Effects of UV/H_2O_2 process conditions on Ames fluctuation assay response

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Introduction

UV processes are widely applied in full scale water treatment processes for disinfection purposes. Furthermore, advanced oxidation processes, e.g. based on UV in combination with hydrogen peroxide, are becoming more popular as efficient techniques to convert organic micropollutants. However, previous research (Heringa et al., 2011) showed that during UV/H_2O_2 processes by-products may be formed, which may cause a response in Ames fluctuation assays. Based on these results, it was decided to investigate this by-product formation at full scale processes applying relatively low UV doses.

Positive responses in the Ames assay are an indication for mutagenicity which has to be confirmed by in vivo tests. The only way to assess human mutagenicity is by identifying the compound(s) causing the positive response. However, due to the very low concentrations involved, identification will be very elaborate and expensive. If the circumstances are known that may result in the formation of such compounds, it may also be possible to prevent their formation. Therefore, the effect of various parameters was studied using a Collimated Beam set-up. In order to be able to vary the water composition, the original water was concentrated by means of membrane filtration. The effect of this procedure on the response in Ames fluctuation assays is described.

Materials and Methods

Experimental set-up

In various full scale plants samples were taken of pre-treated ground- and surface water or river bank filtrate, before and after UV disinfection. For one production plant it appeared to be possible to significantly vary the UV dose applied from 40 to 200 mJ/cm^2.

A collimated beam (CB) set-up was used to study the formation of by-products, as experiments can be carried out under well-defined conditions. However, the small volumes

usually applied limit the types of experiments that can be carried out, as for some analyses, like Ames fluctuation assays, a larger volume is required.
Bolton (Bolton and Linden, 2003; Bolton, 2010) has developed a model to calculate the fluence and irradiation time for a sample, taking into account water quality aspects, like UV transmittance, and the depth of the water layer. From experiments carried out at KWR it was concluded that it is possible to apply this model to water layers up to 10 cm, which enabled us to use larger volumes, suitable for e.g. Ames fluctuation assays (Hofman-Caris et al., 2012).
In order to be able to vary the water composition without affecting the DOC composition, a large sample of the original water of this plant was concentrated by a factor of about 5, using a 4x40 inch NF270 (DOW Filmtec) membrane. Subsequently, the water was diluted with Milli-Q water. Thus we obtained the possibility to vary the water composition. The effect of this procedure on the NOM composition is shown in table 1.

Table 1 NOM composition (according to LC-OCD) of original water from the production plant and of water that had been filtrated and diluted with Milli-Q water.

NOM Fraction		Original water (%)	filtrated water (%)
Hydrophobic		5.2	4.9
Hydrophilic	Biopolymers (MW >> 20,000)	4.6	3.9
	Humic compounds (MW ≈ 1000)	46.3	55.9
	Building blocks (MW ≈ 300-500)	22.6	19.9
	Neutral components (MW < 350)	20.8	15.4
	Acid components (MW < 350)	0.6	--

A collimated beam apparatus equipped with a low pressure (LP) or a medium pressure (MP) lamp was used.

Ames fluctuation assays

Research into the optimal extraction procedure showed that extraction with Oasis®HLB in combination with Ames fluctuation assays is a suitable method to detect mutagenic activity. In vitro Comet tests were carried out, but did not show a positive response.
Ames fluctuation assays were carried out using two different bacterial strains: TA98 and TA100, with and without liver extract S9. The TA98 strain detects frame shift mutations, whereas the TA100 strain can detect strain base pair substitutions. Liver extract S9 was added in order to mimic the effect of metabolic activation by the liver. All experiments were carried out in triplicate, and the total number of positive wells per sample was determined. This procedure was repeated in a replicate experiment. In every series a positive and negative control were applied, as well as a procedure control.

Results

UV disinfection processes in full scale production plants

In this research groundwater, surface water and river bank filtrate were tested. Samples of full scale processes, with LP and MP UV lamps, before and after UV disinfection, were taken, and Ames fluctuation assays were carried out. It was found that at UV doses applied for disinfection purposes (25-70 mJ/cm^2) no significant positive response could be detected in any case.
However, when in one case the UV dose (MP lamps) was significantly increased from 40 to 200 mJ/cm^2, the number of positive wells also seemed to increase (Figure 1, left graph). This experiment was repeated with the same water sample in the CB set-up. Here a positive relation between the number of positive wells in the Ames fluctuation assay and the UV-dose applied could clearly be observed. The fact that the absolute number of positive wells was higher in the CB-test than in the full scale plant may be caused by a difference between the UV spectrum of the lamps in the full scale plant and of the lamp in the CB set-up.

