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Assessment of microbial activity in water based on hydrogen peroxide decomposition rates

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ABSTRACT

This study proposes a new and simple assay that allows rapid assessment of microbial activity in water samples. The assay consists of standardized hydrogen peroxide (H_2O_2) addition to a water sample and subsequent spectrophotometric determination of H_2O_2 reduction over time. The H_2O_2 decomposition rate constant reflects the level of enzymatic activity from planktonic and particle-associated bacteria as well as algae and protozoans. The proof of concept was verified on water samples from recirculating aquaculture systems (RAS), showing that the vast majority of H_2O_2 decomposition was related to microbial activity. Only 3 % of the total H_2O_2 decomposition was related to abiotic processes when 0.20 µm sterile filtered RAS water was compared with unfiltered RAS water. Planktonic bacteria (size range 0.20-1.6 µm) accounted for 16% of H_2O_2 decomposition, while bacterial aggregates, particle-associated bacteria and microbiota above 1.6 µm were responsible for the remaining 81%. H_2O_2 decomposition rate constants were positively correlated to BOD_5 ($r = 0.893$; $p < 0.001$; $n=18$) and to the number of 1-30 µm micro particles ($r = 0.909$; $p < 0.001$; $n=72$) in RAS water, substantiating the biologically mediated decomposition processes in the water phase. The H_2O_2 decomposition assay thus represents a new alternative to existing methods that allows rapid (1-2 h) and simple quantification of microbial activity in fresh- and saltwater samples from aquaculture systems. Potential applications of the assay are discussed.

Key words
Water quality, microbial activity, bacteria, organic matter, micro particles, BOD_5, hydrogen peroxide assay

1. INTRODUCTION

There is a widespread need for cultivation-free methods to quantify the viability of microbial communities in aquatic environments (Hammes et al., 2010). This demand also exists within aquaculture where rapid and reliable methods for measuring bioavailable organic matter in water are increasingly required.
Microbial water quality assessment has the potential to provide insights into the temporal and spatial dynamics of bacterial communities within aquaculture systems as a supplement to general chemical water quality parameters (Timmons et al., 2002; Lekang, 2011; Dalsgaard et al, 2013; Boyd, 2017). This is of particular relevance to recirculating aquaculture systems (RAS) that are characterized by a continuous input of bioavailable substrates, high nutrient levels and long retention times all favoring heterotrophic bacterial growth (Blancheton et al., 2013; Rojas-Tirado et al., 2018).

While large particles are easily removed from RAS (Cripps & Bergheim, 2000; Piedrahita, 2003), micro particles, bioflocs, and bacterial aggregates are not. Therefore, they accumulate in RAS and provide substrate and surface area for bacteria to colonize (Wold et al., 2014; Fernandes et al., 2016; Pedersen et al., 2017). Assessment of bacterial and the broader microbial activity in water (Liu et al., 2013; Proctor & Hammes, 2015; Vadstein et al., 2018) is a method for gaining additional knowledge of potential effects and interaction of various factors (i.e. feed composition, feed loading, hydraulics and mechanical, biological and chemical treatments) on bacteriology and growth potential in RAS (Blancheton et al., 2013; Bentzon-Tilla et al., 2016). Easy and reliable methods are therefore crucial to the ongoing development of RAS because they can provide data for baseline conditions as well as detection of sudden unforeseen deviations.

Determination of biological oxygen demand over five days (BOD_5) is a common method to quantify the bioavailable organic matter in aquaculture water (Sindilariu et al., 2009). The method is simple, reliable, and informative but is inexpedient with respect to time. Related, indirect assessments of organic matter include chemical oxygen demand (COD), total and dissolved organic carbon (TOC and DOC), total and volatile suspended solids (TSS and VSS), turbidity, UV transmission (UVT), and particle size distribution (Fernandes et al., 2017). Each of these measurements has inherent advantages and flaws. Promising online measurements to monitor microbial related parameters include excitation-emission (EEM) fluorescence (Hambly et al., 2015) real-time optical bacterial detection (Højris et al., 2016) and real-time flow cytometry (Besmer & Hammes, 2016).

Enzymatic assays developed to evaluate bacterial activity in water include ATPase activity (Boe-Hansen et al., 2002; Berney et al., 2008; Hammes et al., 2010) and BactiQuant (Reeslev et al., 2011; Rojas-Tirado et al., 2018). Both methods rely on specific enzyme measurements that reflect bacterial numbers and activity. The BactiQuant assay includes activity measurements from both free living and particle associated bacteria and thereby takes activity related to particle size into account (Pedersen et al., 2017).
Traditional methods, such as plate counting to obtain colonies forming units (CFU)are used to predict changes in relative numbers of bacteria in aquaculture water samples (Leonard et al., 2000; Leonard et al. 2002; Brambilla et al., 2008; Hess-Erga et al., 2010). The plate counting method includes various agars and incubation regimens, and hence results in culture dependent conditions where only a very low number of the bacterial species can actually grow and thus be detected. Beside its time-consuming process, in the order of days, another disadvantage of the CFU method is that clumps of bacteria are not differentiated and can be miscounted as single colonies (Hazan et al., 2012).

