Authors' reply to comments referee A. Magic-Knezev

The thorough review is highly appreciated. Most of the suggestions were adopted. Thanks to the referees' suggestions additional literature was studied, resulting in new ideas. The conclusion that bioregeneration was 'not likely' in the (biological) granular activated carbon filters, was changed into that it was 'likely' that bioregeneration occurred.

Specific comments referee A. Magic-Knezev, including authors' reply in italic.

Abstract: Page 1. row 20. Please indicate by how much percent was the theoretical ratio between oxygen consumption and DOC removal exceeded.
 # Both ratios were quantified.

Page 1. row 24. Bioregeneration of large NOM molecules was considered not to occur, due to sequestration. Please indicate whether this assumption is still valid and what other mechanism may explain the excess of oxygen consumption at high temperatures.
 # The results from additional literature research indicate that bioregeneration of large NOM molecules is possible. Therefore, all sections about bioregeneration were revised:

In the section 'Summary':
"The production and loss of biomass, the degassing of (B)GAC filters, the decrease in the NOM reduction degree and the temperature effects on NOM adsorption could only partly explain these excesses and the non-correlation between DOC and AOC removal and oxygen consumption and carbon dioxide production. It was demonstrated that bioregeneration of NOM could explain the excesses and the non-correlation. Therefore, it was likely that bioregeneration of NOM did occur in the (B)GAC pilot filters."

In the section 'Results and discussion':
"Bioregeneration
Bioregeneration of AC is biodegradation of (previously) adsorbed NOM, which results in a decrease in the NOM loading on the AC (Sontheimer et al., 1988). Several authors described two possible mechanisms for bioregeneration. The first hypothesis is that biomass on the external AC surface takes up substrate. Therefore, the concentration of the substrate on the external AC surface becomes smaller than the internal equilibrium concentration. This causes diffusion of the substrate from the internal pores towards the external AC surface, where it is biodegraded. The concentration inside the pores decreases, which results in desorption. The AC is available for adsorption again: it has been bioregenerated. The second hypothesis is that the biomass releases extracellular enzymes that enter the meso-pores of the AC; micro-pores are believed to be too small for the exo-enzymes to enter. The exo-enzymes convert part of the adsorbed substrate into less adsorbable products. These products desorb and diffuse from the internal pores towards the external AC surface, where they are biodegraded. Again, the AC is bioregenerated. In both hypotheses, both desorption and biodegradation are conditions for bioregeneration (Aktas and Çeçen, 2007; Klimenko et al., 2003; Walker and Weatherley, 1998).

During bioregeneration, oxygen is consumed and carbon dioxide is produced. The NOM that is biodegraded originates from the adsorbed phase. An increase in oxygen consumption and carbon dioxide production is possible, without any effect on the measured DOC and AOC concentrations in the filter effluent. During complete oxidation of 1 g C 2.6 g oxygen is needed and 3.7 g carbon dioxide is *produced. Assume that in winter, during a period of 6 months, 1.0 g $C\cdot m^{-3}$ NOM adsorbed onto the AC and that 0.2 g $C\cdot m^{-3}$ NOM was biodegraded. The oxygen consumption and the carbon dioxide production, per amount of NOM removed from the water phase, would have been $0.2\cdot 2.6/1.2 = 0.4$ g $O_2\cdot g\ C^{-1}$ and $0.2\cdot 3.7/1.2 = 0.6$ g $CO_2\cdot g\ C^{-1}$. Assume that in summer, during a period of 6 months, no NOM adsorbed, that 1.2 g $C\cdot m^{-3}$ NOM was biodegraded from the water phase and that all adsorbed NOM from the previous winter period was biodegraded. The oxygen consumption and the carbon dioxide production, per amount of NOM removed from the water phase, would have been $(1.2+1)\cdot 2.6/1.2 = 4.8$ g $O_2\cdot g\ C^{-1}$ and $(1.2+1)\cdot 3.7/1.2 = 6.8$ g $CO_2\cdot g\ C^{-1}$. These figures correspond well to the measured results from the pilot experiment, as seen in Figure 5.*

