed. The ice bath was manually controlled (by continuously adding more ice to the bath), which in turn allowed the controlled temperature of the water inside the Petri dishes to be maintained below 20 °C. The measured of the water temperature in the Petri dish was assumed to be equivalent to the temperature of the ice bath, as they were in thermal equilibrium. In this way, the effect of UV disinfection was separated from the temperature, and solar disinfection was attributed to the effect of germicidal UV radiation only. Furthermore, small lateral holes were made in the trays so that, when the water cooling the dishes reaches a certain level, it flowed out of the tray, thus preventing the water from reaching the surface of the experimental dish and interfering with the wastewater samples. Figure 1 illustrates the experimental set-up used, together with a schematic of the control and recording of climatic conditions and water sample temperature.

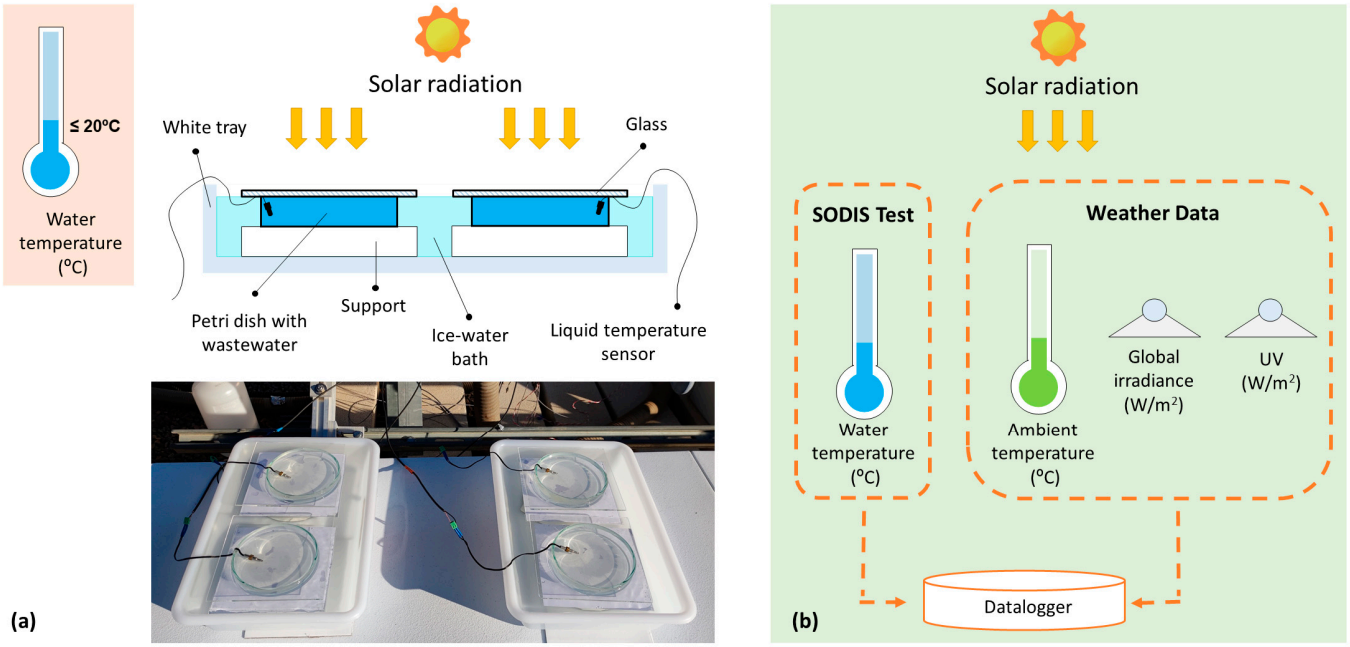

Figure 1. Experimental setup: (**a**) Scheme of solar disinfection during the experimental trials and (**b**) scheme of control and recording of climatic conditions and water sample temperature.

After the solar exposure time had elapsed, each sample was carefully removed from the container, without shading the rest of the samples. First, the borosilicate glass, placed on the Petri dish, was carefully removed. Next, the sample was pipetted and transferred to a sterilized bottle. Later, the sterile bottle was stored, protected from light, and then refrigerated until further analysis (within 24 h).

In conjunction with the research analysis, microbiological analyses were carried out at the beginning (raw water) and after the experimental trials, using *Escherichia coli (E. coli)*, *Enterococcus faecalis* (*E. faecalis*), and *Clostridium perfringens* (*C. perfringens)* as microbiological indicators. In test #1, *E. coli* and *C. perfringens* bacteria were analysed; in test #2, *E. coli* and *E. faecalis* were analysed; and finally, in test #3, all three bacteria studied, *E. coli*, *E. faecalis*, and *C. perfringens*, were included. During the experiment, the physicochemical parameters of pH, conductivity, and turbidity were also analysed. Horizontal global irradiance, horizontal UV irradiance, ambient temperature, and water temperature in the Petri dishes were also monitored. These data were processed using Origin Pro 2021 Data Analysis and Graphing software from OriginLab. Additionally, the absorbance in the UV/Vis wavelength range of each of the initial wastewater samples was determined (see Figure A2 in Appendix A), mainly due to the presence of organic matter and turbidity. The absorbance data of the samples were analysed using UV–Vis Analyst software.

2.2. Control of Climatic Conditions and Electrical Parameters

The climatic conditions were monitored using a Keysight data logger (22-bit); the measured data were: global solar irradiance in the horizontal plane (280–3000 nm), measured with a Kipp & Zonen CMP 21 pyranometer, UV irradiance in the horizontal plane (280–400 nm), measured using a Kipp & Zonen CUV5 radiometer, and ambient temperature obtained using Young sensors. The water temperature inside the Petri dishes was measured by an NTC immersion sensor (10 K), located on the side wall of each Petri dish. All data were recorded every 60 s.

2.3. Microbiologic Analysis

Wild strains of *E. coli*, *E. faecalis*, and *C. perfringens* (from real samples of secondary wastewater effluent from a WWTP) were used as microbial indicators of faecal contami-

nation. The membrane filtration technique (UNE-EN ISO 8199:2018) [21], using 0.45 µm cellulose nitrate membrane filters, was employed to detect bacterial concentration. The filtration ramp was initially cleaned with Milli-Q water, and after use, a disinfectant spray was applied to remove any contaminants. Additionally, controls were also cleaned with Milli-Q water to ensure a valid method. The material used was previously sterilised. Samples were filtered in triplicate and transferred to Petri dishes with appropriate culture media. Microinstant® Chromogenic Coliforms agar (Scharlau 01-797-500) was used for *E. coli*. The Petri dishes were incubated at (36 ± 2) °C for (21 ± 3) h. Dark blue to violet colonies were counted as *E. coli*. UNE-EN ISO 9308-1:2014 [22]. Slanetz and Bartley agar (Scharlau 01-579-500) + sterile 1% TTC solution (Scharlau 06-023) was used for *E. faecalis*. The Petri dishes were incubated at (36 ± 2) °C for (44 ± 4) h, followed by a confirmation step for the considered typical red, brown, or pink colonies in the centre or throughout the colony. Membranes with these colonies were transferred to other Petri dishes with Bile Esculin Azide agar. These dishes were incubated at (44 ± 0.5) °C for 2 h. Colonies displaying a typical brown to black coloration were considered as displaying a positive reaction and were counted as *E. faecalis*. UNE-EN ISO 7899-2:2000 [23]. *C. perfringens* used ChromAgar™ Chromogenic as the culture medium. The Petri dishes were incubated anaerobically at (37 ± 1) °C for (21 ± 3) h. Characteristic colonies were orange in colour. UNE-EN ISO 14189:2013 [24].