In this study, we propose a rapid, simple, inexpensive and reproducible method that reflects the microbial enzymatic activity of both free living and particle associated bacteria, as well as potential contributions from other microbiota (Boaventura et al., 2018).
The underlying principle takes advantage of H_2O_2 decomposition (also referred to as degradation, elimination, reduction, decomposition or decay) which is primarily a biological process and hence related to the microbial composition of the water.

H_2O_2 decomposition is governed by microbial enzymatic activity, primarily bacteria, but also eukaryotic microbiota, documented in previous studies (Cooper et al., 1989; Cooper & Zepp, 1990; Richard et al., 2007; Abucayon et al., 2013). The primary enzymes responsible for H_2O_2 decomposition are catalases and peroxidases (Hossetti & Frost, 1994; Mishra & Imlay, 2012; Iwase et al., 2013). H_2O_2 decomposition follows exponential first order decay (Richard et al., 2007).

Hypotheses examined here are that the rate of H_2O_2 decomposition is positively correlated to i) the composition and quantity of microorganisms, ii) the total enzymatic activity (catalase and peroxidase) of the microbiota, and iii) temperature. Furthermore, we assumed that the low H_2O_2 concentration used (10 $mg \cdot L^{-1}$) and the short duration of the assay did not impact enzymatic activity (Arvin & Pedersen, 2015).

The H_2O_2 decomposition assay is described and verified in this paper including results from experiments conducted with different RAS water matrixes. Possibilities of the H_2O_2 decomposition assay are discussed and suggestions for practical applications are given.

2. MATERIALS AND METHODS

2.1. General description of the assay

The assay is based on adding a well-defined quantity of H_2O_2 to a raw water sample and quantifying the decomposition of H_2O_2 over time under controlled conditions. Water volumes, H_2O_2 concentration and sampling frequency are modifiable as long as consistent mixing and temperature control are provided. An applied fixation reagent terminates the H_2O_2 decomposition and forms a stable colour complex allowing flexible sampling and subsequent spectrophotometric analysis. The specific features and details of the assay are outlined in the following sections.

2.2. Procedure for the H_2O_2 decomposition assay

A representative water sample was collected and regulated to room temperature. Homogenous subsets of 40 mL were transferred to two different 50 mL Sarstedt® screw-cap plastic tubes (Nümbrecht, Germany) and placed in racks in a water bath (Julabo® SW22 shaking water bath; JULABO, Seelbach, Germany) with rotation (100 rpm) at 22 °C. Meanwhile, aliquots of 300 µL of the 4A reagents (described in section 2.3) were dispensed into individually marked cuvettes (10-12 per water sample). The tempered water samples were gently stirred and 2.70 mL was transferred to each of two cuvettes with predisposed reagent (Fig. 1)

A volume of 400 µL of 1000 mg· L^{-1} H_2O_2 stock solution was then added to each of the plastic tubes to reach a concentration around 10 mg $H_2O_2 \cdot L^{-1}$ (time = 0 min). Following gentle stirring of the water with H_2O_2 in the capped tubes, sample volumes of 2.70 mL were then transferred to cuvettes before H_2O_2 addition, and after 1, 15, 30, 45 and 60 minutes (Fig. 1).

A prompt colour complex reaction takes place in the cuvettes with formation of stable yellow oxo-peroxo-pyridine-2,6-dicarboxylato-vanadate(V) complex, OPDV. The colour complex was stable after 15 minutes and remained stable for several hours provided that it was not exposed to direct sunlight.

2.3. Reagents and analytical methods

The H_2O_2 concentration was measured spectrophotometrically by the method described by Tanner and Wong (1998). The modified reagent (4A) was prepared by mixing 1.2 g NH_4VO_3 with 5.2 g dipicolinic acid (recrystallized), 60 mLl Milli-Q water and 60 mL concentrated H_2SO_4 followed by heating to dissolution while stirring. The mixture was diluted to 1000 mL after cooling to room temperature.

The stock solution of hydrogen peroxide (1000 mg $H_2O_2 \cdot L^{-1}$) was made from a 30 % technical grade hydrogen peroxide following exact concentration analysis by autotitration analysis with ceriumsulphate ($CeSO_4$) and sodiumthiosulphate ($Na_2S_2O_3$).

Standard curves were made with Milli-Q water and several H_2O_2 concentrations (0, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 10.0 and 25.0 mg $H_2O_2 \cdot L^{-1}$) with new 4A reagent.

