Photoreactivation and subsequent solar disinfection of *Escherichia coli* in UV-disinfected municipal wastewater under natural conditions

F. Schmidtlein, M. Lübken, I. Grote, H. Orth and M. Wichern

ABSTRACT

Photoreactivation of ultraviolet (UV)-disinfected wastewater of different qualities was experimentally assessed. Photoreactivation ability of secondary effluent and microstrained inflow was analyzed in different samples of 50 mL (Petri dish) and 7,000 mL volume to describe open channel effluent situations of wastewater treatment plants in a more realistic approach. The small sample of secondary effluent revealed a total $\log_{10}$ inactivation of 1.8 units and the small sample of microstrained inflow a total $\log_{10}$ inactivation of 3.2, with an applied UV-254 fluence of 84 and 253 J/m^2, respectively. Maximum net photoreactivation for secondary effluent and microstrained inflow was in the order of 1.2 $\log_{10}$ and 0.37 $\log_{10}$ units, respectively, for both sample sizes. However, significantly faster photoreactivation performance was generally determined for small sample volumes. The photoreactivation processes were completely compensated for by solar disinfection within a 120 min exposure time. Solar disinfection processes were negligible in the larger sample volumes of microstrained inflow. For municipal wastewater treatment systems with open channel effluents, it is essential to take into consideration the dependence of solar UV-365 fluence rate on water depth and wastewater characteristics.

Key words | municipal wastewater, photoreactivation, solar disinfection, UV disinfection, water reuse

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INTRODUCTION

Reuse of appropriately treated municipal wastewater becomes increasingly significant in sustainable water management concepts, especially for irrigation purposes in arid and semi-arid countries. For most applications within the context of water reuse, a sufficient level of pathogen reduction during the wastewater treatment process is necessary. A broad variety of different treatment options have been investigated over the last decades. Membrane technologies have proved to produce a high-quality effluent with respect to minimizing concentrations of *Escherichia coli*, protozoa or helminth eggs (Gómeza *et al.* 2006). However, membrane technologies are often considered to feature high operating costs, with the need to control biofouling. Thermophilic anaerobic digestion can achieve 4–6 $\log_{10}$ removal efficiency for fecal coliforms and intestinal enterococci, besides both nutrient and energy recovery of the wastewater treated (Lübken *et al.* 2007a, b). Sand filtration is a low-cost alternative, which has been demonstrated to be applicable for tertiary wastewater treatment and water reuse (Hamoda *et al.* 2014). The main challenge for municipal wastewater treatment is clogging arising from biofilm growth within the filter pores (Wichern *et al.* 2008). In most of the different technological methods, chlorination is still necessary to ensure an effluent quality to comply with international standards for treated wastewater reuse for irrigation.

Ultraviolet (UV) disinfection gains more and more acceptance as an alternative treatment technology to conventional chlorination (Bischoff *et al.* 2012). Nowadays, UV disinfection in wastewater treatment is mainly applied to water of at least secondary effluent quality (Hijnen *et al.* 2006). For some reuse concepts, especially for small applications, it might also be conceivable to apply UV disinfection to only physical treatment (e.g. microstraining) and not fully biologically treated wastewater still containing considerable dissolved nutrients and therefore fertilization capacity. In those application scenarios, UV disinfection efficiency is strongly affected by a rather limited UV transmittance and a much higher amount of suspended solids,

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resulting in a high energy demand. Linden & Darby (1998) examined the negative influences of high suspended solid concentrations in marginal effluents on UV absorbance, resulting in a decreasing overall available UV dose for disinfection. Madge & Jensen (2006) analyzed the effects of particle size distribution on UV disinfection efficiency and found a slower disinfection rate of fecal coliform associated with particles over 20 µm than those associated with particles of smaller size.

One difficult topic of concern with UV disinfection of municipal wastewater is the ability of many microorganisms to repair DNA damage, at least to some extent (Hijnen *et al.* 2006). Photoreactivation of *E. coli* has mainly been analyzed in pure culture solutions of very small volumes (Petri dishes) and for specific *E. coli* strains (Sommer *et al.* 2000). Results from photoreactivation experiments regarding the influences of different wastewater qualities are rather limited. Within this paper, results from photoreactivation experiments with municipal wastewater of different qualities under natural conditions are presented. Both UV disinfection efficiency and photoreactivation are assessed for marginal effluents, e.g. only physical treatment is applied for municipal wastewater. A fluence-based approach is compared with a time-based approach to interpret results of photoreactivation experiments when different wastewater characteristics influence the photorepair ability.

