Hydrogen Peroxide Can Be a Plausible Biomarker in Cyanobacterial Bloom Treatment

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Abstract

The effect of combined stresses, photoinhibition and nutrient depletion, on the oxidative stress of cyanobacteria was measured in laboratory experiments, to develop the biomass prediction model. *Phormidium ambiguum* was exposed to various photosynthetically active radiation (PAR) intensities and phosphorous concentrations with fixed nitrogen concentration. The samples were subjected to stress assays by detecting hydrogen peroxide (H_2O_2) concentration and antioxidant activities of catalase (CAT) and superoxide dismutase (SOD). H_2O_2 concentration decreased to 30 $\mu molm^{-2}s^{-1}$ of PAR, then increased further with higher PAR intensity. Regarding phosphorus concentration, H_2O_2 concentration generally decreased with increasing phosphorus concentration. SOD and CAT activities were proportionate to the H_2O_2 $protein^{-1}$. No H_2O_2 concentration detected outside of cells indicated the biological production of H_2O_2, and the accumulated H_2O_2 concentration inside cells was parameterized with H_2O_2 concentration $protein^{-1}$. Over 30 $\mu molm^{-2}s^{-1}$ of PAR, H_2O_2 concentration $protein^{-1}$ had a similar increasing trend with PAR intensity, independently of phosphorous concentration. Meanwhile, with increasing phosphorous concentration, H_2O_2 $protein^{-1}$ decreased in a similar pattern regardless of PAR intensity. Protein content decreased with increasing H_2O_2 gradually up to 4nmol H_2O_2 mg^{-1}protein, which provides a threshold to restrict the growth of cyanobacteria. With these results. an empirical formula was developed to obtain the cyanobacteria biomass.

Introduction

Cyanobacteria blooms often produce toxic metabolites and are harmful for other organisms as well as humans. Hydrogen peroxide (H_2O_2) is often endorsed to reduce the cyanobacterial abundance as it is more effective in application with cyanobacteria as compared to other phytoplankton[1-2]. However, H_2O_2 is also produced by other factors. First, hydrogen peroxide is generated photochemically from organic material exposed to UV, and H_2O_2 distribution was observed in natural lakes[3-5]. At the same time, H_2O_2 is biologically produced in cells, exposed to environmental stresses, including metal ion toxicity, salinization, temperature, PAR conditions, eutrophication, allelopathy, and pathogens. though its contribution with respect to the total concentration is unknown[5].

Under a stress environment, endogenous reactive oxygen species (ROS) production, including superoxide, hydroxyl radicals and H_2O_2 exceeds its scavenging capacity[6-8]. Reactive oxygen species are important for growth regulation and signaling mechanisms in photosynthetic organisms[9-10] and those organisms in turn are capable of controlling excess ROS production with their inherent scavenging enzymes and non-enzymatic components[9,11]. Accumulation of an excessive amount of ROS inside cells causes harmful impacts on cyanobacteria such as disrupting the cellular homeostasis, causing membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damages, etc., and affects the photosynthetic apparatus, finally leading to cell mortality as the concentration exceeds the threshold value[12].

Cyanobacteria are sensitive to even a minor change in light intensity as they usually expose relatively weak light, and thus even a moderate solar radiation may cause stress[13]. Collecting solar energy at photosystem II (PSII) in the thylakoid membrane results in the oxidation of water molecules and the reduction of plastoquinone, a molecule involved in the electron transport chain. The produced electrons are transported to PSI, where they are consumed in the synthesis of carbohydrates. An overabundance of solar energy, however, results in the generation of ROS, including superoxide radicals, as the energy transfer rate is limited due to the underutilization of energy absorbed by the PSII antenna complex in the PSII reaction center[11,14-16]. Superoxide dismutase (SOD) catalyzes superoxide radicals into hydrogen peroxide (H_2O_2), before being detoxified into water by antioxidant activities[17]. However, the high oxidation potential of ROS can lead to the destruction of proteins, which otherwise recover the photosystem activities[18]. Thus, excessive solar radiation inhibits the proliferation of cyanobacteria. The shortage of nutrient conditions is also identified as a dominant stressor that suppresses the growth of cyanobacteria[19]. The combined effect of various abiotic stresses on the production rate is often reported[20-21]. Some combinations inhibit growth due to contradicting effects of stressors; however, significant reduction of biomass is also reported as caused by simultaneous exposure to multiple stressors as compared to a single source of stress[22].

The concentration of H_2O_2 and activity of antioxidant enzymes are some of the biomarkers employed in stress detections; and the role of H_2O_2, in plants and how they respond to environmental stress has been a focus throughout the literature[23-25] suggesting a potential to develop ROS-based strategies for the prediction of cyanobacterial bloom formation and H_2O_2 concentration[26]. Thus, this research was designed to study (1) effects of the PAR regime and phosphorus concentration on the stress of cyanobacteria, particularly H_2O_2 concentration, (2) the combined effects of the PAR regime and phosphorous concentration on H_2O_2 concentration, and (3) the relationship between H_2O_2 concentration and antioxidant enzyme activities of cyanobacteria, aiming at the possibility of applying H_2O_2 concentration as a proxy to detect stress intensity in algal management and the contribution rate of biological H_2O_2 production rate in the treatment.