2.4. Physicochemical Analysis

This study performed a physicochemical analysis of turbidity (NTU) using a Lovibond TB 211 IR turbidimeter; conductivity (σ) and pH were evaluated with a HACH SensION + MM374 Multimeter + 5014 electrode (pH) + 5070 cell (electrical conductivity); biological oxygen demand (BOD_5) and chemical oxygen demand (COD), as well as absorbance analysis of the wastewater, were performed with a UV/Vis UV-3100PC spectrophotometer; these analyses were performed within 24 h after sampling of the secondary wastewater effluent at the WWTP. Turbidity, pH, and conductivity were also analysed after each experimental sampling.

3. Results and Discussion

The results of the experimentation corresponding to the three experimental tests are shown below.

3.1. Weather Conditions

Figure 2 shows the climatic conditions versus the time spent during the solar disinfection treatments. The experiments were carried out under sunny conditions; only in experiment #1b, some small clouds were observed after the start of the experimentation (time period: 13:45–14:15 h).

Experiment #1a was carried out between 10:30 h and 13:05 h, under a total sun exposure of 2 h and 35 min (2.58 h). In contrast, experiment #1b was conducted between 13:30 h and 15:31 h, under a total sun exposure of 2 h and 1 min (2.02 h). This led to a cumulative UV dose of 37.02 Wh/m^2 and 37.06 Wh/m^2, respectively. The maximum value of global irradiance in the horizontal plane reached in Exp. #1a was 780.63 W/m^2, with an average of 628.64 W/m^2, together with a maximum UV irradiance of 36.32 W/m^2 and an average value of 28.47 W/m^2. Exp. #1b exhibited higher values, with maximum global solar irradiance of 816.16 W/m^2 and a UV irradiance of 37.87 W/m^2, with respective average values of 793.25 W/m^2 and 36.45 W/m^2. Similarly, experiment #2a also took place between 10:30 h and 13:05 h (2.58 h duration). Experiment #2b was conducted between 13:30 h and 15:26 h, under a total sun exposure of 1 h and 56 min (1.93 h). Thus, a cumulative UV dose of 29.54 Wh/m^2 and 29.45 Wh/m^2 was achieved, respectively. Exp. #2a obtained a maximum global irradiance of 653.13 W/m^2 and a UV irradiance of 30.00 W/m^2. Respectively, the average values were 511.46 W/m^2 and 22.72 W/m^2. In contrast, Exp. #2b reached higher values for the maximum global irradiance of 681.01 W/m^2 and an average of 661.30 W/m^2,

as well as for the maximum UV irradiance of 31.41 W/m^2 and an average of 30.20 W/m^2. Finally, experiment #3a was carried out between 10:30 h and 13:05 h (like tests #1a and #2a, with a total duration of 2.58 h), while experiment #3b was conducted between 13:30 h and 15:33 h, under a total sun exposure of 2 h and 3 min (2.05 h). In this test, the highest UV doses were reached with 47.15 Wh/m^2 and 47.08 Wh/m^2, respectively. Exp. #3a showed a maximum global irradiance of 950.09 W/m^2 and an average value of 787.94 W/m^2. During the spring test, the highest UV irradiance values were reached with a maximum of 44.75 W/m^2 and an average value of 36.63 W/m^2. On the other hand, Exp. #3b reached higher values for the maximum global irradiance of 1000.42 W/m^2 and an average of 980.66 W/m^2, as well as the maximum UV irradiance of 47.09 W/m^2 and an average of 45.91 W/m^2.

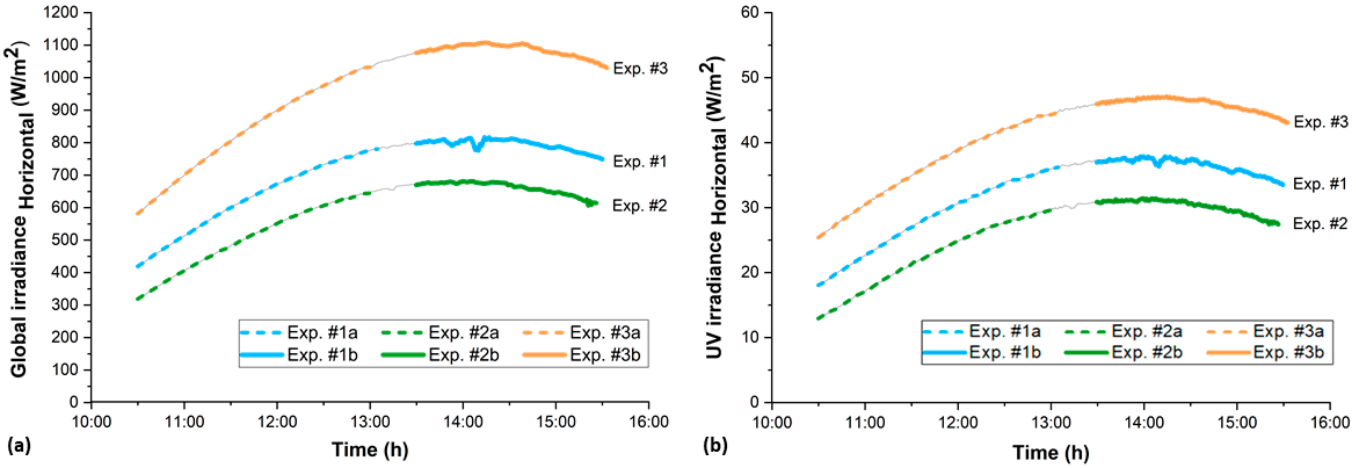

Figure 2. Climatic conditions of global irradiance (**a**) and UV irradiance (**b**) versus solar exposure time of experiments #1, #2 and #3 performed on the rooftop of the E.P.S. of Linares (Jaén, Spain).