METHODS

Municipal wastewater treatment

The experiments were performed on a pilot municipal wastewater treatment plant (WWTP) located in Rabat, Morocco. Figure 1 shows a typical schematic process flow diagram of the pilot plant, which is treating wastewater for 1,000 people equivalents.

The wastewater flows directly after screening with a bar width of 10 mm into the activated sludge tank. In addition to regular operation, the inflow of the treatment plant was microstrained during the investigation period using a microsieve with a 0.08 mm mesh size. Microsieving as a very simple technical operation system was investigated in order to evaluate the efficiency of only mechanical wastewater treatment against the background of potential water reuse concepts. It is of special interest if the water quality achieved is sufficient for a direct downstream operated UV disinfection step. The sand filtration was operated with a flow rate of 0.5–1.0 m^3/h. Quartz sand was used as filter media, with a grain size between 0.7 and 1.2 mm and a layer thickness of 80 cm. The investigation period extended over 4 months. Standard parameters to characterize the wastewater qualities were analyzed according to German standard methods for the examination of water, wastewater and sludge (DEV 2014). For the measurements of UV transmittance at 254 and 365 nm wavelength a spectrophotometer was used (model DR 5000 UV/VIS, Hach-Lange, Germany) and for the determination of turbidity a nephelometer (Model 2100P ISO, Hach-Lange, Germany) was used.

UV disinfection, photoreactivation and subsequent solar exposure

For proof of *E. coli* (according to DIN EN ISO 9308-3:1999) a microplate method (BioRad Laboratories, Inc., Hercules, USA) was chosen as a miniaturized multi-tube (most probable number) procedure which is based on the detection of a specific substrate MUG/EC (*E. coli* medium with 4-methylumbelliferyl-β-D-glucuronide) for *E. coli* present in dehydrated

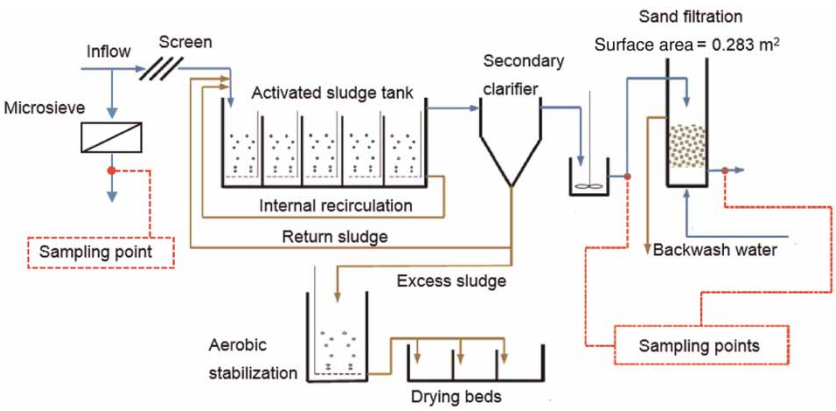

Figure 1 | Flow scheme of the investigated pilot municipal WWTP.

form in 96 wells of each plate. The MUG/EC substrate is rehydrated by adding the water sample to the wells, and in the presence of the appropriate enzyme it releases blue fluorescent 4-methylumbelliferone which is visible under UV light (366 nm) after an incubation period of 36–72 hours at 44 °C. All photoreactivation samples were analyzed immediately after sample taking. Sterile tubes were taken for the respective dilution of samples using special microplate diluent (dehydrated DSM, BioRad Laboratories, Inc.) also containing synthetic sea salt. To minimize interfering impacts of surrounding light on the photoreactivation results all tubes were wrapped in aluminum foil to keep the samples in darkness. Using this test method for *E. coli*, typical detection limits ranged from 15 to 38 cfu/100 mL.