AC bioregeneration was reported for different specific compounds in (industrial) waste waters (Aktas and Çeçen, 2007; Klimenko et al., 2003; Walker and Weatherley, 1998). Although no hard evidence was found, some researchers suggested that bioregeneration of NOM in drinking water is possible (Sontheimer et al., 1988). Both desorption and biodegradation of the compounds are conditional for bioregeneration. It is obvious that a part of the NOM in the pilot (B)GAC filters was biodegradable and adsorbable (Figure 2 and Table 3). For batch experiments, desorption of 4% to 58% of previously adsorbed NOM was reported. The percentage of NOM desorption depended on the type of NOM and on the type of AC (Yapsakli et al., 2009), Therefore, in theory, the conditions for biodegradation and for desorption can be met. Because in summer the reported ratio between oxygen consumption and DOC removal exceeded 2.6 g $O_2\cdot g\ C^{-1}$ and the ratio between carbon dioxide production and DOC removal exceeded 3.7 g $CO_2\cdot g\ C^{-1}$, it was likely that bioregeneration of NOM did occur in the (B)GAC pilot filters."

In the section 'Conclusions':
"Bioregeneration of NOM could explain the excesses and the non-correlation. Therefore, it was likely that bioregeneration of NOM did occur in the (B)GAC pilot filters.

It is recommended that adsorption, desorption and biodegradation experiments be performed with labeled ^{14}C-glucose (Servais et al., 1994), or if possible with larger (both biodegradable and non-biodegradable) ^{14}C-NOM molecules. This will make it possible to determine the fate of NOM and to quantify relevant processes in BGAC filtration. Possibly, hard evidence for bioregeneration of NOM will be found."

Introduction: Page 2. row 2. Activate carbon is used worldwide, mainly for same reasons. Suggestion is to remove "In the Netherlands…"
 # "In the Netherlands…" was removed and "precursors for disinfection byproducts" was added.

Page 2. row 3. please replace …"and the removal of substances"... by …"and organic compounds"
 # The suggestion was adopted.

Page 2. row 15. I suggest starting a new paragraph that describes benefits of biodegradation with the sentence "When a part of …".
 # The suggestion was not adopted, because also in the previous sentences biodegradation is discussed.

Page 2. row 26 -30… Please consider shortening the text to: "A part of NOM can be used by heterotrophic bacteria for growth and maintenance as a source of

carbon and energy. In this process bacteria consume oxygen and produce carbon dioxide and water.
 # The suggestion was partly adopted. Some parts were not shortened to provide additional information.

Page 2. rows 30-32. “The oxygen consumption per amount of biodegraded NOM is constant (Urfer and Huck, 2001)...”. Urfer and Huck, 2001 measured biological activity as oxygen consumption after the addition of defined substrate to a sample. Oxygen consumption for this particulate substrate is constant. However, the amount of oxygen used for metabolism of various organic compounds varies with the compound. Therefore this sentence may be misleading. Please consider removing or changing. Also the purpose of next sentence is not clear. Please consider reformulating ex.: Aerobic biodegradation can be assessed by measuring oxygen consumption and CO2 formation.
 # The suggestion was adopted.

Page 2 row 32 - page 3 row 1-2. Please consider deleting text: “Each bacterial species...while others are hardly biodegraded” It does not add much to the information that is stated in the following sentence: “NOM in natural waters…”.
 # The suggestion was partly adopted. The second part was not deleted to provide additional information.

Page 3 row 2-7. Please consider changing the sentences “NOM in natural waters... …(... Yavich et al., 2004).” to: NOM in natural water sources that are used for drinking water production is usually not easily biodegradable. Pre-oxidation of this water during the treatment process increases biodegradability of NOM, resulting in increased concentrations of ... ... (...Yavich et al. 2004)”
 # The suggestion was partly adopted. Some parts were not shortened to provide additional information.

Page 3 rows 9-15. Please consider starting a new paragraph with the sentence: “Biodegradation increases...”. This paragraph is about biodegradation in activated carbon filters and previous about biodegradation in general. Please change sentence: “Biodegradation increases....” into “Biodegradation in (B)GAC increases ....”
 # Both suggestions were adopted. Also two sentences about the limiting factor for biodegradation were moved to the previous paragraph, to make a clear distinction between biodegradation in (B)GAC filters and biodegradation in general.