3.2. Physicochemical Tests

The most relevant physicochemical parameters (pH, turbidity, and conductivity) were monitored throughout each experiment for each of the samples analysed. The results did not show significant variations before and after the SODIS treatment, nor among the experimental trials of each experiment (see Table 1). However, it is true that the analyses of the initial turbidity of the wastewater samples from tests #1 (8.70 NTU) and #2 (8.61 NTU) were almost twice as high as that in test #3 (4.77 NTU). This may have hindered UV penetration and thus, the inactivation of bacteria, regardless of the UV dose applied. The values of pH, turbidity, and conductivity during the experimentation ranged from 7.64–8.81, 3.79–9.98 NTU, and 555–1026 µS/cm, respectively. In addition, only BOD_5 and COD measurements were performed on the raw wastewater samples before SODIS. These analyses showed values in the range of 19–23 mg/L for BOD_5 and 44–54 mg/L for COD, which indicated a significant organic load.

3.3. Control of the Thermal Effect in SODIS

As previously mentioned, for the analysis of the reciprocity law in SODIS, it was performed only under the optical effect of UV radiation, avoiding the influence of the thermal effect by controlling the temperature of the water samples. These temperatures were maintained (manually) in a controlled manner using the ice-water bath for the Petri dishes containing the water samples, with cold temperatures maintained below 20 °C. The average temperatures of the different water samples in each experiment were: 18.64 °C in Exp. #1a and 18.88 °C in Exp. #1b; 18.80 °C in Exp. #2a and 18.12 °C in Exp. #2b; and 17.62 °C in Exp. #3a and 17.63 °C in Exp. #3b. Figure 3 shows the temperatures of the treated water during the experimental sampling and the ambient temperature of experiments #1 (Figure 3a), #2 (Figure 3b), and #3 (Figure 3c).

During the experimentation, the temperatures of the water samples were kept below 20 °C, which is far from the optimal growth temperatures of the tested faecal bacteria: 37 °C for *E. coli*, 35 °C for *E. faecalis*, and 43–47 °C for *C. perfringens* [25–27]. Therefore, it is argued that the antagonistic effect of temperature did not affect during the water disinfection process.