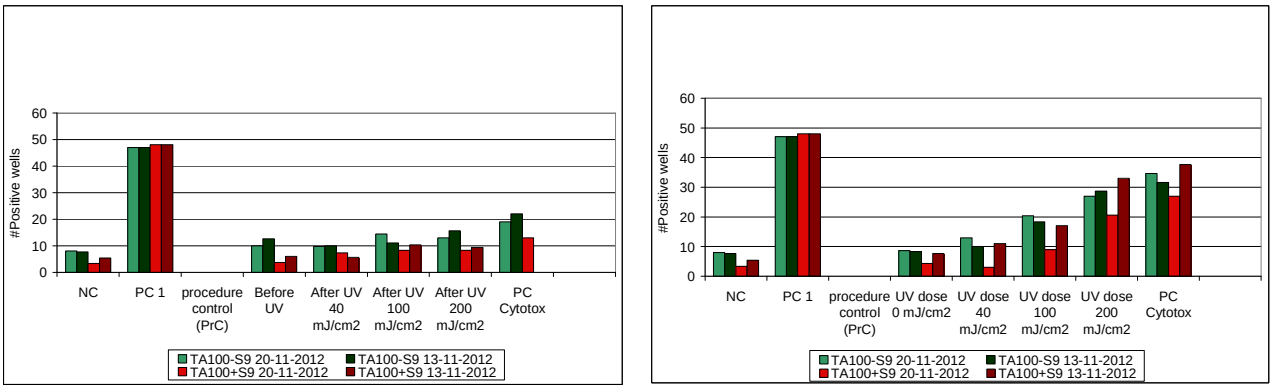

Figure 1. Formation of possibly mutagenic by-products as a function of UV dose in a full scale UV disinfection system (left graph) and in a collimated beam set-up (right graph).

CB testing of filtrated water

In some cases UV irradiation of NOM present in water can result in the formation of by-products which cause an increase in the response of Ames fluctuation assays (Martijn and Kruithof, 2012). From the results shown in figure 1 it can be concluded that the NOM present in this water is capable of forming such by-products under the influence of UV irradiation.
By means of membrane filtration it became possible to vary the concentrations of compounds in this water. However, it had to be established whether or not this procedure affects the NOM composition, and thus the behavior of the water in UV experiments. Characterization of the NOM (table 1) showed that filtration resulted in the loss of some small (acidic) compounds. It is not to be expected that such compounds are involved in the formation of mutagenic by-products. This was confirmed in CB experiments with both original and filtrated water (Figure 2). The NOM concentration in the filtrated water had been adjusted to the original value.