Corrections for potential background interference were made on all water samples (2.70 mL raw water before adding H_2O_2 + 300 µ 4A reagent). This apparent H_2O_2 concentration was subtracted from the values measured in H_2O_2-spiked samples. Distilled water or Milli-Q water was used to dilute the stock solution and to blank the spectrophotometer.

Chemical oxygen demand (COD) was measured using the Hach-Lange® (Brønshøj, Denmark) test kit LCK 314 (15–150 mg $O_2 \cdot L^{-1}$) and LCK 414 (5–60 mg $O_2 \cdot L^{-1}$). Biological oxygen demand over 5 days at 20 °C (BOD_5) was measured according to ISO 5815, using a WTW Oxi 340i oxygen probe (Rojas-Tirado et al., 2018). Micro particle numbers and distribution between 1 and 30 µm in diameter were quantified with a Coulter counter Multisizer 4e (Beckman Coulter, Indianapolis, USA) with a 50 µm aperture using a 40 µm sterile nylon cell strainer (VWR, Søborg, Denmark) to prefilter the samples.

For all batch experiments, the H_2O_2 and COD concentrations as well as BOD_5 were measured in at least duplicates and the average values were used.

2.4. Theory and calculation

The method is based on addition of H_2O_2 to a water sample with subsequent analysis of H_2O_2 decomposition over a short period of time. Under constant conditions (nominal H_2O_2 concentration, temperature and mixing), the decomposition rate reflects the amount of bacteria and any eukaryotic microbiota present (planktonic and particle bound) and their total enzymatic activity.

The first-order decomposition reaction rate constant (k) is calculated from the exponential decay equation:

$$C_t = C_0.e^{-kt} \quad (1)$$

where C_t indicates the concentration of H_2O_2 at time t, and C_0 represents the initial concentration, and k represents the rate constant in time^{-1}. The magnitude of the reaction rate constant (k) is considered directly proportional to the total bacterial activity in the water sample.

The reaction rate constant is deducted as

$$k = - (\ln (C_T / C_0))/t \quad (2)$$

and the half-life (T½) is calculated as

$$T_{½} = \ln 2/k \quad (3)$$

Temperature coefficients, θ, were calculated based on the van't Hoff-Arrhenius equation (eq. 4), where k is the H_2O_2 decomposition rate constant (h^{-1}), k_{20} is the rate coefficient at the reference temperature of 20 °C, θ is the dimensionless temperature coefficient, and T is temperature in °C (Zhu and Chen, 2002).

$$k = k_{20}\, \theta^{T-20} \quad (4)$$

2.5. Description of RAS system used for water sampling

The water samples were all collected from 1.7 m^3 pilot scale freshwater RAS´s operated at 16-18 °C with rainbow trout (*Oncorhynchus mykiss*) at different densities and feed loadings. To investigate effects of filtration on H_2O_2 decomposition kinetics, water was collected from a RAS operated at constant conditions for a period of 4 months (Spiliotopoulou et al, 2018). To compare H_2O_2 decomposition rate constants with BOD_5 analysis, water samples were collected from six different RAS over a three week period as described by Rojas-Tirado et al. (2017). Finally, H_2O_2 decomposition rate constants were compared with micro particle numbers based on water samples from 12 different RAS operated at constant conditions as described by Rojas-Tirado et al. (2018)

2.6. Statistics

A two-way analysis of variance (ANOVA) was performed with temperature and filtration as fixed factors and H_2O_2 decay rate as the dependent variable followed by a pairwise comparison procedure (Holm-Sidak) to test for significant differences ($P < 0.05$). Pearson product moment correlation analyses were performed between calculated decomposition reactions rate constants and i) BOD_5 and ii) micro particles. SigmaPlot version 13.0 (Systat Software Inc., CA, USA) was used for both types of tests.

3. RESULTS AND DISCUSSION

3.1. Determination of H_2O_2 concentration and decomposition rate

A linear standard curve based on H_2O_2 standard solutions from 0.5 to 25 mg $H_2O_2 \cdot L^{-1}$was described by y = 0.0132x + 0.0022 (R^2= 0.999) where y was absorbance (Abs) measured at 432 nm and x was [H_2O_2] in mg$\cdot L^{-1}$. The H_2O_2 concentration was then calculated as [H_2O_2] = (Abs_{432} - 0.0022)/ 0.0132 with that particular reagent. The level of detection (LOD) of the applied method was 0.16 mg$\cdot L^{-1}$ H_2O_2, corresponding to ≤ 0.002 Abs units. Table 1 illustrates a set of measurements from one water sample spiked with H_2O_2 where high reproducibility was observed. The current assay implies that background levels (Abs measured in raw sample + 4A reagent) are subtracted to obtain an adjusted nominal H_2O_2 concentration. Background Abs is a product of the reagent colour, dissolved organic compounds (e.g., lignin and humic substances) absorbing at 432 nm, and any H_2O_2 potentially present.