Analyses

Total soluble protein content analysis

Total soluble protein concentration was determined by using the same method that was mentioned in[27] with minor modifications. Cyanobacterial cells were extracted from 1 mL of culture media by centrifugation at 4 °C for 10 min at 10,000 rpm, and the pellet was washed once with distilled water. Then, the cell pellet was subjected to a freeze-thaw cycle. Total soluble protein was extracted using 0.5 M NaOH solution, and the extraction was centrifuged at 4 °C for 20 min at 10,000 rpm. The supernatant was used as crude protein extract, and the protein content was quantitatively analyzed with the aid of Coomassie Bradford protein assay kit. Crude protein extract was stained with Coomassie (G-250) dye and

incubated for 10 min at room temperature, and then the absorbance was measured at 595 nm using a UV-Vis spectrometer (Shimazu, Japan). Protein content was determined using a known concentration series of Albumin.

Stress assay

H_2O_2 Assay

Cellular H_2O_2 contents were estimated according to the titanium chloride method[28]. A total of 750 µL of 0.1% titanium chloride in 20% H_2SO_4 (v/v) was then added to initiate the reaction. The optical absorption after 1 min was measured at 410 nm using a spectrophotometer (UVmini-1240). However, the absorption at 410nm includes the effect of other soluble compounds[29-31]. Thus, the H_2O_2 concentration was calculated from the slopes of the standard curve obtained from known H_2O_2 concentration, which was offset derived by the intercept absorption rate with zero H_2O_2 concentration samples[30]. The results were compared with those of the e-FOX method and the suitable agreement was obtained[31].

CAT assay

The CAT activity was measured by reacting 15 µL of 750 mM H_2O_2, 920 µL of potassium phosphate buffer, and 65 µL of extract supernatant. Optical absorption was measured at 240 nm using UV mini-1240. The measurements were recorded every 20 s for 3 min, and CAT activity was calculated using an extinction coefficient of 39.4 $mM^{-1}cm$[32]. Scavenging rate of H_2O_2 by enzyme extract per minute was defined as one unit of CAT per 1 µg of protein.

APX assay

For APX assay, the reaction mixture contained 100 µL of enzyme extract, 200 µL of 0.5 mM ascorbic acid in 50 mM potassium phosphate buffer (pH 7.0), and 2 mL of 50 mM potassium phosphate buffer (pH 7.0) was mixed with 60 µL of 1 mM H_2O_2. The decrease in absorbance at 290 nm was recorded every 20 s for 3 min. The APX activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1} [33].

SOD assay

Total SOD activity was determined by using methods as described by[34]. The reaction mixture contained 50mM phosphate buffer (pH 7.8), 0.66 mM EDTA, 10 mM methionine, 33 µM NBT, 0.0033 mM riboflavin and 50 µL cyanobacterial enzyme extract. The reaction was allowed to proceed under a fluorescent illumination, after that the absorbance of the reaction mixture was read at 560 nm. One unit of SOD activity was defined as the amount of enzymes required to cause 50% inhibition of the NBT photo-reduction, and the results were expressed as unit per $µg^{-1}$ of total soluble protein.

Statistics

Variance (ANOVA) and the bivariate analysis were used and Pearson's correlation method was followed to evaluate the relationship among parameters. Statistical analyses were performed with the help of IBM SPSS V25.

H_2O_2 concentration showed correlations with increasing PAR intensities and decreasing phosphorus concentrations, independently. The fitted curve patterns were different for each PAR intensity or each phosphorous concentration.

Therefore, the most fitted curves of H_2O_2 concentration with respect to phosphorus concentration, and with respect to PAR intensity, 30 to 200 $\mu molm^{-2}s^{-1}$, were obtained for each PAR intensity, and the phosphorus concentration, 0.1 to 1000mgL^{-1}, respectively, then the effect of PAR intensity and phosphorus concentration in the H_2O_2 concentration was estimated.

The variance (ANOVA) and the bivariate analysis were used and Pearson's correlation method was followed to evaluate the relationship among parameters. Statistical analyses were performed with the help of IBM SPSS V25.

Results

The effect of PAR intensity and phosphorous concentration on the H_2O_2 concentration

Protein content and H_2O_2 concentration are shown in Fig1. Protein content for different PAR intensity levels and for each phosphorus concentration level (mgL^{-1}). Vertical bars indicate standard deviation and Fig2. H2O2 concentration for different PAR intensity levels and for each phosphorus concentration level (mgL^{-1}). Vertical bars indicate standard deviation. with respect to PAR intensity.