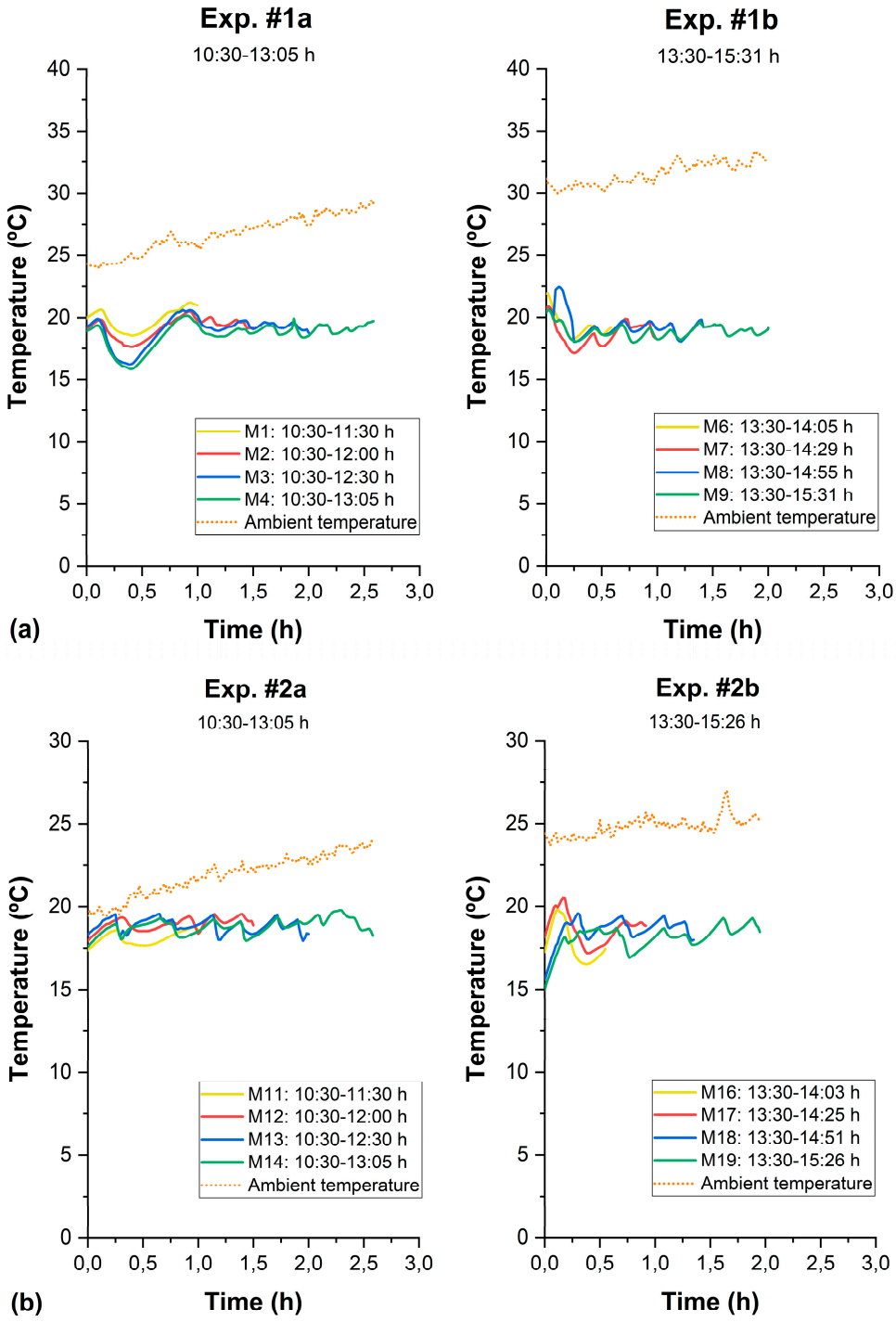

Figure 3. *Cont.*

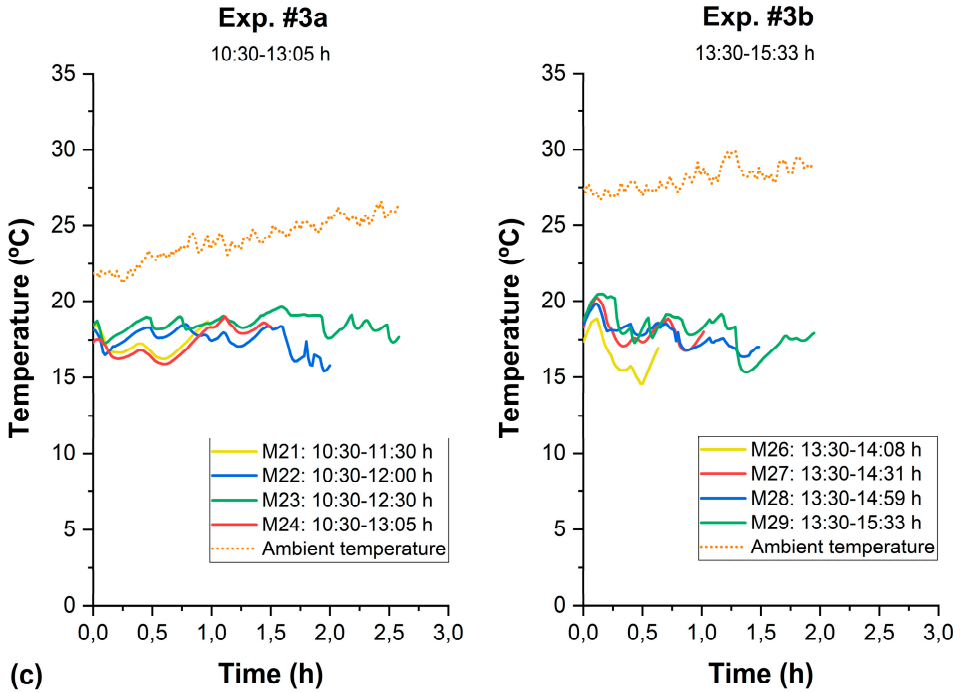

Figure 3. Temperature control during SODIS treatment for tests #1 (**a**), #2 (**b**), and #3 (**c**). Right, the first trial with, high exposure time and low solar irradiance, and left, the second trial, with lower exposure time and high solar irradiance. The water samples taken during sampling are indicated by the letter "M" and are numbered; the exposure time of each is indicated in the graph.

3.4. Solar Disinfection: Exclusively UV (Optical Effect)

Figure 4 shows the inactivation kinetics of *E. coli*, *E. faecalis*, and *C. perfringens* during the solar water disinfection treatments studied. These results are complemented by Table 1, which shows the percentage of bacterial inactivation and includes global solar irradiance, UV irradiance, UV dose, and physicochemical parameters analysed during the experimental sampling of each experiment.