A collimated beam device (ITT Wedeco, Herford, Germany) with four low-pressure UV lamps (Type NLR 2036) was used for disinfection. Intensity UV-C irradiance of the low-pressure lamps was continuously controlled throughout the experiments by a highly selective UV-C sensor on the surface of the liquid suspension as well as by uridine actinometry. All samples, with a sample volume of 150 mL, were irradiated in sterile glass Petri dishes of 19 cm diameter and were placed under the low-pressure UV lamps with a distance of 54.7 cm between sample surface and UV lamps. Respective radiant exposure was adjusted by changing the exposure time and was calculated according to the standard protocol published by Bolten & Linden (2003). UV fluence of 84 J/m² for secondary effluent and of 253 J/m² for microstrained inflow was preliminarily applied to photoreactivation. These UV fluences were selected on the basis of preliminary laboratory experiments (data not shown), using wastewater from two different treatment facilities (WWTP Düsseldorf-Süd, WWTP Bochum-Ölbachtal). With the respective fluences for secondary effluent and microstrained inflow, *E. coli* was reduced to a level below 1.0×10^3 cfu/100 mL, which is the WHO (1989) standard for reused wastewater. In general, the recommended doses for disinfection of treated wastewater are significantly higher. According to the California and Florida disinfection treatment-based standards for tertiary recycled water and high level disinfection, the recommended dose is, for example, 100 mJ/cm² (1,000 J/m^2) following sand or cloth filtration (US Environmental Protection Agency (EPA) 2012).

For photoreactivation, UV disinfected samples were exposed to solar light on sunny days without cloud cover in Rabat, Morocco (34.025 °N latitude, 6.836 °W longitude). The samples were exposed over midday. The exposure time was limited to 180 min in order to avoid influence of evaporation. The temperature of samples was 26 ± 2 °C. Solar irradiance in the range of 310–490 nm wavelength was recorded using a UV-visible radiometer (RM12, Dr. Gröbel UV-Elektronik GmbH, Germany) provided with a UVA sensor with a maximal spectral sensitivity at 365 nm wavelength. Average solar irradiance (365 nm) measured during the exposure time was 24.6 W/m². Photorepair fluence was calculated similarly to the procedure used for UV disinfection fluence by integrating the incident irradiance over the depth of the sample, then dividing by sample depth. According to Bohrerova & Linden (2007) we adjusted the generally accepted standard protocol of Bolten & Linden (2003) using a new reflection factor for wavelength of 365 nm ($1-R = 0.978$) and a water factor that takes UV transmittance at 365 nm into account.

For this study, photoreactivation ability of secondary effluent and microstrained inflow was analyzed in two different sample volumes. To stick with the laboratory setups of most photorepair studies carried out so far, small sample volumes of 50 mL were placed under sunlight in Petri dishes of 92 mm diameter, resulting in very thin sample layers of 0.75 cm. To describe open channel effluent situations in WWTPs in a more realistic approach, measurements in larger volumes were carried out as well. Therefore, samples of 7,000 mL volume, resulting in 25 cm water depth, were irradiated by sunlight in canisters of opaque plastic material to allow only light transmission from the surface. For each photoreactivation experiment two further samples were kept in complete darkness over the time of solar exposure as reference controls.

Data analysis

The photoreactivation reaction was assumed to follow a saturation-type first-order reaction (Kashimada *et al.* 1996), and the function of time was computed as follows:

$$S = (S_m - S_0) \cdot (1 - e^{-k_{\text{time}} \cdot t}) + S_0$$

where S is the survival at time t, k_{time} is the first-order reaction rate constant of photoreactivation (min^{-1}), S_m is the maximum survival and S_0 is the survival immediately after UV irradiation. $(S_m - S_0)$ expresses the maximum net photoreactivation. S_m and k_{time} were determined using the least square method after measuring S_0, S and t experimentally. To compare time-based and fluence-based approaches of evaluating photoreactivation ability we adjusted the above equation with a fluence-based term, resulting in

$$S = (S_m - S_0) \cdot (1 - e^{-k_{\text{fluence}} \cdot F}) + S_0$$

where $k_{fluence}$ is the fluence-based first-order reaction rate constant (m^2/J) and F is the fluence (J/m^2).

RESULTS AND DISCUSSION

Wastewater qualities and UV disinfection efficiency

Standard parameters to characterize the wastewater qualities at different treatment units of the municipal WWTP are presented in Table 1.

Evaluating filtered secondary effluent and secondary effluent without filtration, we did not observe significant differences considering the UV transmittance (at 254 and 365 nm), turbidity, bacterial concentration before disinfection and bacterial concentration after UV disinfection (same UV fluence of 84 J/m^2 was applied). A slight reduction of the amount of suspended solids as well as of the chemical oxygen demand (COD) was reached by filtration. Photoreactivation experiments and reference measurements to quantify the influence of solar disinfection showed rather similar results for filtered and unfiltered secondary effluent. Therefore, the subsequently presented results and the following discussion concentrate on a comparison between photoreactivation of secondary effluent and photoreactivation of microstrained inflow (0.08 mm mesh) under natural conditions. For the disinfection of secondary effluent an average UV-254 fluence of 84 J/m^2 was applied; a UV-254 fluence of 253 J/m^2 was used for microstrained inflow.