Protein content was higher in less than 50 $\mu molm^{-2}s^{-1}$ of PAR, and was observed to increase with increasing PAR intensity; H_2O_2 concentration per protein shows the similar variational trend with respect to the PAR intensity Fig. 3. H2O2 content per protein for different PAR intensity levels and for each phosphorus concentration level (mgL^{-1}). Vertical bars indicate standard deviation. Regardless of phosphorous concentration, it declined with low PAR intensities down to 30 $\mu molm^{-2}s^{-1}$ of PAR intensity and then increased gradually with decreasing enhancement rate. At values higher than 50 $\mu molm^{-2}s^{-1}$ PAR, no significant difference with different phosphorus concentration levels was detected.

H_2O_2 per protein was generally lower with higher phosphorus concentration ($p<0.03$) Fig. 4. H2O2 content per protein for different phosphorus concentration level (mgL^{-1}) and for each PAR intensity level ($\mu molm^{-2}s^{-1}$). Vertical bars indicate standard deviation. Dotted lines show the approximate relation for each light intensity.

H_2O_2 per protein, which was measured as high as 2.5 up to 10 $\mu molm^{-2}s^{-1}$, with 100 to 200 $\mu molm^{-2}s^{-1}$ of PAR intensities, declined with higher phosphorus concentration Fig. 4. H2O2 content per protein for

different phosphorus concentration level (mgL^{-1}) and for each PAR intensity level ($\mu molm^{-2}s^{-1}$). Vertical bars indicate standard deviation. Dotted lines show the approximate relation for each light intensity.

Fig. 3. H2O2 content per protein for different PAR intensity levels and for each phosphorus concentration level (mgL^{-1}). Vertical bars indicate standard deviation denotes the relationship between H_2O_2 per protein and PAR intensity for higher than 30 $\mu molm^{-2}s^{-1}$of PAR intensity. For all phosphorus concentration levels, H_2O_2 per protein values had a similar pattern with respect to PAR intensity ($p<0.01$).

Antioxidant activities with respect to H_2O_2 concentration per protein

SOD activity was uniquely proportionate to H_2O_2 per protein Fig. 5. SOD activity per protein for different phosphorus concentration level (mgL^{-1}) and for each PAR intensity level ($\mu molm^{-2}s^{-1}$). The approximate relation is shown by the diagonal line, where H_2O_2/protein($nmolmg^{-1}$) = 0.176(min)*SOD ($nmolmg^{-1}$ min^{-1}), (R^2=-0.805, $p<0.01$).

CAT activity is shown as a function of H_2O_2 concentration per protein, separately shown by each phosphorous concentration Fig. 6. CAT activity per protein for different phosphorus concentration levels (mgL^{-1}). and for each PAR intensity level ($\mu molm^{-2}s^{-1}$). Dotted lines indicate the approximate lines for each phosphorus concentration. Overall CAT activity has a high correlation with H_2O_2 (n=90, R^2=0.60, $p<0.01$).

For each phosphorus concentration level, CAT activity per protein linearly increased with H_2O_2/protein, and the increasing rate was higher based on PAR intensity (18.73CAT/H_2O_2, R^2=0.573 for 1000$mgPL^{-1}$, 13.82CAT/H_2O_2, R^2=0.977 for 100 $mgPL^{-1}$; 12.89CAT/ H_2O_2, R^2=0.793 for 10 $mgPL^{-1}$; 14.53CAT/ H_2O_2, R^2=0.949 for 1$mgPL^{-1}$; and 9.22CAT/H_2O_2, R^2=0.766, for 0.1$mgPL^{-1}$), and the proportional coefficient was found to have a significant positive correlation with the logarithmic scale of the phosphorus concentration ($p<0.01$).

For each PAR intensity level, on the other hand, CAT activity did not have significant positive correlation with the phosphorous concentration level.

Discussion

The effect of biologically produced H_2O_2 on the suppression of cyanobacterial blooms

The artificial endorsement of H_2O_2 has a high potential to suppress cyanobacterial blooms with less effects on other organisms compared to other controlling methods[35-39]. Researchers obtained the lethal H_2O_2 concentration of cyanobacteria by laboratory incubations under different concentrations of H_2O_2; cyanobacterial chlorophyll declined to nearly half after 18 h with the dose of approximately 30 mmol of H_2O_2 L^{-1} [1,40] and in 4 h with 100 mmol of H_2O_2 L^{-1} [2]. H_2O_2 delayed fluorescence decay with 0.1mmol of

H_2O_2 L^{-1} [37], while the Fv/Fm value substantially declined with 100 mmol of H_2O_2 L^{-1} [41], dead cells increased with 275 mmol of H_2O_2 L^{-1} [39]. Cyanobacteria were in the lethal condition[1-2] and sub-lethal at concentrations exceeding 50 mmol of H_2O_2 L^{-1} [39]. All experimental results reveal that cyanobacterial biomass is degraded with higher H_2O_2 concentration; however, the threshold H_2O_2 concentration varies widely from 1 to 1000 mmol L^{-1}.