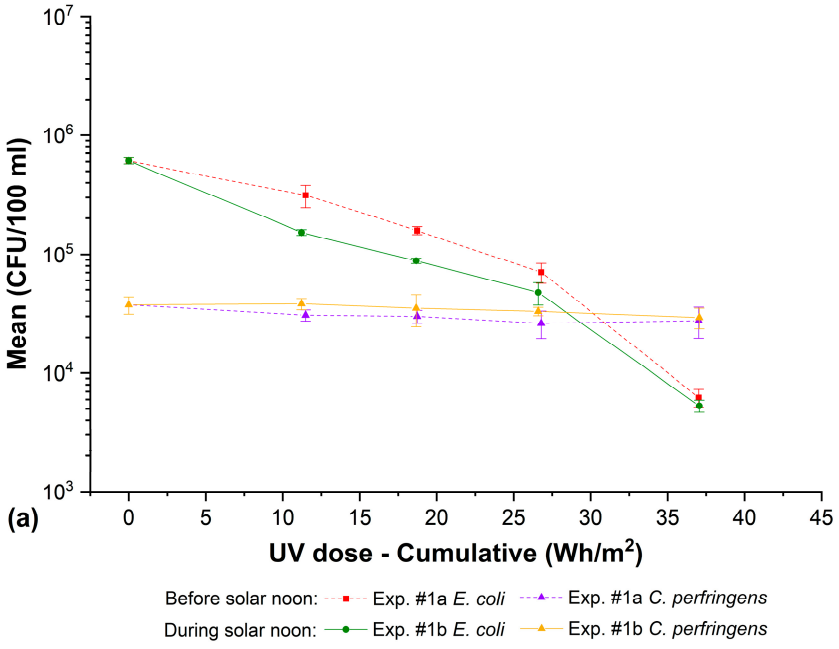

Figure 4. *Cont.*

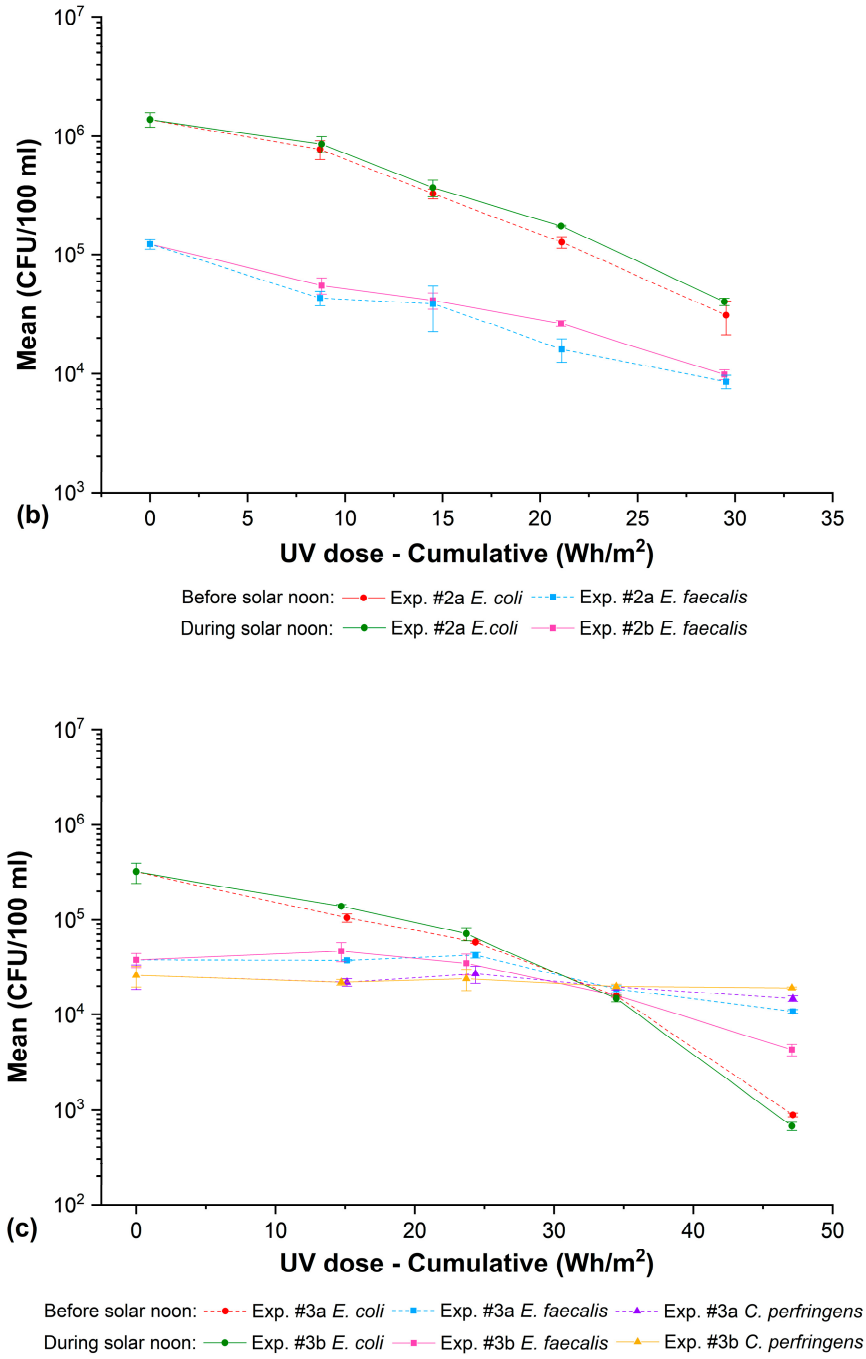

Figure 4. SODIS disinfection kinetics for *E. coli*, *E. faecalis*, and *C. perfringens* during tests #1 (**a**), #2 (**b**), and #3 (**c**), before solar noon and during solar noon, with controlled temperature (<20 °C). The reciprocity law was valid for *E. coli* and underwent deviations for *E. faecalis*. *C. perfringens* showed no significant results. The detection limit (100 CFU/100 mL) was not reached in any test.

The highest initial concentrations were obtained in test #2, with $1.37 \times 10^6 \pm 1.81 \times 10^5$ CFU/100 mL for *E. coli* and $3.8 \times 10^4 \pm 6.43 \times 10^3$ CFU/100 mL for *E. faecalis*, and in test #1, with $3.73 \times 10^4 \pm 6.03 \times 10^3$ CFU/100 mL for *C. perfringens*. Similarly, the lowest concentrations were acquired in test #3 for all three bacteria, with $3.17 \times 10^5 \pm 7.57 \times 10^4$ CFU/100 mL, $3.80 \times 10^4 \pm 6.43 \times 10^3$ CFU/100 mL, and $2.57 \times 10^4 \pm 6.43 \times 10^3$ CFU/100 mL, respectively. (Table A1 in Appendix A).

In the first experimental test (Figure 4a), *E. coli* achieved a 2.0 log reduction in Exp. #1a, with a UV dose of 37.02 Wh/m^2. In Exp. #1b, *E. coli* showed a 2.1 log reduction under

a similar dose of 37.06 Wh/m^2. *C. perfringens* did not reach significant inactivation levels, with respective reductions of 0.13 log and 0.10 log, mainly due to the strong resistance of its spores to solar disinfection, which makes their elimination difficult. This is further corroborated in the graph and Table 1, where the inactivation kinetics encompass the statistical error of the initial bacterial population (standard deviation). In the second experimental test (Figure 4b), *E. coli* achieved a 1.6 log reduction in Exp. #2a, with a UV dose of 29.54 Wh/m^2. In Exp. #2b, *E. coli* showed a 1.5 log reduction under a similar dose of 29.45 Wh/m^2. For *E. faecalis* the reduction levels were 1.2 log and 1.5 log, respectively. In the third test (Figure 4c), *E. coli* achieved a 2.6 log reduction in Exp. #3a, under a high UV dose of 47.15 Wh/m^2, and a 2.7 log reduction in Exp. #3b, under a similar dose of 47.08 Wh/m^2. Correspondingly, *E. faecalis* achieved a reduction of 0.6 log and 1.0 log. Finally, *C. perfringens* again achieved low reductions, which were similar to those in test #1, although with a lower initial concentration and a higher UV dose. The final inactivation values can be considered significant, with a 0.24 log inactivation in Exp. #3a and a 0.13 log inactivation in Exp. #3b.

The results indicate that no experiment achieved complete inactivation, but significant levels of disinfection were achieved for *E. coli* and *E. faecalis*. After the experimental studies, better quality water was obtained, although its possible uses as reclaimed water were not analysed, but the findings obtained were used to verify the law of reciprocity. Figure 4a–c shows how pure UV disinfection was accelerated under the higher UV doses used for *E. coli* and *E. faecalis*, while for *C. perfringens*, this effect was negligible.

Another finding observed was that the inactivation curves for *E. coli* and *E. faecalis* showed an analogous effect. In addition, in the three studies, *E. coli* was shown to be the test organism most sensitive to UV, followed by *E. faecalis*; and *C. perfringens* was the most resistant. These facts were also corroborated by Kamel et al. [15] for all three bacteria in real wastewater samples, during a similar experimentation for the reciprocity law with UV LEDs. On the other hand, the slow inactivation of *C. perfringens* (vegetative + including spores) indicated the low susceptibility of this strain to solar disinfection, mainly due to its high resistance as a bacterial spore-forming species. In addition, the temperatures in this study, controlled below 20 °C, might not be optimal for bacterial inactivation. In future research, it is recommended to focus exclusively on the spores, excluding the vegetative elements.

Moreover, the physiological response between the microorganisms studied and the SODIS treatment was observed to differ between the bacteria analysed, concluding that it is natural to expect anomalies in the law of reciprocity for different bacterial species. In addition to microbial susceptibility, other possible factors that could influence the different degrees of inactivation during solar disinfection are considered, such as the initial concentration of bacteria, suboptimal temperatures in the water samples, and the presence of organic matter and turbidity in the water. These variables need to be further analysed in future research in regards to real wastewater samples.

In general, the final results obtained argued that *E. coli* does comply with the Bunsen–Roscoe reciprocity law for each of the tests performed, as reflected in Table 1, while deviations in reciprocity were observed for the *E. faecalis* strains. More specifically, when looking further into the *E. coli* disinfection results, it was found that during the experimental sampling for low UV doses (in this work, low UV doses below 20 Wh/m^2 were considered), the reciprocity law was not always fulfilled or suffered slight deviations. For example, the deviation fron this law could be observed in the first experimental test, a logarithmic inactivation of 0.29 log was shown for a dose of 11.49 Wh/m^2 in Exp. #1a, compared to a 0.60 log inactivation under an irradiated UV dose of 11.22 Wh/m^2 in Exp. #1b. In addition, it was also observed in the third test, where a disinfection of 0.48 log was achieved in Exp. #3a versus a 0.36 log in Exp. #3b, for corresponding UV doses of 15.14 Wh/m^2 and 14.73 Wh/m^2. However, in the second test, although theoretically, a logarithmic inactivation of 0.25 log was achieved in Exp. #2a versus an inactivation of 0.20 log (8.80 Wh/m^2) in Exp. #2b, these limits were within the margin of error of the standard deviation for *E. coli*, so it is considered that the law of reciprocity is fulfilled.

On the other hand, when analysing the impact of solar disinfection on *C. perfringens*, a practically flat and stable disinfection curve was observed, with no significant changes. As a consequence, this strain requires further study to achieve bacterial inactivation and thus to be able to analyse the law of reciprocity, with longer exposure times and/or higher irradiance values, in order to obtain relevant information on its disinfection by solar energy.