Before explaining the objectives of the research please indicate why we still need more research on biodegradation in GAC. What was the purpose of quantifying effects of ozonation and temperature on the biodegradation?
 # The following sentences were added: ”Nowadays, many drinking water treatment plants (DWTPs) have installed (B)GAC filters. Because influent water quality and operations vary, the need for dynamic models for optimization of operational conditions has increased (Bosklopper et al., 2004; van der Helm et al., 2008). Because biodegradation of NOM is a key process in BGAC filtration, it should be incorporated in such models.”

Methods and Materials:

Page 3 row 30. Please indicate residence time in reservoir.
 # The residence time was included.

Results and discussion:

Page 5 rows 15-20. Please indicate if the applied method for CO2 calculation is sufficiently sensitive to detect reduction of 0.17 g C m-3 of DOC.
 # The analytical method for DOC removal is not accurate enough to detect a reduction of 0.17 g C·m^{-3}, based on a single measurement. However, the dataset was large enough (n=28) to determine that the DOC removal was significant during ozonation with 2.5 g O_3·m^{-3}. For this specific case the p-value of the anova-test was 2·10^{-9}. Not all statistical information was included in the paper, because it is the authors' opinion that this would disturb the readability. In the paper the following sentence was added to emphasize that ozonation caused a significant change in the DOC concentration and in other water quality parameters: "All target parameters after ozonation also correlated significantly with the ozone dose."

Page 6 rows 26-30. You indicate that due to ozonation AOC concentration in the influent is increased and that none of the filters receiving ozonated water were able to reduce AOC concentration to the level before ozonation. Table 3 suggests that the GAC filter receiving non ozonated water is producing AOC, while other 3 filters are removing the AOC. As GAC filtration is not employed for bio-stability, but for other purposes, please clarify what was the effect of GAC and (B)GAC on bio-stability.
 # The following sentences were added: "The GAC effluent on average contained more AOC than the influent, as seen in Table 3. Apparently, the GAC filter produced AOC." Also the GAC was included in the next sentence: "Therefore, both GAC filtration and ozonation followed by BGAC filtration were expected to have a negative effect on biostability."

Page 8 row 2. Please ad "of" before "...DOC, AOC and ..."
 # The suggestion was adopted.

Page 8 rows 1-9. Note that oxygen may also be consumed for microbial oxidation of inorganic compounds (ammonium, nitrite, iron).
 # In section 3.2 "Effects of ozonation and water temperature on biodegradation of NOM in (B)GAC filters" an extra assumption was made "It was also assumed that biodegradation of NOM was the only biodegradation process in the filters. From experiences in the full-scale plant, it was known that there was no microbial oxidation of inorganic compounds because ammonium, nitrite and iron were absent due to pre-treatment by rapid sand filtration (see Figure 1)."

Figure 5 shows that there is also oxygen consumption and CO2 production before day 194. This should also be considered in the discussion about oxygen consumption and CO2 production.
 # The suggestion was not adopted. It was specifically the objective to study the influence of ozonation and temperature on biodegradation of NOM. To avoid interaction with adsorption, the authors chose to use data from day 194 onwards only. This is specifically explained in section 3.2 "Effects of ozonation and water temperature on biodegradation of NOM in (B)GAC filters"

Page 8 paragraph 3.2.1 In table 3 average oxygen removal per g DOC is presented for different ozone doses. Please consider presenting the difference in oxygen consumption in the filters receiving water after different ozone doses at different temperatures by plotting dO2 gC-1 with temperature for 4 different ozone doses in a new figure.
 # The suggestion was adopted, see Figure 5.