Natural H_2O_2 formation has been identified in aquatic ecosystems as a photolysis of dissolved organic carbon (DOC) exposed to UV[3,42-44] reported that the H_2O_2 production varies with the nutrient content of the water body. However, the H_2O_2 concentration of these waters was in the magnitude of mmol L^{-1} [4,26,45]. The comparison of these results indicates that the photolysis of organic carbon in natural water only is not sufficient to control cyanobacterial biomass.

H_2O_2 is also produced biologically and is accumulated in cells, subject to high levels of environmental stress. In the present experiment, UV was limited. Accordingly, measured H_2O_2 was considered a biologically produced component in cells or cell surfaces, which was then released into the ambient water. In the present experiment, protein content was measured as a reference of biomass of cyanobacteria. Cell biomass is two to three times larger with the protein content[46].

As the buoyancy of the cells is nearly neutral, the H_2O_2 content per protein, ~1 mmol of H_2O_2 kg^{-1} was generated and contained in the cell before release. This constitutes more or less the same level of the lethal H_2O_2 concentration in water.

The protein content in water declined with increasing H_2O_2 concentration per protein up to 2 nmol mg^{-1} protein. A higher protein level was not observed with higher H_2O_2 concentration levels in the present study. The growth of cyanobacteria is suppressed by the generation of higher H_2O_2 levels.

The lethal H_2O_2 concentration obtained here corresponds well with about 5 mmol of H_2O_2 g^{-1} FW of a threshold condition to grow *Egeria densa* in natural water[25], considering the weakness of cyanobacteria to H_2O_2 rather than other plant species[35].

The possible indicator of environmental stress and the effect of combined stress factors

The accumulation of ROS is reported to augment in parallel fashion to increased abiotic stress[47-49]. In the present experiment, two types of abiotic stresses, phosphorous deficiency and high or low PAR intensities were applied with different intensities of each.

Though H_2O_2 is produced under normal environmental conditions, their production is accelerated under high stress intensity. In natural water, cyanobacteria often suffer from a shortage of nitrogen and phosphorus. Stoichiometrically, the ratio of nitrogen and phosphorus of cyanobacterial cells is approximately 16:1 [50]. Waters with an N:P ratio of < 15 are most susceptible to cyanobacterial

dominance[51-52]. In the present experiment, the phosphorus concentration was changed with the fixed amount of nitrogen concentration of 3000mg L^{-1}. Thus, phosphorous concentration becomes restrictive, except for 1000mg P L^{-1} in the present study's conditions. A significant increasing trend was observed in H_2O_2 per protein with decreasing phosphorus concentration. The deficiency of restrictive nutrients may increase the oxidative stress and then deteriorate the growth rate.

Under all tested phosphorus concentrations, H_2O_2 per protein content decreased with increasing PAR intensity until 30 $\mu molm^{-2}s^{-1}$, taking the lowest value there, then increased at higher PAR intensities though increasing rate gradually decreased. The enhanced production of H_2O_2 under prolonged low PAR conditions has not been reported upon sufficiently, though the production of superoxide in dark conditions is reported[26]. With submerged macrophytes, *Egeria densa*, H_2O_2 concentration was empirically the lowest under the prolonged exposure of a PAR intensity level of approximately at 60 $\mu molm^{-2}s^{-1}$ [53] and increased both with decreasing or increasing PAR intensities. However, the underlying mechanisms are unknown.

Increasing H_2O_2 concentration per protein over 30 $\mu molm^{-2}s^{-1}$ of PAR intensity is attributed to the excessive harvesting of PAR energy[17-18]. In the thylakoid membrane, electrons are produced by solar energy and transmitted to plastoquinone in PSII, which are partially accepted for the carbon dioxide fixation. More electrons are generated, when exposed to higher levels of solar radiation, and consequently, the photoinhibition of photosystem-II (PS-II) is induced, leading to oxidative damage because of the generated ROS, such as superoxide, hydroxyl radical, and H_2O_2. It damages cellular components, such as D1 protein, which otherwise mends the damaged photosynthesis apparatus[18].

The process is composed of the direct reduction of O_2 by PS-I resulting in the production of singlet oxygen, followed by superoxide, which is converted to H_2O_2 by the activities of the enzyme SOD.

In the present study, H_2O_2 per protein was proportionate with SOD activity, which generates H_2O_2 from superoxide. CAT activity was far higher than other major antioxidant activities to decompose H_2O_2, and linearly increased with H_2O_2 concentration.

Though SOD and CAT activities demonstrated different dependencies on PAR intensity levels and the phosphorus concentration, their activities were evaluated by the single function with H_2O_2 per protein. The steady H_2O_2 concentration is sustained by the balance of the generated H_2O_2 by different type of stresses and these antioxidant activities, as a single function of H_2O_2 content per protein.