Photoreactivation

Apparent $\log_{10}$ inactivation results as a function of UV disinfection and time-based photorepair under sunlight are presented in Figure 2. Data were obtained from experiments with small (50 mL, 0.75 cm sample depth) and larger (7,000 mL, 25 cm sample depth) volumes. At a reactivation time of 0 min, the readings within the figures represent the UV disinfection efficiency according to Table 1.

Figure 2 shows that a maximum net photoreactivation of 1.2 $\log_{10}$ units as a function of time-based photorepair occurred in both sample sizes of secondary effluent. This is equivalent to a degree of photoreactivation of 21% of the previously inactivated *E. coli*. Within 20 min reactivation time this maximum net photoreactivation was reached in the small volume Petri dish samples, whereas 60 min of reactivation time was required in the samples of 25 cm depth. Concerning microstrained inflow, a maximum net photoreactivation of 0.37 $\log_{10}$ units was measured within the first 20 min of reactivation time, corresponding to 0.1% degree of photoreactivation. In the samples of 25 cm depth, photoreactivation was observed as well, but it is distinguished by a slighter increase in the beginning and by a required reactivation time of 180 min to reach maximum value of 0.53 $\log_{10}$ units corresponding to 0.2% degree of photoreactivation. Especially in the small volume samples, a significant further increase in inactivation was observed for both kinds of wastewater qualities after the maximum photoreactivation level was reached.

Solar disinfection

Inactivation data as a function of time-based solar disinfection for undisinfected reference samples of secondary effluent and microstrained inflow quality are shown in Figure 3. Even though the solar disinfection efficiency is rather limited during the first 20 min of exposure, a strong solar disinfection was observed subsequently – especially for the secondary effluent and the small volume samples. With a total $\log_{10}$ inactivation of 2.55 units for small sample secondary effluent and 0.37 for small sample microstrained inflow (see Figure 2), the photoreactivation

Table 1 | Wastewater quality parameters used to compare the effectiveness of different treatment units in respect to UV disinfection

Suspended solids (mg/L)	Transmittance 365 → 254 nm (–)[a]	Turbidity (NTU)	COD (mg/L)	*E. coli* (undisinfected) (cfu/100 mL)	*E. coli* (disinfected) (cfu/100 mL)
Microstrained inflow (0.08 mm mesh)					
33	21.4 → 54.8	38.4	71.6	3.25×10^6	2.3×10^3
Secondary effluent					
4.3	59.5 → 77.4	4.7	40.1	2.37×10^4	3.42×10^2
Filtered secondary effluent					
1.3	60.4 → 77.9	4.2	32.3	1.76×10^4	5.09×10^2

[a]% transmittance measured in a 1 cm path length.

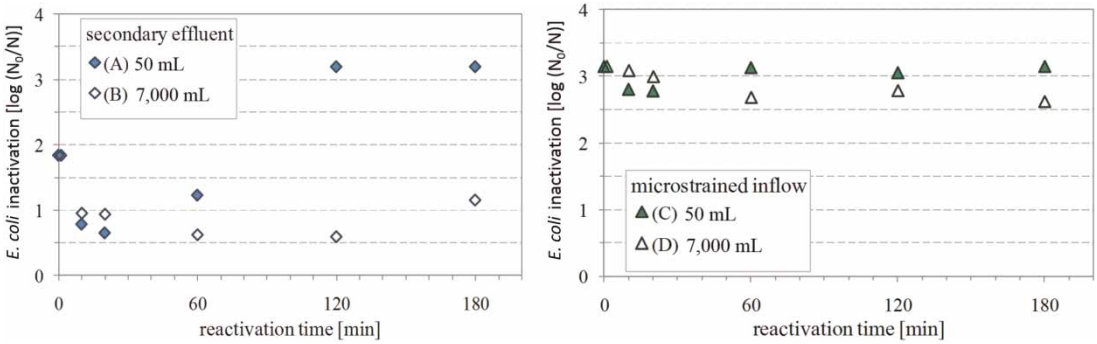

Figure 2 | Apparent $\log_{10}$ inactivation for secondary effluent (left) and microstrained inflow (right).

processes were completely compensated for by solar disinfection within a 120 min exposure time. In contrast, solar disinfection processes were negligible in the larger sample volumes of microstrained inflow.