Calculation of H_2O_2 decomposition rate constants

Based on the adjusted H_2O_2 concentration in Table 1, the first order decomposition rate constant, *k*, was 0.306 h^{-1} (corresponding to a decomposition half-life of ln2/0.306 h^{-1} = 2.27 h). This was deduced from an exponential regression analysis (see Equation 1) where y = 9.408$\cdot e^{-0.3055\ t}$ (R^2 = 0.992), , or similarly by linear regression of the ln transformed data with the equation: ln y = - 0.3055 t + 2.242 (R^2 = 0.992).

3.2. *Effects of filtration*

H_2O_2 decomposition rate constants derived from raw and pre-filtered RAS water confirmed that the majority of H_2O_2 decomposition was related to particle associated bacterial activity. The rate constant in 0.2 µm sterile filtered RAS water at 22 °C was 0.038 h^{-1} (Fig. 2) with a corresponding H_2O_2 half-life of $T_{½}$=18.2 h. Planktonic bacteria and small aggregates in the size range from 0.2 to 1.6 µm (Gerardi, 2006) had a seven-fold higher decomposition rate constant with k = 0.263 h^{-1} ($T_{½}$= 2.6 h), while the H_2O_2 decomposition rate constant in unfiltered RASwater at 22 °C was 1.425 h^{-1} ($T_{½}$= 0.49 h). Similar size-fraction specific H_2O_2 decomposition demonstrating biologically mediated H_2O_2 decomposition was described in previous studies by Cooper et al. (1994) and Richard et al. (2007).

Cooper et al. (1994) filtered lake-water samples in four size ranges from 0.2 µm to 64 µm and found that microorganisms in unfiltered water decomposed naturally present H_2O_2 with a half-life at 4.4 h. The fraction without small algae [0.2-1.0 µm] decomposed H_2O_2 with $T_{½}$= 19 h

markedly faster compared to 0.2 µm sterile filtered water with an estimated half-life of 58.7 h. We further substantiated the biologically mediated H_2O_2 removal by autoclaving (120°C for 15 min) RAS water to exclude biological activity without removing dissolved and particulate organic matter. In that sterile water matrix, the resulting H_2O_2 decomposition rate was negligible (< 0.01 h^{-1}). This was also the case when H_2O_2 was added to Milli-Q water (*data not presented*).

The COD content of the unfiltered RAS water samples was 88.5 ± 0.1 mg $O_2 \cdot L^{-1}$, while 1.6 µm and 0.2 µm filtration reduced the COD content to 40.1 ± 0.2 mg $O_2 \cdot L^{-1}$ and 35.9± 0.2 mg $O_2 \cdot L^{-1}$, respectively. These values, along with accumulated nitrate and phosphate, indicates that RAS are nutrient rich environments with both dissolved and particulate organic matter favoring heterotrophic bacterial growth (Rojas-Tirado et al., 2018) as opposed to the oligotrophic conditions in some natural waters (Egli, 2010). The direct correlation between biodegradable organic matter and rate of H_2O_2 decomposition was recently confirmed in RAS with different feed loadings (Rojas-Tirado et al., 2018), in RAS with acetate supplements (Rojas-Tirado et al., 2019) and in a survey of seven commercial Danish model trout farms (Gregersen et al., 2019). The abundance and distribution of planktonic bacteria versus particle associated bacteria and small eukaryotic microorganisms are likely to differ among individual RAS and over time. The exact causes, mechanisms, and potential implications require further research. Site specific conditions and associated microbial composition may also reveal interesting enzymatic contributions from bacteria, algae, and protozoa (Coopers et al., 1994).

The H_2O_2 decomposition rate constants were significantly affected by temperature in the unfiltered RAS water sample (Fig. 2). With values of 0.979 h-1 at 17°C and 1.425 h^{-1} at 22°C, a temperature coefficient of 1.078 (7.8 % increase per °C) was calculated and was similar to values previously reported by Arvin and Pedersen (2015).

3.3. H_2O_2 decomposition rates, BOD_5 and micro particle correlations

The proof of concept of the assay was tested in two separate studies. Fig. 3 shows a highly significant positive correlation (r= 0.893; p = $6.2 \cdot 10^{-7}$, n=18) between H_2O_2 decomposition rate constants and bioavailable organic matter measured as standardized oxygen consumption over five days (BOD_5). A similar positive correlation was also found by Pedersen et al. (2007) in a study where a H_2O_2 liberating product was added to different freshwater RAS. The decomposition of H_2O_2 took place in a closed RAS, and hence included contributions from both suspended and surface attached bacteria.