In natural water, cyanobacteria are exposed to various types of abiotic stresses that enhance the oxidative stress, producing H_2O_2, and it may deteriorate cyanobacterial biomass. Significant negative correlation was recognized for protein content with respect to H_2O_2 concentration ($n=90$, $R^2=-0.712$, $p<0.01$), irrespective of stress types.

The production rate of H_2O_2 is not necessarily cumulative for different types of abiotic stresses[22]. However, H_2O_2 concentration was enhanced with increasing PAR intensity and decreasing phosphorus concentration, respectively, and the enhancement of H_2O_2 concentration was independent of each other ($p<0.01$). The total H_2O_2 per protein is empirically given as the sum of the amount of H_2O_2 produced by the intensity of each stress component, at least as a practical use level. Thus, for the combined stresses, the total H_2O_2 concentration is approximately provided by the sum of the H_2O_2 concentration attributed to each stress. The same trend was obtained for submerged macrophytes[25,53]. Consequently, the potential to use the H_2O_2 concentration to estimate the cyanobacterial biomass exists.

The estimation of H_2O_2 concentration produced by cyanobacteria under abiotic stresses

For the application of the empirically obtained results to practical use in the prediction of algal blooms in the environment where PAR and phosphorous concentration, P, are restricted factors for the growth, the trend of the H_2O_2 per protein (nmol/mg) is obtained as a function of PAR ($\mu molm^{-2}s^{-1}$) and the phosphorous concentration, P (mgL^{-1}), as formulated by

$$H_2O_2/protein = -312*PAR^2/(50^2+PAR^2)*((25/PAR)^4+1)*Log(P/133100) \quad (1)$$

Where $0.1mg\ P\ L^{-1}<P<1000mg\ P\ L^{-1}$, and $30\ \mu molm^{-2}s^{-1}<PAR$, and protein represents the amount of protein in mgL^{-1}.

The relationship is shown in Fig. 8. The simulated results of H_2O_2/protein by equation (1) compared with experimental results and a significant similarity was obtained ($R^2=0.953$, $p=0.012$, for 1000mg P L^{-1}, $R^2=0.696$, $p=0.0065$ for 100 mg P L^{-1}; $R^2=0.927$, $p=0.023$ for 20 mg P L^{-1}; $R^2=0.982$, $p=0.00289$ for 1 mg P L^{-1}; $R^2=0.024$, $p=0.024$ for 0.1 mg P L^{-1}).

The protein content (mgL^{-1}), as shown in Fig. 9. The simulated results of protein content by equation (2), as a function of H_2O_2 per protein was shown to possess significant negative correlation ($R^2=-0.675$, $p<0.01$), which is empirically formulated by

$$protein = -192*Log((H_2O_2/protein)/4.1) \quad (2)$$

($R^2=-0.71$, $p<0.01$).

With equation (1) and (2), protein content is estimated as a function of PAR and the phosphorous concentration.

The estimated protein contents are denoted in Fig. 7. Protein content in water for different phosphorus concentration levels (mg/L) and for each PAR intensity level ($\mu molm^{-2}s^{-1}$). The concentration uniquely increased with increasing phosphorus concentration.

The cellular growth rate gradually decreased with light intensity[54] , and the growth rate of cyanobacteria reached a maximum at 30 to 50 $\mu molm^{-2}s^{-1}$ [55]. *P. ambiguum* prefers relatively low light intensity, at ~18 $\mu molm^{-2}s^{-1}$ [56]. The diagram seems to provide reasonable results.

Conclusions

Endogenous H_2O_2 concentration is an effective tool to detect the stress level of cyanobacteria.

Both PAR regimes and shortage of phosphorus concentration are shown to enhance H_2O_2 concentration in a cyanobacterial culture.

H_2O_2 per protein content declines in low PAR conditions, then increases when exposed to higher PAR intensity levels while generally increasing as phosphorus concentration decreases.

H_2O_2 per protein for combined stresses is given by the sum of the amount produced by each stress. Protein content decreases uniquely following the value of H_2O_2 per protein in a cyanobacterial culture. The prediction model was developed for the protein content, to design management criteria for excessive cyanobacterial blooms in freshwaters.

Methodology

Culture and Incubation

Phormidium ambiguum, which is an odor-forming benthic cyanobacterial species, was obtained from the National Institute of Studies (NIES), Japan. The strain was cultured and acclimatized for 30 days in an autoclaved BG 11 medium[57], maintained at 20°C under controlled PAR conditions with white fluorescent light, having flux of 20 $\mu molm^{-2}s^{-1}$ in a light-and-dark cycle of 12h:12h. The cultures were manually shaken twice a day. Cells were subcultured by diluting with new BG 11 medium as needed[27-28].