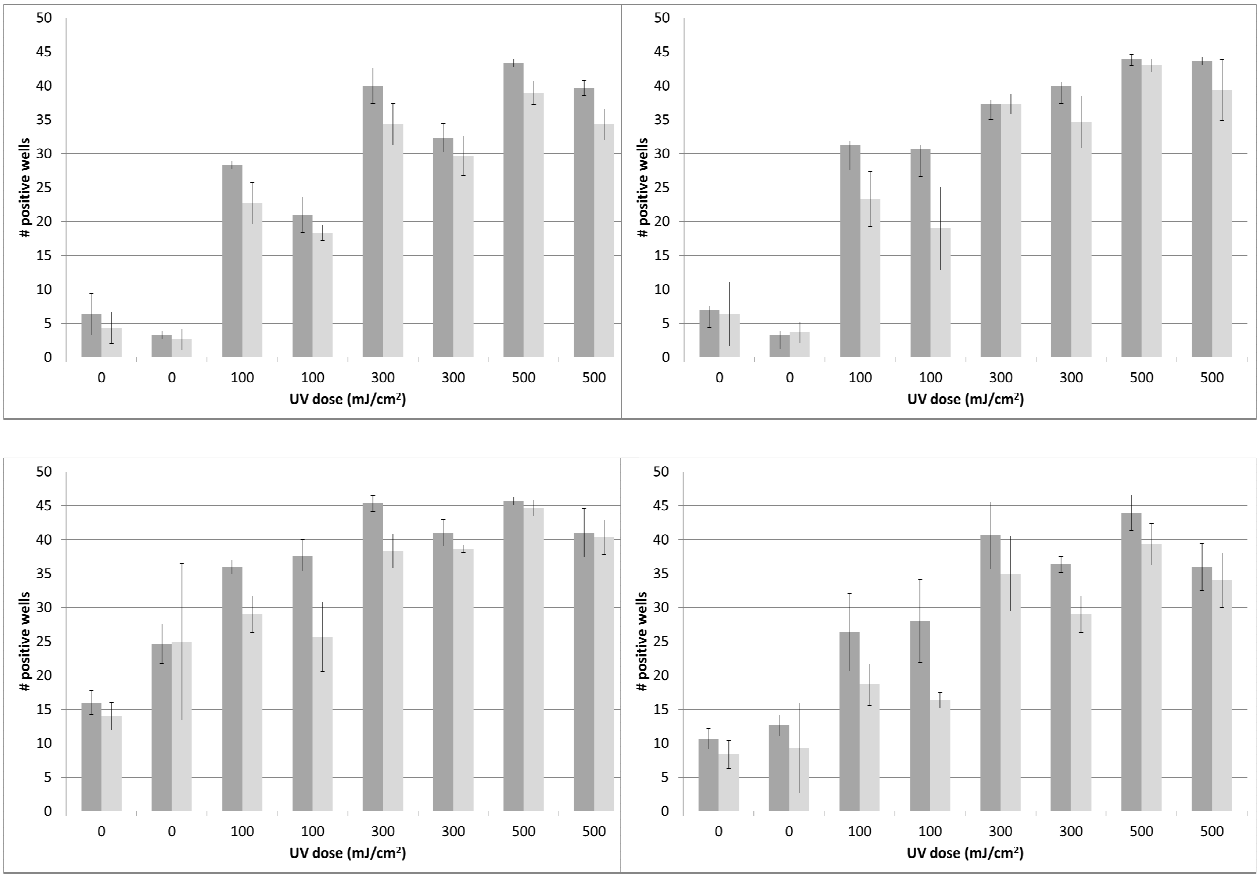

Figure 2. Number of positive wells in replicate Ames fluctuation assays as a function of UV dose, using TA98 – S9 (upper left), TA98 + S9 (upper right), TA100 – S9 (lower left) and TA100 + S9 (lower right). Dark grey bars: original water. Light grey bars: filtrated water. Experiments carried out in a CB-set-up equipped with an MP UV-lamp in the absence of H_2O_2.

The results show an increase in the number of positive wells with increasing UV dose. There does not seem to be a significant difference in response to the Ames fluctuation assay between original and filtrated water. Similar results were obtained with addition of 10 mg/L H_2O_2, although in that case in general the number of positive wells appeared to be lower. This is in accordance with previous research (Heringa 2011). As part of the UV irradiation will be used for the photolysis of H_2O_2, addition of this compound results in a less efficient photolysis of other compounds. In general, addition of H_2O_2 results in a more efficient process, but this is due to the oxidation by hydroxyl radicals, that are formed during photolysis of H_2O_2. Apparently, this oxidation process is not significantly involved in the formation of by-products that cause an increase in response in the Ames assay.
When LP lamps were used, also no difference between original and filtrated water could be observed. However, both in the presence and absence of H_2O_2, the number of positive wells was notably lower than with MP lamps. As LP lamps, contrary to MP lamps, only emit at 253.7 nm, in general they are less efficient in causing photolysis. Therefore, these results also indicate that the formation of mutagenic by-products is caused by the photolysis of compounds present in the water.

Conclusions

It was found that in full scale disinfection UV processes, at common disinfection UV doses of 25-70 mJ/cm^2, no significant positive response in the Ames fluctuation assay could be observed, independent of the type of water and of the type of UV-lamp (LP or MP).
However, after significantly increasing the UV dose in case of an MP UV lamp an increasing positive response was found in Ames fluctuation assays. The water was filtrated over a membrane, rendering it possible to adjust concentrations while maintaining almost the same NOM composition. This procedure was shown not to affect the response in Ames assays.
CB-tests showed that increasing the UV dose, using MP UV lamps, results in an increase in the positive response in the Ames fluctuation assay. Addition of H_2O_2 in general gives a lower response, indicating that photolysis is involved in the formation of mutagenic by-products rather than oxidation processes. Here too no significant difference was observed between the response in original and in filtrated water.
When LP UV lamps were applied, a much lower response was observed, which is in accordance with the conclusion that the process causing the formation of mutagenic by-products is photolysis.

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