In contrast to the findings of this study, in previous research by Giannakis et al. [17], discussed in the introduction, it was argued that in simulated solar disinfection for synthetic wastewater samples with *E. coli* strains at a treatment temperature (SODIS) of 20 °C (800 vs. 1200 Wh/m^2) for concentrations of 10^3–10^6 CFU/mL, the same UV radiation dose was more effective at lower irradiation intensities. However, in the case of *E. coli* and *E. faecalis*, this effect was only slightly observed in test #2, a result that did not agree with those of the rest of the tests, which led to the consideration of this effect as not significant. The main reason for this lies in the disinfection technique used. Giannakis et al. used simulated sunlight with a specific radiation intensity, while in this study, natural sunlight was used. Natural light contains a wide range of wavelengths and its intensity can vary according to climatic conditions and environmental bacteria strains (non-synthetic water). Moreover, in this investigation, the law of reciprocity was also fulfilled for *E. coli* concentrations around 10^6 CFU/100 mL at a temperature of 20 °C.

On the other hand, pure UV disinfection kinetics was also performed following a first order logarithmic linear model: Log (N_t) = $-$ K_{UV} · UV dose + N_0, where N is the concentration of bacteria (CFU/100 mL) at time t, K_{UV} is the inactivation constant/rate, UV dose is the cumulative UV radiation (Wh/m^2) at time t, and N_0 the initial microbial population (CFU/100 mL). Table 2 shows the calculated KUV inactivation rates for each experiment and bacteria, the coefficient of determination R^2, and the turbidity value for each experiment.

Table 2. Kinetics of the purely UV disinfection processes with controlled temperature control (<20 °C) in the experiments performed during tests #1, #2, and #3 for *E. coli*, *E. faecalis*, or *C. perfringens*. A first order logarithmic linear decay model was followed.

Bacteria	Experiment	Turbidity (NTU) Average	$k_{ultraviolet}$	R^2
E. coli	#1a	8.55	0.044 ± 0.005	0.997
	#1b	8.99	0.050 ± 0.003	0.999
C. perfringens	#1a	8.55	0.027 ± 0.002	1
	#1b	8.99	$0.039 \pm 5.662 \times 10^{-4}$	1
E. coli	#2a	8.48	0.051 ± 0.003	0.999
	#2b	8.44	0.046 ± 0.004	0.999
E. faecalis	#2a	8.48	0.040 ± 0.002	1
	#2b	8.44	0.035 ± 0.001	1
E. coli	#3a	4.34	0.045 ± 0.005	0.995
	#3b	4.66	0.046 ± 0.006	0.993
E. faecalis	#3a	4.34	0.008 ± 0.002	0.999
	#3b	4.66	0.014 ± 0.004	0.996
C. perfringens	#3a	4.34	$0.004 \pm 9.949 \times 10^{-4}$	1
	#3b	4.66	$0.003 \pm 3.744 \times 10^{-4}$	1

The kinetics of *E. coli*, *E. faecalis*, and *C. perfringens* were fitted to a first order model for purely UV disinfection processes, with coefficients of determination (R2) high and very close to 1, indicating an adequate fit to the experimental data. In general, the inactivation rates (K_{UV}) for *E. coli* (0.044–0.051 m^2/Wh) were higher than for the other two bacteria tested under the same experimental conditions and with a higher initial bacterial population, again leading to the conclusion that *E. coli* is more sensitive to UV. The results showed almost identical inactivation rates for *E. coli* in each test, despite the differences in UV conditions in the different seasons and times of day, which were also very similar in all three tests

performed. Thus, the law of reciprocity was again justified for *E. coli*. Furthermore, the reciprocity law was valid for *E. faecalis* when intensities < 700 W/m^2 were applied in test #2, reaching similar inactivation rates of 0.04 m^2/Wh (Exp. #2a) and 0.035 m^2/Wh (Exp. #3b); however, when higher irradiation intensities (700–1000 W/m^2) were applied, deviations from this law were shown in test #3, with rates of 0.008 m^2/Wh (Exp. #3a) and 0.014 m^2/Wh (Exp. #3b). This finding was in agreement with those of Bosshard et al. [19], who demonstrated the importance of reciprocity in SODIS when using simulated sunlight, where their findings argued that this law suffered some deviations for *Shigella flexneri* and *Salmonella typhimurium* enteric bacteria with exposures to very high irradiance intensities (>700 W/m^2). Finally, for the *C. perfringens* strain, this law was also not accepted, since the inactivation rates obtained in each experimental test were discrepant and not significant.

Concerning variations throughout the day in the intensity of solar radiation for solar disinfection, it has been reported that solar disinfection is most effective when UV radiation is most intense, which tends to occur during the hours of the day when the sun is at its highest point in the sky, that is, around solar noon. For example, Sichel et al. [28] and Ubomba-Jaswa et al. [29] both conducted experiments at different times of the day and concluded that experiments starting near solar noon, with higher UV irradiance values, achieve faster disinfection rates than those starting early in the morning. These results have also been supported in this study.

Lastly, the main conclusion of this research suggests that the law of reciprocity in solar disinfection would be difficult to use for the estimation of solar water disinfection as a function of irradiance and exposure time, since deviations from this law are observed in at least one species (*E. faecalis*). Further studies are therefore required to fully understand and determine the validity of this law and its potential application for predicting solar water disinfection.

From another perspective, in terms of the research carried out in this paper, the scientific literature has studied the reciprocity law for water disinfection under UV LEDs for different wavelengths and power levels [14,15], under exposure to sunlight (SODIS) [16,18,19] or simulated sunlight [17]. Bacterial inactivation can be equivalent under the same UV dose (reciprocity law), regardless of whether (a) low radiation is applied over a prolonged time or (b) high radiation is employed over a shorter time. However, studies have shown that disinfection may be higher in either case. The results show an important controversy for this law which, together with the results obtained in this research, have been discussed due to the influence of the following several factors:

(1) The significant influence of temperature on solar disinfection (thermal effect), which is a key factor.
(2) The use of synthetic water samples (with greater control over variables and a more controlled and reproducible study) versus natural samples (exhibiting the complexity and variability of the real environment).
(3) The importance of differences in the intensity and spectrum of UV radiation emitted. For example, UV LEDs generally emit UV radiation in a specific wavelength range, and its intensity can be controlled, whereas solar radiation contains a broader mix of wavelengths, and its intensity varies according to climatic conditions and geographical location.
(4) The fact that bacteria may be affected differently under UV radiation, depending on the wavelength applied and the type of specific species. Some bacterial species may be less sensitive to certain wavelengths and more sensitive to others. This is due to the individual characteristics of each species and the defence and repair mechanisms of the genetic material, which may also be influenced by environmental factors such as the availability of nutrients or the intensity of UV radiation.