Long-term exposure experiment

After 30 days, well-grown cyanobacterial cells were collected by centrifugation, washed once with distilled water, and then re-suspended in modified BG 11 media. All experiments were conducted by using incubators (MIR-254, Sanyo, Tokyo, Japan) with a nutrient level of BG-11 medium, consisting of $NaNO_3$ 17.6 mM, K_2HPO_4 0.2296 mM, $MgSO_4.7H_2O$ 0.0146 mM, Na2CO3 0.0189mM, Citric acid 0.0031 mM, Ferric ammonium citrate 0.0023 mM, EDTA (Na_2 salt) 0.0297 µM, H_3BO_3 4.6253µM, $MnCl_2.4H_2O$ 0.9145 µM, ZnSO4.7H2O 0.0765 µM, $Na_2MoO_4.2H_2O$ 0.1611 µM, $CuSO_4.5H_2O$ 0.0316 µM, and $Co(NO_3)_2.6H_2O$ 0.0023 µM in 1 L of deionized water[57], adjusted for nitrogen and phosphorus concentrations respectively at 3000 mgL^{-1} of nitrogen and 0.1, 1.0, 10, 100, and 1000 $mgL^{-1}$of phosphorus. Six different PAR intensities, namely - 10, 20, 30, 50, 100 and 200 $\mu molm^{-2}s^{-1}$ by white fluorescent light (Toshiba, Japan) and VBP-L24-C2 PAR source (Valore, Kyoto, Japan) were used with

12h:12h PAR and dark cycle. The PAR intensities were measured using a quantum sensor (EKO Instruments Co., Ltd., Japan) and adjusted uniformly in the media. The temperature was kept constant (20 ^{o}C) throughout the experiment. At 12:00, after 7 days, samples were taken for the subsequent stress response analysis. Collected samples were subjected to bioassays that are described later.

Short-term (one day) exposure experiment

Another experimental setup was conducted to check the transition within a day of the culture. Above culture was exposed to 0, 30, 100, and 300 $\mu molm^{-2}s^{-1}$ of PAR intensity with BG-11 medium, except for nitrogen and phosphorous concentrations, 250 mgL^{-1} and 5.5 mgL^{-1}, respectively. A part of samples was taken at 6:00 (initial), 9:00, 12:00, 15:00, 18:00, 21:00, then was subjected to the analyses.

For both types, experimental units per each PAR and nutrient condition were triplicated in the same procedure, in order to confirm reproducibility.

Declarations

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Author contribution statement

T.A. contributed to the design of the experiment, analyses, and writing the manuscript.

M.R. contributed to the revision of the manuscript.

H.D.L.A. carried out the experiment and writing the experiment part.