The H_2O_2 decomposition rate constants reached 3.7 h^{-1} at 15 °C. As BOD_5 reflects the amount of bioavailable dissolved and particulate organic matter, this parameter gives a more exact description of the bacterial growth potential than COD, which is nevertheless often used as a proxy due to its faster processing time (Arvin & Pedersen, 2015; Fernandes et al., 2017). The H_2O_2 decomposition assay described here has potential to become a supplementary or alternative method to BOD_5 and COD measurements since it correlates well with both parameters. The H2O2 assay has a high reproducibility and allows fast measurements in both fresh and saline water samples where dilutions are often needed for BOD_5 and COD (Vyrides & Stuckey, 2009; Li et al., 2018). The H_2O_2 decomposition assay describes the actual microbial activity in a sample as opposed to an indirect measure of organic matter for potential bacterial growth provided by BOD_5 and COD.

The H_2O_2 decomposition rate constant was also strongly correlated ($r=0.909$; $p<0.001$; $n=72$) with the quantity of micro particles in RAS water samples (Fig. 4). Micro particles in size range from 1 to 30 µm were found in concentrations from 1×10^6 to 9.5×10^6 particles· mL^{-1}. The micro particle distribution was dominated by small particles, with 50-60 % smaller than 3 µm. Pedersen et al. (2017) recently described the importance and implications of particle concentration and particle surface area on bacterial activity. The study showed strong correlations between micro particles and bacterial activity measured by both Bactiquant® (Reeslev et al, 2011) and BOD_5 in different RAS water matrices. The Bactiquant® assay applied did not, however, correlate well with particle surface area in intensive RAS, which was explained as a shortcoming of only quantifying larger than 5 µm. Data from Rojas-Tirado et al. (2019) provides additional information to those findings by extending the size range to include micro particles down to 1 µm and thereby including planktonic bacteria containing catalase and peroxidase.

3.4. *Potential applications of the H_2O_2 decomposition assay*

Bacteria in the water are suspended, aggregated and attached to particles, and they potentially challenge the performance of fish and RAS (Michaud et al., 2006; Summerfelt et al., 2009; Becke et al., 2018). The factors affecting microbial composition and dynamic, their virulence and associated causal relationships are likely to become a research area of high importance for the aquaculture industry in the near future.

The assay presented here provides direct information on microbial activity in the water with a short-duration measurement. Fig. 5 conceptualizes how to apply the method in a practical way where an approximate rate constant is assessed based on time-flexible single measurements. In oligotrophic water, or water from advanced RAS with UV, ozone disinfection and efficient solids removal, increasing exposure time will improve resolution of the assay.. At the other end of the

spectrum, the H_2O_2 assay can be made with shorter duration and increased H_2O_2 addition when assessing microbial rich eutrophic water from natural water bodies or open aquaculture pond systems with high feed loading and long water retention time (Hargraves, 2006; Ray et al., 2010).

As a fast new tool to describe microbial activity, H_2O_2 decomposition assay are applicable for:

- Evaluating microbial activity in an aquaculture facility on a regular basis. This will give new information, increase system understanding and make it possible to establish baseline conditions and detect deviations.
- Quantifying and evaluating the effect of a given treatment component (foam fractionator, mechanical and biological filters etc.) and changes in operational practices (disinfection, new feed, altered fish densities, hydraulic, etc.).
- Improving planning and optimizing water treatment and sanitation (disinfection demand).
- Providing measurements of microbial activity and dynamics in field- and laboratory trials.
- Replacing COD (and BOD_5) measurements particularly for saline samples.

The assay could also be modified to quantify bacterial activity in biofilms or on system level. Modifications of the method might include simplifications (strip sticks), automation (e.g. using an online H_2O_2 sensor), use of other reagents, or use of plate reader assays to increase throughput.

In conclusion, the study has demonstrated that hydrogen peroxide decomposition is a rapid, efficacious and feasible indicator of microbial activity in water samples. The H_2O_2 assay has several potential applications in aquaculture from pond farming to intensive RAS and might also be applied in other areas.

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Figure legends

Fig. 1. Illustration of the hydrogen peroxide decomposition assay procedure. A temperature controlled water sample is spiked with H_2O_2 with subsequent temporal quantification of H_2O_2 residuals by use of a fixating color forming reagent.

Fig. 2. H_2O_2 decomposition rate constant (*k*) derived from freshwater RAS samples. Bars represent mean ± std. dev. (n=3) based on spiking H_2O_2 trials with unfiltered and prefiltered (0.20 µm sterile filter and 1.6 µm GFA filters) water samples at 17° and 22 °C. Organic matter measured as COD was 88.5±0.1 mg O_2/l (unfiltered); 40.1 ±0.2 mg O_2/l (1.6 µm) and 35.9±0.2 mg O_2/l (0.2 µm). Different letters denote highly significant differences between groups ($p < 0.01$).