Reactivation time

A time-based comparison of the photoreactivation results gained from samples with different volumes certainly allows for a proper determination of the quantity of maximum net photoreactivation (in this study 1.2 $\log_{10}$ units for secondary effluent and 0.37–0.53 $\log_{10}$ units for microstrained inflow), but the reactivation times required to reach those maximum values turn out to be quite different. The net photoreactivation for wild strains of *E. coli* measured in this study can be considered as relatively low compared to numbers previously reported in literature. Sommer *et al.* (2000) for instance have shown a photorepair in small volumes of pure *E. coli* (ATCC 11229) in the order of 3.4 $\log_{10}$ units after inactivation with UV-254 of 80 J/m² and a 120 min reactivation time under artificial irradiation. Zimmer & Slawson (2002) reported photorepair of 2.6 $\log_{10}$ units for the same strain of *E. coli* (ATCC 11229) after UV-254 exposure of 80 J/m² and laboratory photoreactivation experiments. On the other hand, Kashimada *et al.* (1996) and Bohrerova & Linden (2007) for example emphasized in their studies using small sample volumes irradiated in Petri dishes that in the case of sunlight exposure the photoreactivation of fecal coliforms and *E. coli* saturates in less than 15 min. Regarding our results using larger sample volumes with significant higher sample depths, in order to describe open channel effluent situations in WWTPs in a more realistic approach, those short required reactivation time values cannot be confirmed.

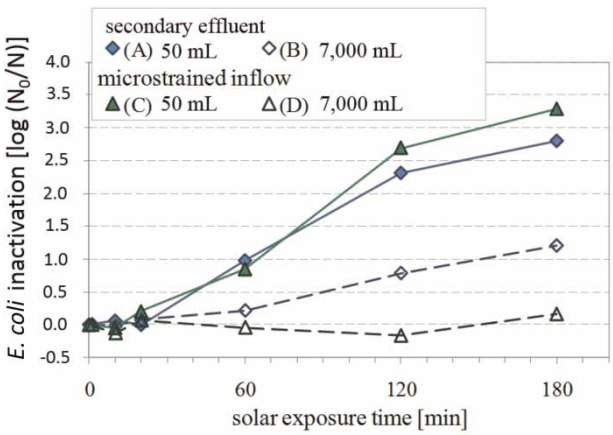

Figure 3 | Apparent $\log_{10}$ inactivation as a function of time-based solar disinfection for undisinfected reference samples of secondary effluent and microstrained inflow.

UV-365 fluence-based approach and reactivation constants

Interpreting the results presented above, it becomes obvious that some important aspects affecting photoreactivation under realistic conditions are not taken into account adequately by using laboratory-scale experimental setups with small sample volumes and by using reactivation time-based evaluation procedures. Especially the influence of wastewater characteristics like UV transmittance and suspended solids concentration growing in proportion to increasing water depth is thereby not considered sufficiently. Figure 4 shows the decrease of average UV-365 irradiance over water depth for both kinds of wastewater quality analyzed in this study. Starting from an incident solar irradiance (365 nm) of 24.6 W/m² at the sample surface, within 12.5 cm sample depth the average irradiance was reduced to 7.2 W/m² for secondary effluent (UV-365 transmittance of 77.4% transmittance measured in a 1 cm path length) and to 3.2 W/m² for microstrained inflow (UV-365

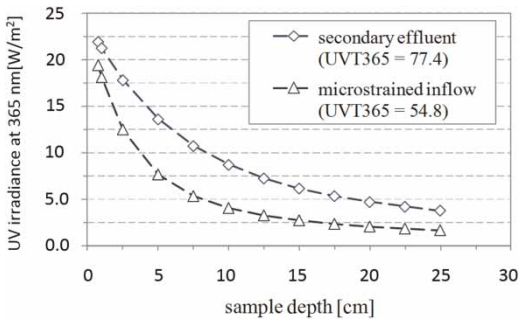

Figure 4 | Decrease of average solar UV-365 irradiance over water depth in dependency of UV transmittance (UVT365).

transmittance of 54.8% transmittance measured in a 1 cm path length). Hence, it becomes evident that it is essential to take this reduction of solar UV-365 irradiance into consideration when results from laboratory-scale studies are transferred to UV applications in WWTPs in practice with open channel effluent systems. Therefore we hypothesize that UV-365 fluence-based approaches to describe photoreactivation processes introduced by Bohrerova & Linden (2007) not only are essential to standardize photoreactivation experiments using different artificial fluorescent lamps, but also allow for a more realistic description of photoreactivation processes under natural conditions, including the specific characteristics of the water body, than a time-based evaluation approach does.