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Figures

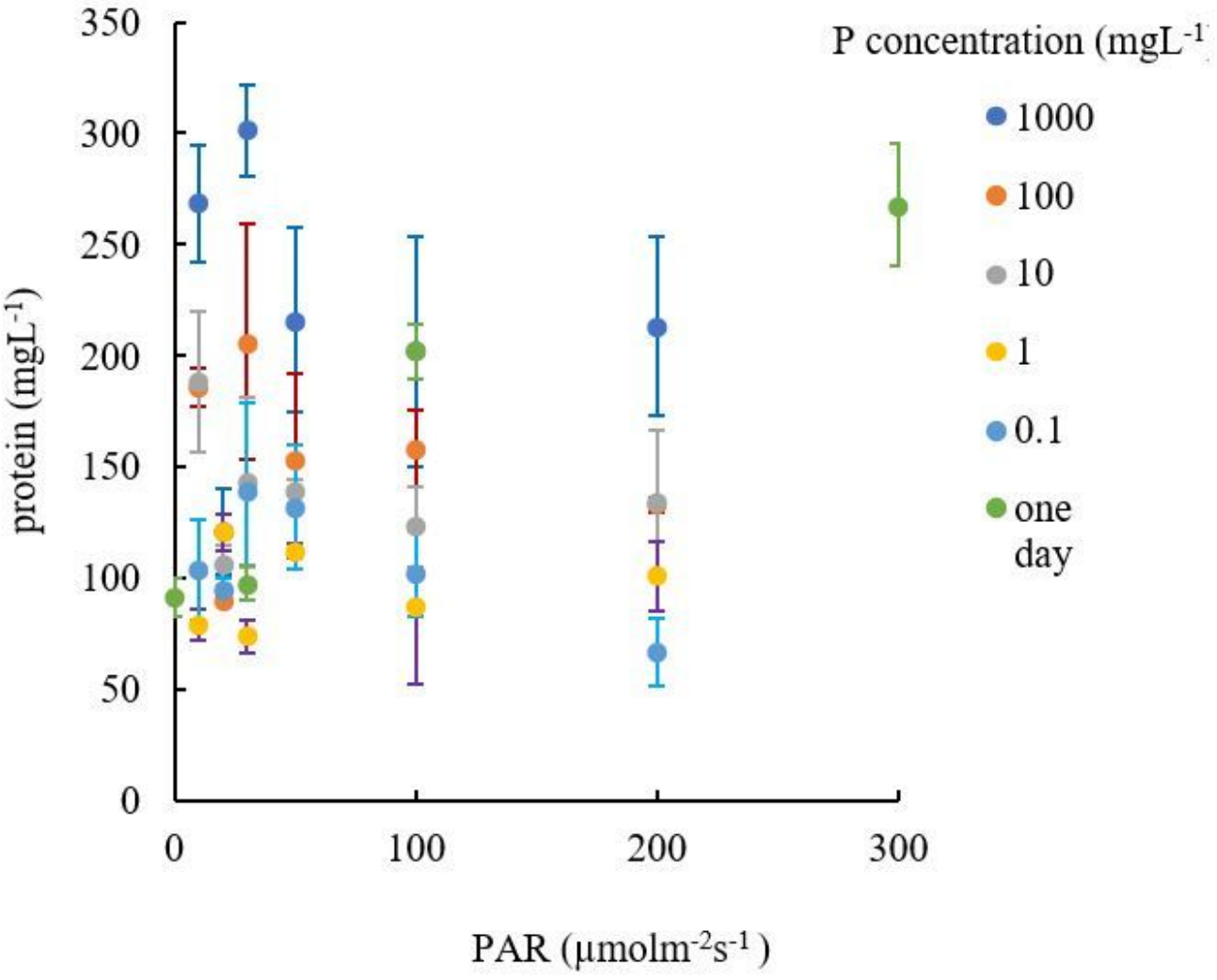

Figure 1

Protein content for different PAR intensity levels and for each phosphorus concentration level (mgL-1). Vertical bars indicate standard deviation.