Fig. 3. Linear correlation between biological oxygen demand (BOD_5; mg O_2/l) and H_2O_2 decomposition rate constant k (in h^{-1}) based on 18 water analysis from six different freshwater RAS operated at 16-17 °C (cf. Rojas-Tirado et al., 2018).

Fig. 4. Linear correlation between numbers of micro particles (size range 1-30 µm) and H_2O_2 decomposition rate constant k (in h^{-1}) based on 72 water analysis from twelve different freshwater RAS operated at 16-17 °C.

Fig. 5. Theoretical/modelled decomposition scenarios of H_2O_2 calculated based on nominal H_2O_2 concentration of 10 mg/L and various decomposition rates (k; in h^{-1}). The figure makes it possible to determine the k-value based on controlled H_2O_2 addition and a time specific measurement of the residual H_2O_2. The green circle exemplifies a decomposition curve where 5 mg H_2O_2 is measured 40 minutes after H_2O_2 addition which corresponds to a decomposition rate constant *k of* 1.0 h^{-1} [half-life $T½ = \ln 2/k \sim 40$ min).

Table 1. Example of absorbance readings (λ = 432 nm) and calculated H_2O_2 concentrations measured in RAS water at 22°C after addition of 10 mg/L H_2O_2 at time 0.

Figures (5)

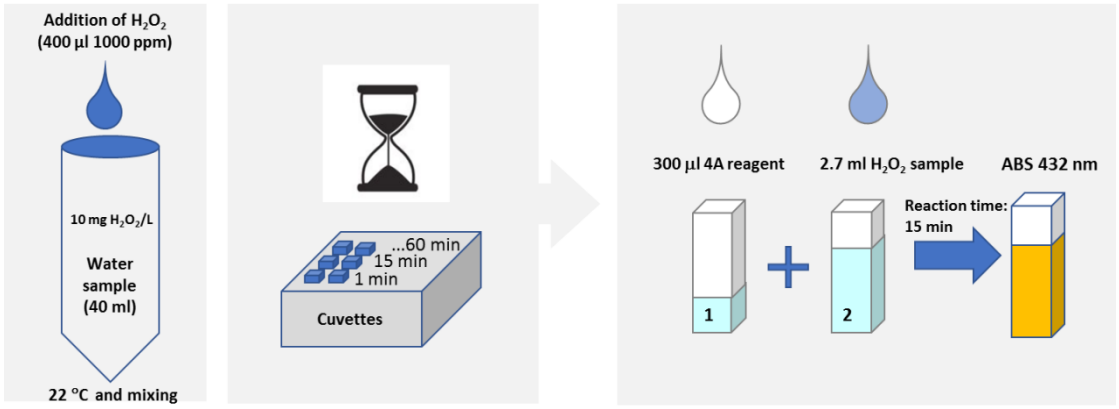

Fig. 1.