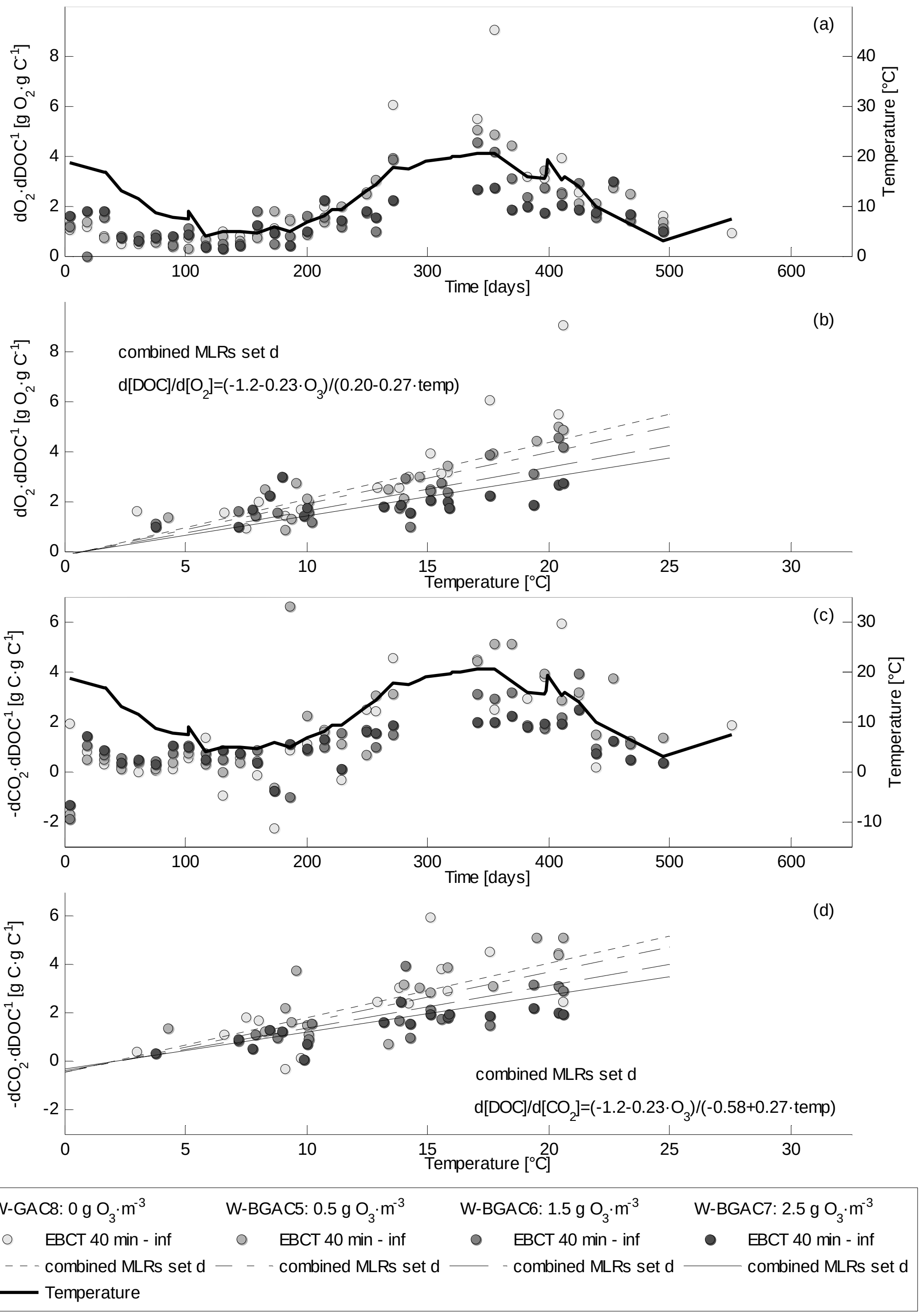

Figure 5: Oxygen consumption to DOC removal ratio (a), oxygen consumption to DOC removal ratio as function of temperature from day 194 to 559 (b), carbon dioxide production to DOC removal ratio (c) and carbon dioxide production to DOC removal ratio as function of temperature from day 194 to day 559 (d).

Figure 5 indicates highest oxygen consumption at maximum temperature in the filter receiving non-ozonated water. Please address this observation too.

 # In section 3.2.1 "Decrease of NOM reduction degree" the following sentences were introduced: "The MLRs from sets d for DOC and oxygen consumption, and for DOC and carbon dioxide production (see Table 4) were combined into equations for the ratio between oxygen consumption and DOC removal and for the ratio between carbon dioxide production and DOC removal, as seen in Figure 5. Both ratios were higher at higher temperatures and lower at higher ozone doses."