Figure 5 (left) presents the net photoreactivation for secondary effluent and microstrained inflow on a reactivation time-based approach. Significant differences between the 50 mL Petri dish samples and the 7,000 mL samples exist. Faster photoreactivation performance is determined in Petri dish samples, which results in big discrepancies regarding the overall repair coefficients – (A) $k_{time} = 0.207\ min^{-1}$ compared to (B) $k_{time} = 0.064\ min^{-1}$ for secondary effluent and (C) $k_{time} = 0.266\ min^{-1}$ compared to (D) $k_{time} = 0.017\ min^{-1}$ for microstrained inflow. Compared to time-based photorepair constants reported in literature of laboratory-scale reactivation experiments with artificial irradiation, both coefficients derived from Petri dish experiments – (A) and (C) – indicate a rather fast repair due to the high incident irradiance from sunlight. It has been clearly shown in previous studies (Bohrerova & Linden 2007) that time-based photorepair rates increase with lamp intensity or incident irradiance, respectively.

Evaluating the net photoreactivation of both wastewater qualities on a UV-365 fluence-based scale (Figure 5, right) the differences between small volume Petri dish samples and samples of 25 cm depth become negligible. Even though the maximum net photoreactivation of secondary effluent samples ((A) $S_m = 1.209$, (B) $S_m = 1.253$) is significantly higher than the photorepair measured in microstrained inflow ((C) $S_m = 0.551$, (D) $S_m = 0.369$), the fluence-based repair coefficients turn out to be almost equal ((A) $k_{fluence} = 0.00016\ m^2/J$, (B) $k_{fluence} = 0.00015\ m^2/J$, (C) $k_{fluence} = 0.00009\ m^2/J$ and (D) $k_{fluence} = 0.00023\ m^2/J$).

The results confirm the hypothesis that a UV-365 fluence-based evaluation of photoreactivation processes including knowledge of water characteristics (especially of UV transmittance) not only enables a precise determination of maximum photoreactivation to be made but also allows for a better and more realistic examination of photoreactivation progresses, which is very essential for any UV application in practice.

CONCLUSION

To assess the efficiency of UV disinfection and the influences of photoreactivation in practice for treatment systems with open channel effluents, it is essential to take the dependence of solar UV-365 fluence rate on water depth and wastewater characteristics into consideration. Small-scale laboratory setups with small sample volumes, as used in most photoreactivation studies so far, combined with a time-based evaluation

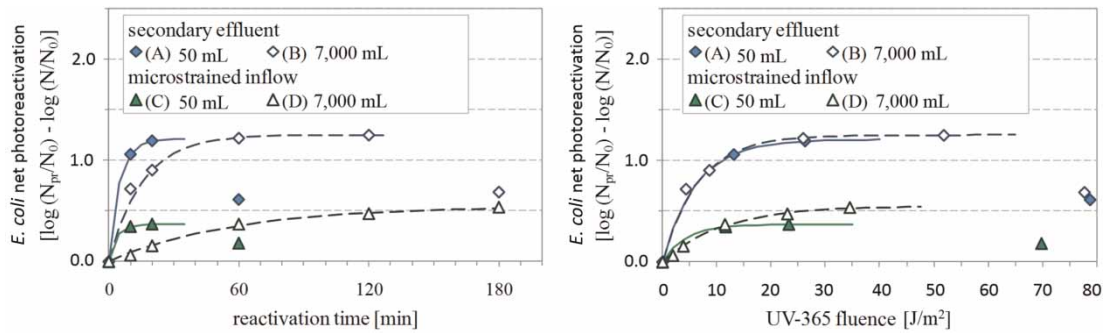

Figure 5 | Left: time-based net photoreactivation for secondary effluent and microstrained inflow. Right: UV-365 fluence-based net photoreactivation for secondary effluent and microstrained inflow.

of results do not reflect these influencing factors sufficiently. In those small sample volumes, the quantity of maximum photoreactivation can be adequately estimated, but the progression of photoreactivation is not described correctly. Reported reactivation time values in the literature of 15 min for a complete saturation of photoreactivation under sunlight were not confirmed for significantly deeper samples in this study. A UV-365 fluence-based interpretation of results not only enables a precise determination of maximum photoreactivation to be made but also allows for a better and more realistic examination of photorepair progresses.

UV disinfection was successfully applied to only physically treated wastewater. While in such treatment concepts a considerable part of the nutrients can theoretically be used as fertilizer, the results of this study show that a regrowth of pathogens is conceivable, and hence a hygienically safe reuse of wastewater is not feasible.

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