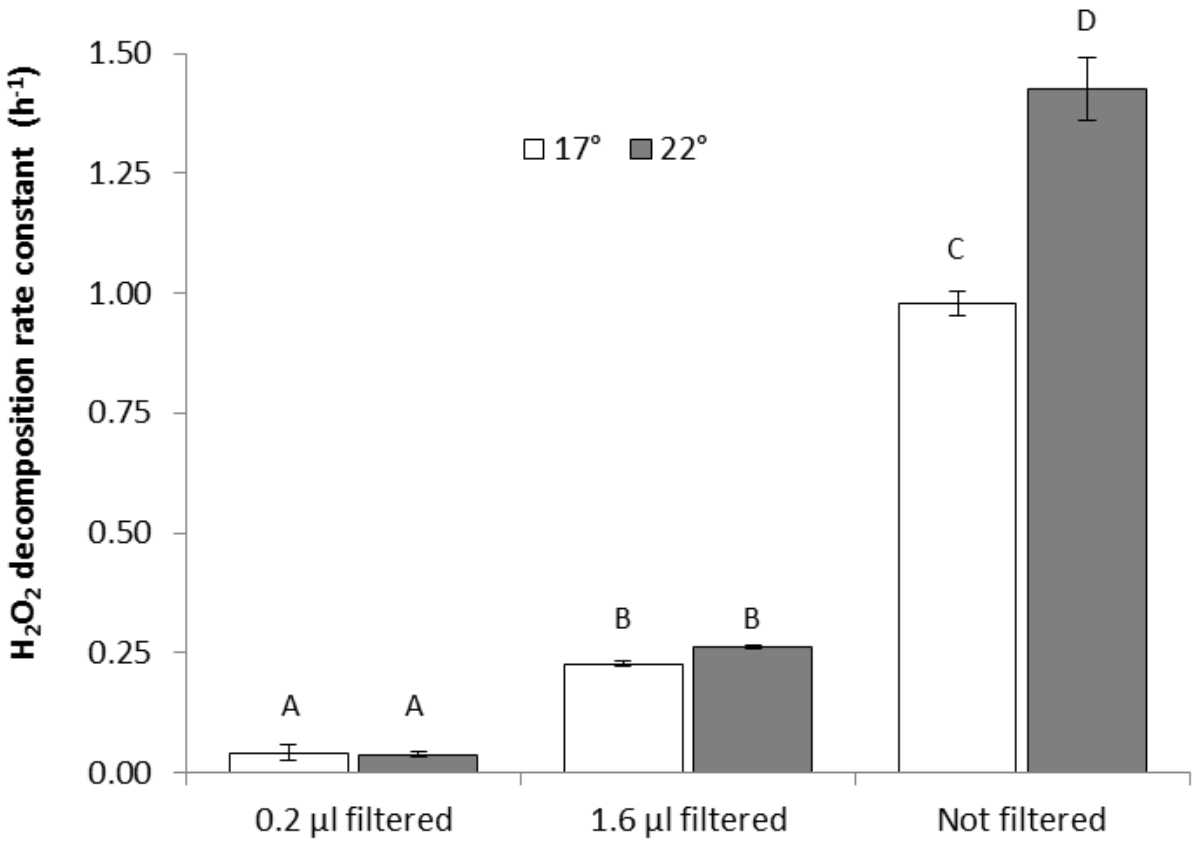

Fig. 2.

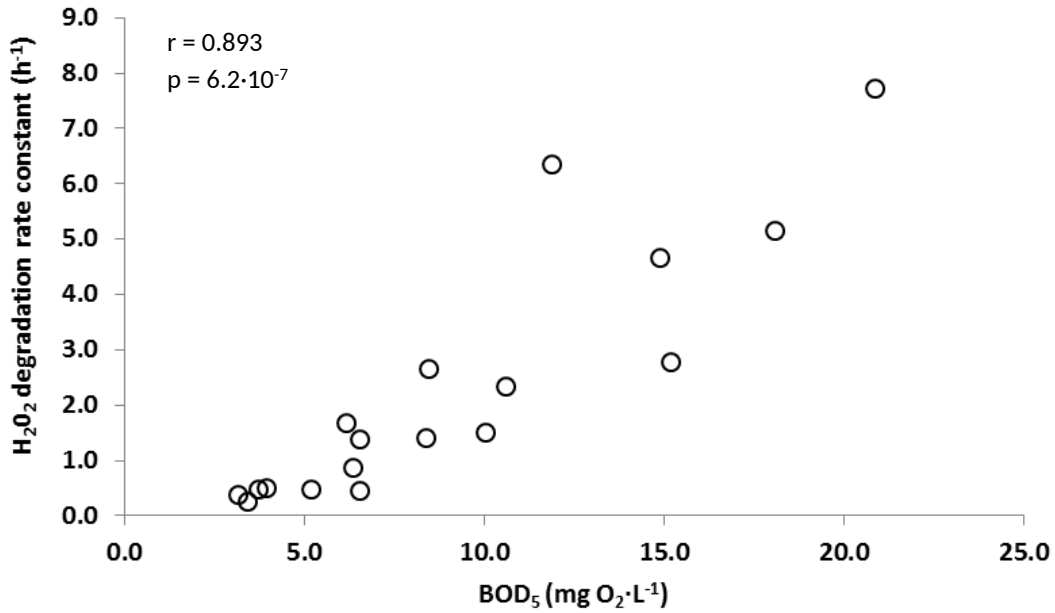
r = 0.893
p = 6.2·10-7
H2O2 degradation rate constant (h-1)
BOD5 (mg O2·L-1)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0

Fig. 3.

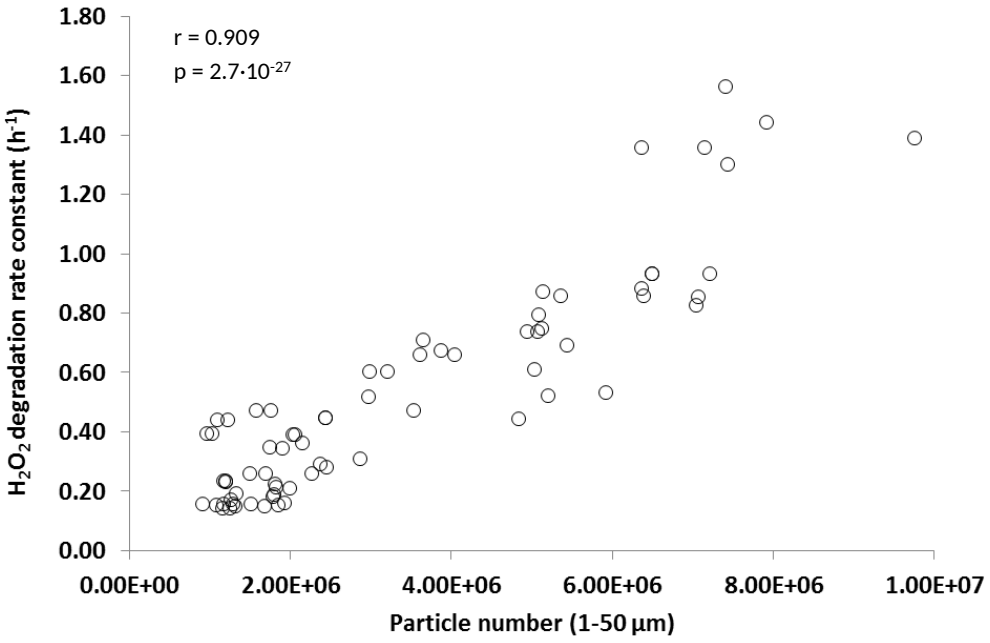
r = 0.909
p = 2.7·10-27
H2O2 degradation rate constant (h-1)
Particle number (1-50 µm)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.00E+00
2.00E+06
4.00E+06
6.00E+06
8.00E+06
1.00E+07