Nature of substrate may have effect on the oxygen uptake.
 # Additional literature was studied about the composition of NOM in freshwaters. In section 3.2 "Effects of ozonation and water temperature on biodegradation of NOM in (B)GAC filters" the following section was rewritten:
"For example, for complete oxidation of glucose ($C_6H_{12}O_6$) 2.7 g O_2·g C^{-1}, for cellulose $(C_6H_{10}O_5)_n$ 2.7 g O_2·g C^{-1} and for ethanol (CH_3OH) 4.0 g O_2·g C^{-1} is needed. Perdue and Ritchie (2004) reviewed different studies on NOM and collected the compositions of NOM in 57 fresh waters. The average NOM of these 57 samples had a C:H:O ratio of 400:660:645. For complete oxidation of this average NOM 2.6 g O_2·g C^{-1} is needed. The oxygen consumption of all the NOM samples always was between 2.1 g O_2·g C^{-1} and 3.4 g O_2·g C^{-1}. Complete oxidation of NOM always results in a carbon dioxide production of 3.7 g CO_2·g C^{-1}."
Also, an extra assumption was made: "It was assumed that in the pilot filters the type of NOM remained fairly constant during the year and that always 2.6 g O_2·g C^{-1} was needed for complete oxidation."

Page 9. Paragraph 3.2.3 Please define the change rate in the biomass activity. This is not clear. Depending on the generation time, biomass in GAC filters is completely replaced each In the summer GAC filters are backwashed more often than in the winter what indicates that in the summer more material is accumulated in a filter than in the winter. This material may partly originate from bacteria. Furthermore, increasing temperature is associated with increasing metabolic activity implying higher turnover. This indicates that biomass is more often replaced per unit of time in the summer than in the winter. However, some researchers found lower bacterial growth efficiency at higher temperatures, thus less bimass production but higher respiration at higher temperatures (G. Daneri, B. Riemann and P.J.leB. Williams. 1994. Journal of Plankton research 16(2): 105-113).
 # Paragraph 3.2.3 "Biomass production and lysis" was changed into:

"Biomass production and losses
A part of the biologically removed NOM carbon is converted into biomass. For aquatic bacteria the bacterial growth efficiency, or yield, varies from less than 0.05 to 0.6 g C·g C^{-1}, depending on type and concentration of the substrate, type of bacteria, growth phase of bacteria, temperature and other water quality parameters (del Giorgio and Cole, 1998). For bacteria growing at a yield of 0.05 g C·g C^{-1}, both the oxygen consumption and the carbon dioxide production per amount of biodegraded NOM is about 2 times higher than for bacteria growing at a yield of 0.6 g C·g C^{-1}. In the pilot (B)GAC filters these figures were more than 5 times higher in summer than in winter (see Figure 5). In theory, this would only be possible at a maximum yield (in winter) of 0.8 g C·g C^{-1} or more, which is not realistic. Variable yields also cannot explain why in summer more than 2.6 g O_2·g C^{-1} was consumed and more than 3.7 g CO_2·g C^{-1} was produced.

The produced biomass can either accumulate in the filter bed, or leave the filter with the effluent or with the backwash water. Besides, it is possible that the biomass dies, disintegrates (lysis) and serves as a carbon source for other micro-organisms. Furthermore, bacteria in a starvation or limitation phase can utilize internally stored carbon. In this study, the concentrations of ATP, as a measure of active biomass, were determined in the filter influents, effluents and backwash water (data not shown). Assuming a biomass carbon/ATP ratio of 250 g C·g ATP^{-1} (Magic-Knezev and van der Kooij, 2004), the amounts of biomass, expressed in carbon (biomass carbon), in the influents and effluents were less than 1% of the NOM that was biologically removed in the (B)GAC filters. During a backwash, 13% of the biomass was removed from pilot filter W-BGAC6 (1 observation). At a maximum frequency of 1 backwash per 4 days, the biomass carbon removed *during backwashing was approximately 1% of the NOM that was biologically removed in the filter during 4 days. The observed changes in biomass activity were always between $+4\cdot10^{-9}$ g ATP·g $AC^{-1}\cdot day^{-1}$ (net biomass growth) and $-4\cdot10^{-9}$ g ATP·g $AC^{-1}\cdot day^{-1}$ (net biomass removal), which corresponded to less than 1% of the NOM that was biologically removed. Therefore, biomass growth, accumulation in the filter bed, lysis and the loss of carbon with biomass in the effluent and backwash water could not explain the discrepancy between biological NOM removal and carbon dioxide production in the BGAC filters."*