Also, in relation to solar water disinfection using natural strains, more detailed research is required, especially for *E. coli* at lower initial irradiance levels, as well as for *E. faecalis*. This implies considering greater variability throughout the year to determine a minimum UV dose, with an appropriate irradiance level at the beginning, in order to ensure complete disinfection of the water. For *C. perfringens*, further research is crucial to determine whether

disinfection by solar exposure alone would be adequate, or whether it would require any complementary pre-treatment.

4. Summary and Conclusions

Based on previous scientific literature, this research represents the first analysis of the law of reciprocity in regards to solar disinfection treatment under real sunlight using natural wastewater samples from a WWTP that contained wild bacterial strains, organic matter, and nutrients. This study was conducted under a controlled temperature below 20 °C to eliminate the significant influence of the thermal effect of solar radiation as a conditioning factor in microbiological inactivation in order to exclusively study the optical effect of solar radiation to evaluate its effectiveness on different microorganisms, including *E. coli*, *E. faecalis*, and *C. perfringens*.

The experimental results of solar disinfection did not achieve the total or effective inactivation of any bacterial strain studied at any time. However, significant disinfection rates were achieved for *E. coli*, with a 1.53–2.68 log reduction, and for *E. faecalis*, with a 0.55–2.16 log reduction. These data did not affect the purpose of the research, which was to verify the law of reciprocity.

The results show that the reciprocity law was only justified for the solar disinfection of *E. coli*, while *E. faecalis* suffered deviations in regards to this law. The inactivation kinetics of *E. coli* revealed almost identical inactivation rates in each trial for the same UV dose, which was also very similar for the three tests performed, under different environmental conditions. However, it was observed that the reciprocity may suffer slight deviations and is not always fulfilled at low UV doses (UV doses < 20 Wh/m^2 have been estimated based on the results obtained in this work). For *E. faecalis*, the law was valid at intensities < 700 W/m^2, and it was rejected at higher solar irradiation intensities (>700 W/m^2). Moreover, *C. perfringens* strains did not show relevant results after disinfection treatment, so the behaviour of this bacteria should be studied in depth. On the other hand, it was observed that the physiological response that occurred between the microorganisms studied and the SODIS treatment differed between the bacteria analysed. Therefore, it was reaffirmed that it is natural to expect anomalies in the reciprocity law for different bacterial species.

In summary, as there is no valid and equal final conclusion for all bacteria studied, without absolute certainty that the reciprocity law is always fulfilled, the reciprocity law could not be used to accurately calculate or estimate the disinfection of wastewater from the received global solar radiation values and the treatment time. Furthermore, taking into account that in real tertiary treatment applications, it will not be possible to control the water temperature, we can conclude that we cannot currently use this law to estimate the solar disinfection of water under different climatic conditions. Further research with different microorganisms, as well as different temperatures, would be needed to determine whether the law holds true and could be used to make daily estimates of tertiary solar water disinfection. It would also be interesting to determine whether the microbial concentration influences the reciprocity law during solar disinfection. Finally, the way in which this law affects natural versus synthetic water samples should also be studied, as the literature expresses differing opinions.

Author Contributions: Conceptualization, J.T., A.M.P., M.F. and M.V.; methodology, J.T., A.M.P., M.F. and M.V.; software, M.F. and M.V.; validation, J.T., M.F. and M.V.; formal analysis, J.T. and M.V.; investigation, J.T., A.M.P., M.F. and M.V.; resources, J.T., A.M.P., M.F. and M.V.; data curation, J.T., M.F. and M.V.; writing—original draft preparation, J.T.; writing—review and editing, J.T. and M.V.; visualization, J.T., M.F. and M.V.; supervision, J.T., M.F. and M.V.; project administration, M.F. and M.V.; funding acquisition, M.F. and M.V. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

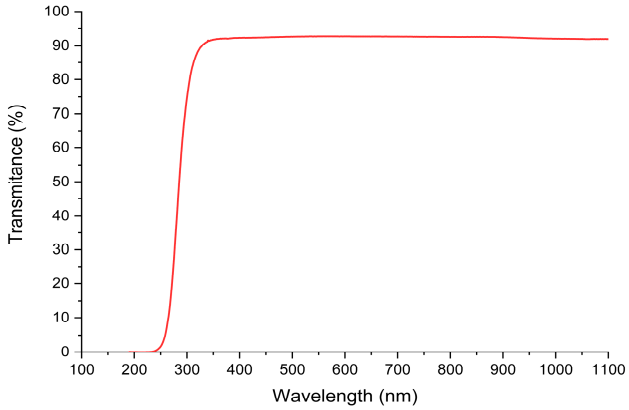

Figure A1. UV–Vis transmission spectrum of borosilicate glass. This material allows for a high transmittance of the UV spectrum (UVA-UVB), being able to reach 90% of the visible spectrum and the infrared spectrum.