Fig. 4.

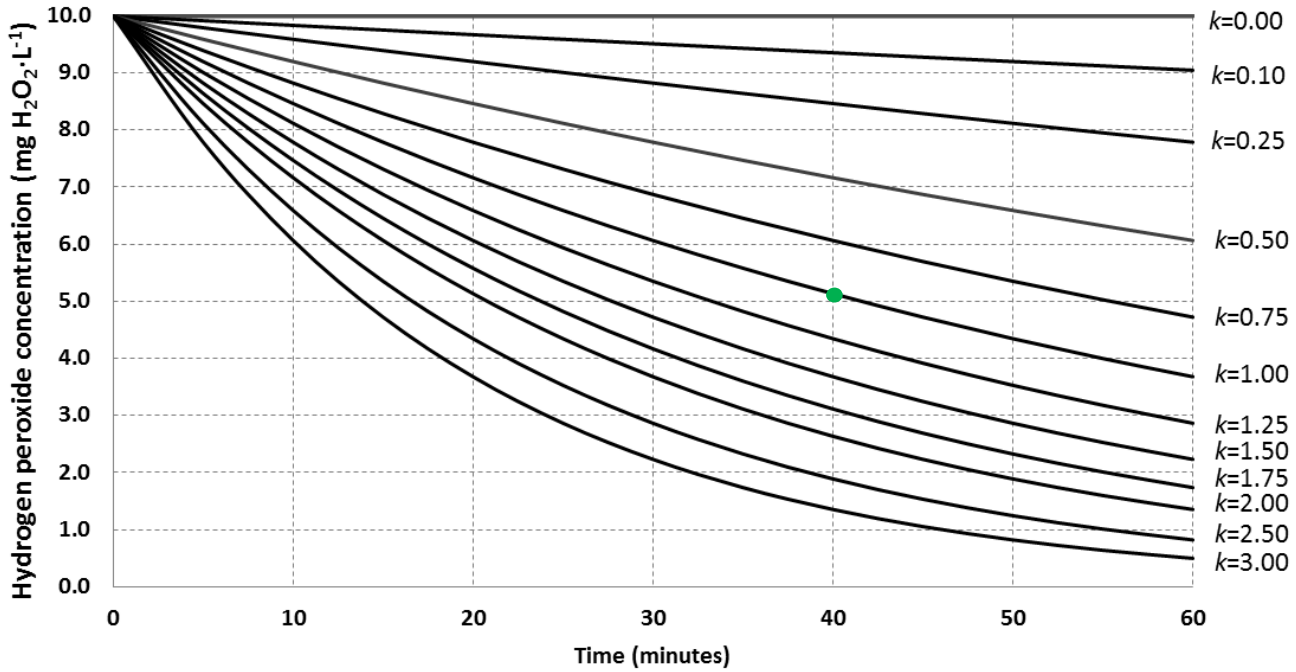

Fig. 5.

Table 1

Time, (min.)	**$Abs_{Sample1}$**	**$Abs_{Sample2}$**	**$Abs_{Sample3}$**	**$Abs_{Average}$**	**$[H_2O_2]_{app}$* (mg/L)**	**$[H_2O_2]_{adj.}$* (mg/L)**
Before	0.036	0.036	0.035	0.036	2.54	-
5.0	0.157	0.159	0.158	0.158	11.80	9.27
15	0.150	0.150	0.151	0.150	11.22	8.69
30	0.140	0.142	0.141	0.141	10.52	7.98
45	0.133	0.133	0.136	0.134	9.98	7.45
60	0.128	0.128	0.128	0.128	9.53	6.99

* App and Adj. refer to apparent (incl. background) and adjusted H_2O_2 concentration