Although the correlation between DOC removal and temperature was not significant, figure 4 suggest that for the high ozone dose temperature may have an effect on DOC removal (a). Was this data set tested separately?
 # The effects of the temperature were not tested separately for the individual filters (ozone dosages) to make general statements on the effects possible. For the specific case of DOC removal at 2.5 g $O_3\cdot m^{-3}$ (individually judged), the linearization with temperature alone resulted in $\Delta[DOC]=-1.3-0.038\cdot temp$, with a p-value of 0.01. The correlation between DOC removal and water temperature in the other three individually judged filters was not significant. The suggestion to include individually tested data sets was not adopted, because it is the authors' opinion that including all statistical information would confuse the readers.

DOC removed in a GAC filter is used for the biomass production and for the maintenance (respiration). The amount of organic C assimilated by bacteria is the sum of bacterial production (BP) and bacterial respiration (BR). Bacterial growth efficiency (BGE) (BGE=BP/(BP+BR)) vary for aquatic bacteria between 0.05 and 0.6 and is determined by the rate of supply of energy, the quality of substrate and the energy demands (P.A. del Giorgio and J.J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst.. 29:503-541). Higher oxygen consumption at higher temperatures may indicate higher respiration rate and lower bacterial growth efficiency resulting in same DOC removal but higher consumption of oxygen. Nature of substrates and availability of nutrients in the summer and in the winter may also contribute to differences in bacterial growth efficiency. Furthermore, bacteria in a starvation/limitation phase can utilize internally stored substrate resulting in increased oxygen consumption.
 # See previous described adjustments to paragraph 3.2.3

The average oxygen consumption is similar for all filters (table 3), while average CO2 production varies much more among filters. Please address this observation.
 # From Table 3 it is clear that the variation in the average carbon dioxide production was larger than the variation in the average oxygen consumption. However, both anova tests and multiple linear regressions (MLRs) showed that both datasets did not correlate significantly with the ozone dosage. The p-values of the anova-tests for the carbon dioxide production and the oxygen consumption in the filters with different ozone dosages were 0.41 and 0.96 respectively. The suggestion was not adopted, because it is the authors' opinion that including all statistical information would disturb the readability.

Page 9. row 28. Please consider adding a sentence after the text: "It is possible that at higher temperatures adsorption decreased… ". This means that more NOM become available for the biodegradation and therefore more oxygen is consumed as more NOM is biodegraded.
 # The following part was added: "Because of the increased biodegradation…"

Page 10 row 26. Explain why bio-regeneration of large NOM molecules is not likely to happen. Degradation of the side chains of adsorbed NOM molecules could be another mechanism of extra carbon supply in the summer.

 # The results from additional literature research indicate that bioregeneration of large NOM molecules is possible. Therefore, all sections about bioregeneration were revised. See previous described adjustments to the abstract.

Conclusions Page 11, row 4. Please explain how is this calculated? AOC production depends also in DOC concentration. It is not clear how is the DOC concentration considered here.
 # The increase of the AOC concentration in the (B)GAC filters with increasing ozone dosage was $35 \cdot 10^{-3}$ g acetate-C·g O_3^{-1}. This was derived from the MLR for the water quality parameters after ozonation. There was no significant correlation between AOC concentration after ozonation and the DOC concentrations in the raw water. Other studies showed that the formation of AOC and BDOC during ozonation does increase with the concentration of NOM in the raw water (Carlson and Amy, 1997; van der Helm, 2007). The limited variation in the DOC concentration in the raw water in this study could be a reason for not finding a significant correlation. All this was mentioned in section 3.1 "Effects of ozonation and water temperature on water quality". The authors chose not to repeat all this information in section 4 "Conclusions".

Page 11, rows 7-10. Please see the comments for page 6 rows 26-30.
 # The following sentence was added: "The GAC filter produced small amounts of AOC." Also the GAC was included in the next sentence: "Therefore, both GAC filtration and ozonation followed by BGAC filtration were expected to have a negative effect on biostability."

Table 3. Please indicate also minimum and maximum value of the removal (production) absolute and relative (%).
 # The suggestion was not adopted, because it is the authors' opinion that including all information would disturb the readability. Besides, no conclusions were derived from the minimum and maximum values.

References:

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