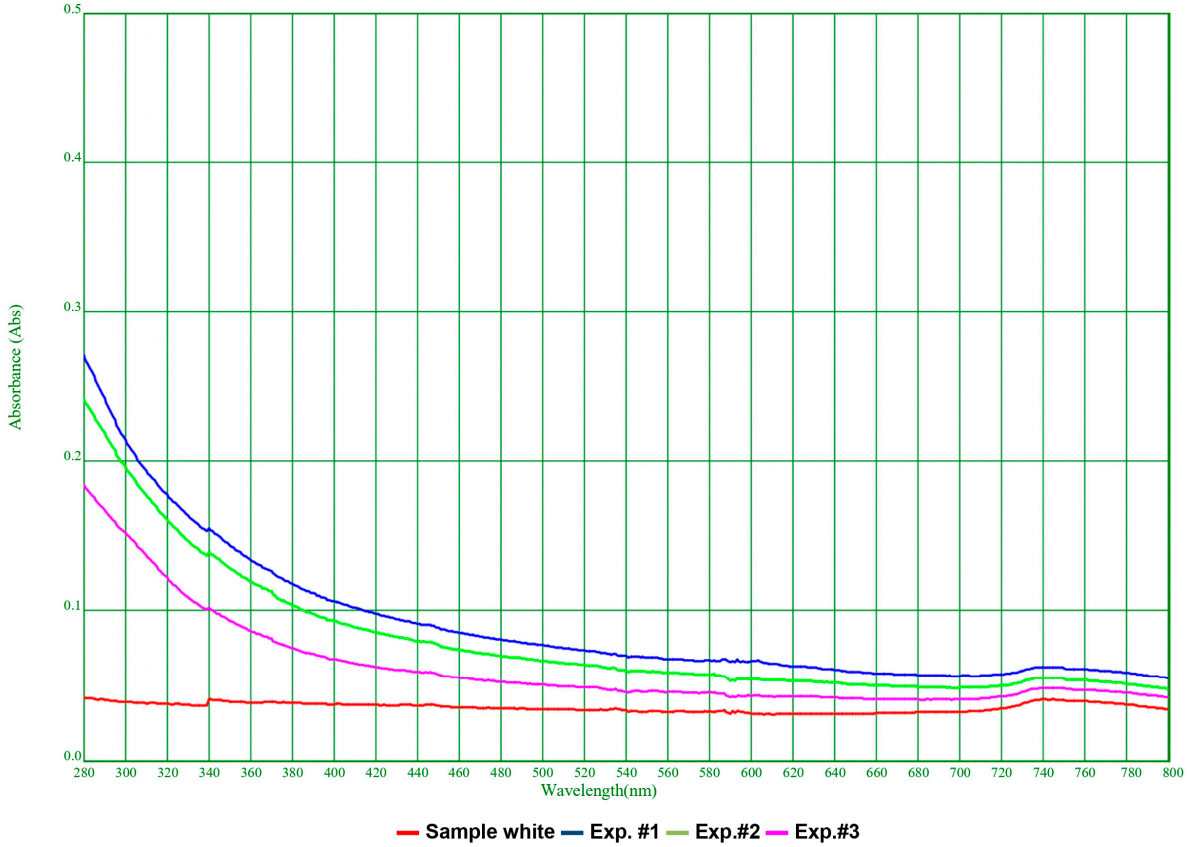

Figure A2. Absorbance (UV–Vis wavelength range) of the samples of the secondary effluent wastewater from the Linares WWTP (Jaén, Spain) used in the experimental trials #1, #2, and #3, with a respective initial turbidity of 7.77 NTU, 7.70 NTU, and 7.64 NTU. The white sample was created using ultrapure water (Milli-Q). Quartz cuvettes were used.

Table A1. Concentration of *E. coli*, *E. faecalis*, and *C. perfringens* (CFU/100 mL) during the inactivation kinetics in each experimental test.

Test	Sample	Time (h)		UV Dose		Mean (CFU/100 mL)			Log_{10} Reduction (N/N_0)		
		Real	Duration	(Wh/m^2)	(KJ/m^2)	*E. coli*	*E. faecalis*	*C. perfringens*	*E. coli*	*E. faecalis*	*C. perfringens*
Exp. #1a	* M0	10:30:19	0.00	0.00	0.00	610,000 ± 36,100	-	37,300 ± 6030	0.00	-	0.00
	M1	11:30:19	1.00	11.49	41.36	313,000 ± 68,100	-	30,700 ± 3210	0.29	-	0.08
	M2	12:00:19	1.50	18.73	67.42	159,000 ± 12,000	-	30,000 ± 3610	0.58	-	0.09
	M3	12:30:19	2.00	26.79	96.43	70,300 ± 13,600	-	26,300 ± 6810	0.94	-	0.15
	M4	13:05:19	2.58	37.02	133.26	6230 ± 1100	-	27,700 ± 8020	1.99	-	0.13
Exp. #1b	* M5	13:30:19	0.00	0.00	0.00	610,000 ± 36,100	-	37,300 ± 6030	0.00	-	0.00
	M6	14:05:19	0.58	11.22	40.37	153,000 ± 8190	-	38,000 ± 4000	0.60	-	0.00
	M7	14:29:19	0.97	18.67	67.20	88,300 ± 4510	-	35,000 ± 10,400	0.84	-	0.03
	M8	14:55:19	1.40	26.60	95.74	47,300 ± 10,200	-	33,000 ± 2650	1.11	-	0.05
	M9	15:31:19	2.02	37.06	133.40	5300 ± 624	-	29,300 ± 5690	2.06	-	0.11
Exp. #2a	* M10	10:30:44	0.00	0.00	0.00	1,370,000 ± 181,000	122,000 ± 11,400	-	0.00	0.00	-
	M11	11:30:44	1.00	8.72	31.39	773,000 ± 142,000	43,300 ± 6030	-	0.25	0.45	-
	M12	12:00:44	1.50	14.51	52.23	323,000 ± 30,600	38,700 ± 16,200	-	0.63	0.50	-
	M13	12:30:44	2.00	21.11	76.00	127,000 ± 14,400	16,100 ± 3610	-	1.03	0.88	-
	M14	13:05:44	2.58	29.54	106.33	31,000 ± 9850	853 ± 1170	-	1.65	2.16	-
Exp. #2b	* M15	13:30:44	0.00	0.00	0.00	1,370,000 ± 181,000	122,000 ± 11,400	-	0.00	0.00	-
	M16	14:03:44	0.55	8.80	31.67	857,000 ± 134,000	55,300 ± 8620	-	0.20	0.34	-
	M17	14:25:44	0.92	14.50	52.21	363,000 ± 58,600	41,300 ± 6510	-	0.58	0.47	-
	M18	14:51:44	1.35	21.08	75.87	174,000 ± 2890	26,300 ± 1530	-	0.90	0.67	-
	M19	15:26:44	1.93	29.45	106.01	40,300 ± 2890	9830 ± 929	-	1.53	1.09	-
Exp. #3a	* M20	10:30:59	0.00	0.00	0.00	317,000 ± 75,700	38,000 ± 6560	25,700 ± 6430	0.00	0.00	0.00
	M21	11:30:59	1.00	15.14	55.53	105,000 ± 10,400	37,700 ± 577	22,000 ± 2000	0.48	0.00	0.07
	M22	12:00:59	1.50	24.36	88.86	58,300 ± 1530	42,700 ± 2890	27,000 ± 5570	0.74	0.00	0.00
	M23	12:30:59	2.00	34.48	125.40	15,700 ± 231	18,600 ± 1620	19,600 ± 1430	1.31	0.31	0.12
	M24	13:05:59	2.58	47.15	171.10	887 ± 40.4	10,700 ± 473	14,700 ± 1000	2.55	0.55	0.24
Exp. #3b	* M25	13:30:59	0.00	0.00	0.00	317,000 ± 75,700	38,000 ± 6560	25,700 ± 6430	0.00	0.00	0.00
	M26	14:08:59	0.63	14.73	54.43	138,000 ± 6110	47,300 ± 10,600	22,300 ± 1530	0.36	0.00	0.06
	M27	14:31:59	1.02	23.70	86.72	71,000 ± 10,800	35,300 ± 8740	22,400 ± 6080	0.65	0.03	0.06
	M28	14:59:59	1.48	34.47	125.46	14,700 ± 1190	15,300 ± 3210	19,900 ± 1140	1.33	0.40	0.11
	M29	15:33:59	2.05	47.08	170.78	665 ± 66.6	4300 ± 600	19,100 ± 493	2.68	0.95	0.13

(*) Raw water samples; (-) analysis not conducted.

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