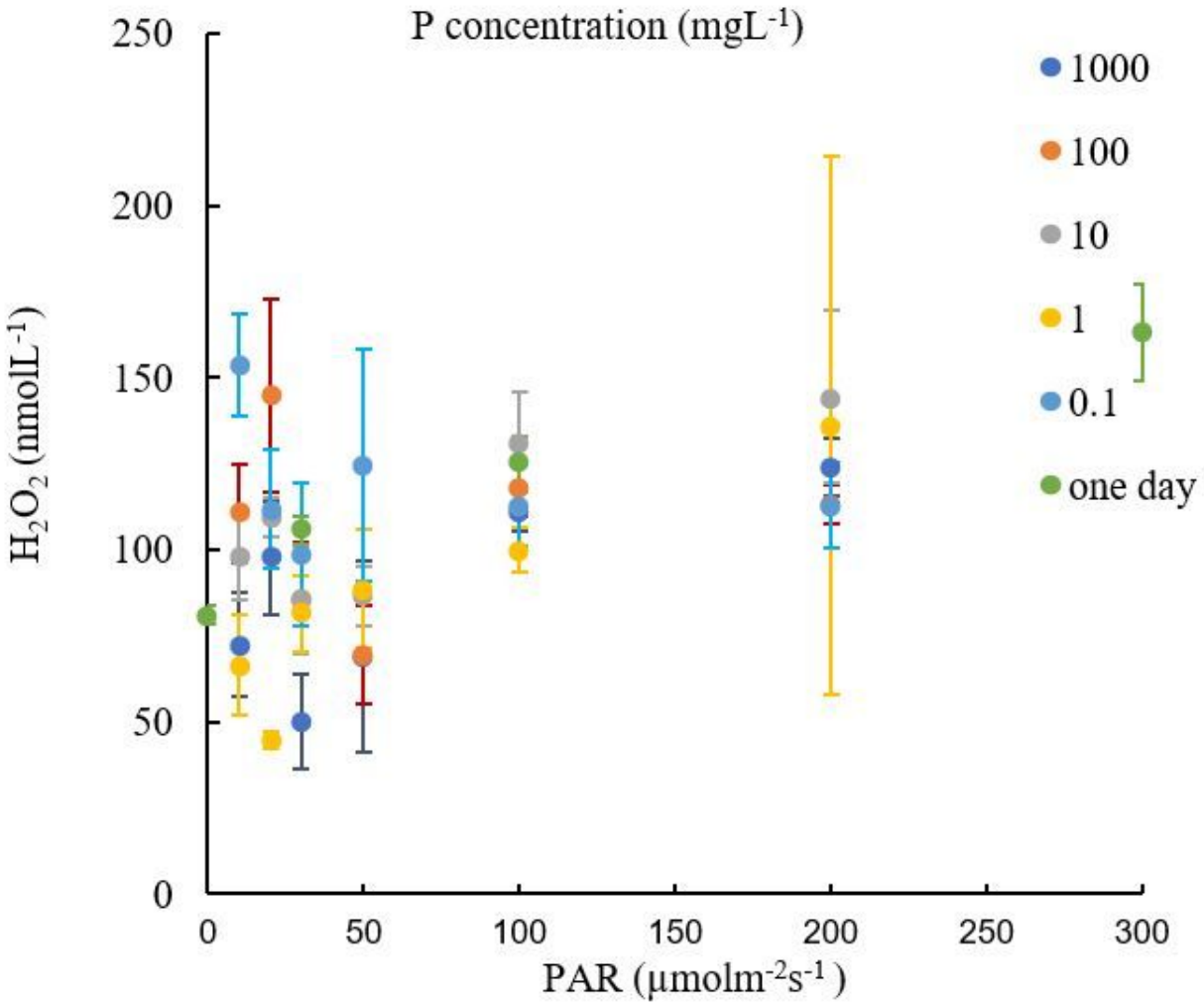

Figure 2

H2O2 concentration for different PAR intensity levels and for each phosphorus concentration level (mgL-1). Vertical bars indicate standard deviation.

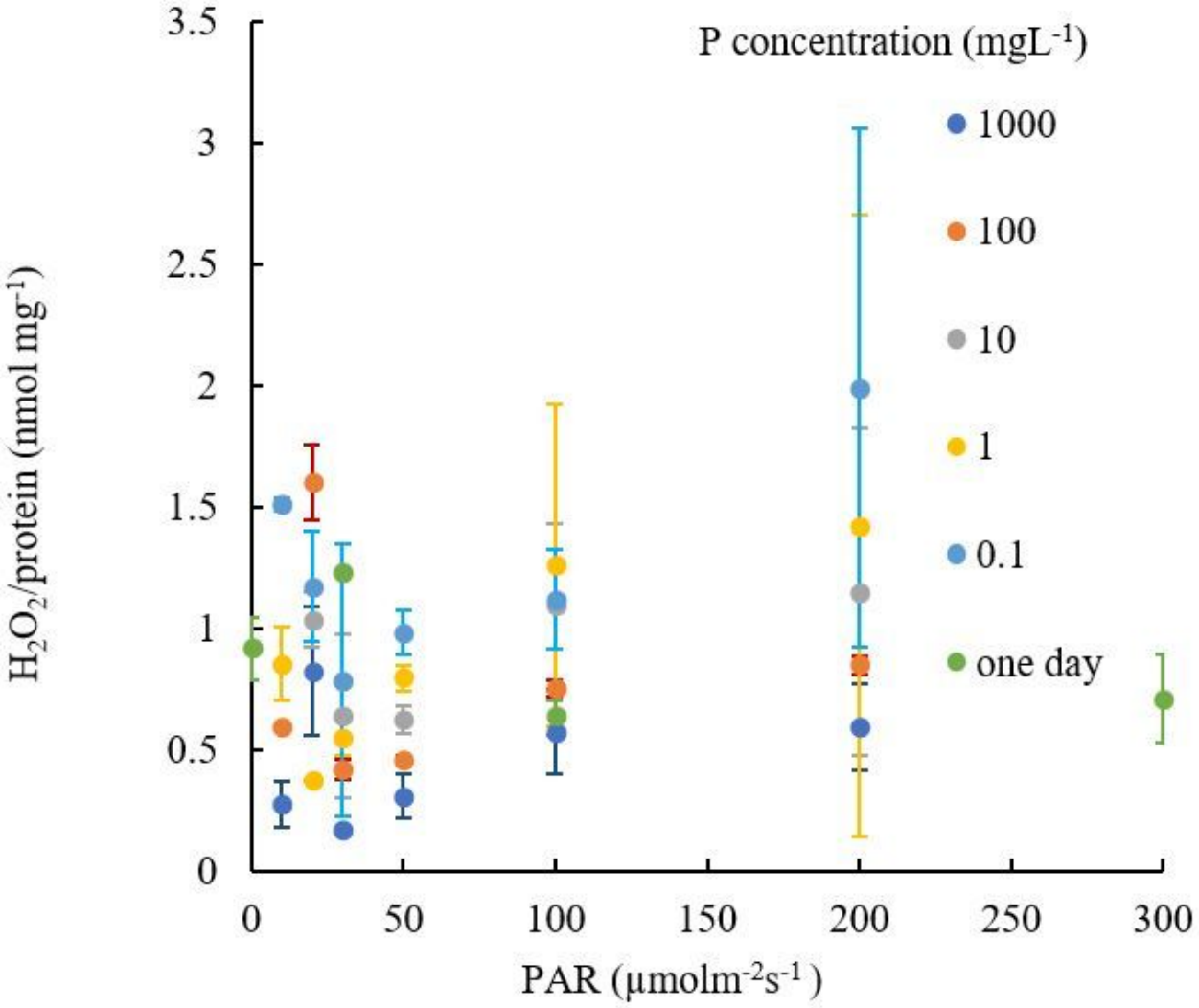

Figure 3

H2O2 content per protein for different PAR intensity levels and for each phosphorus concentration level (mgL-1). Vertical bars indicate standard deviation.

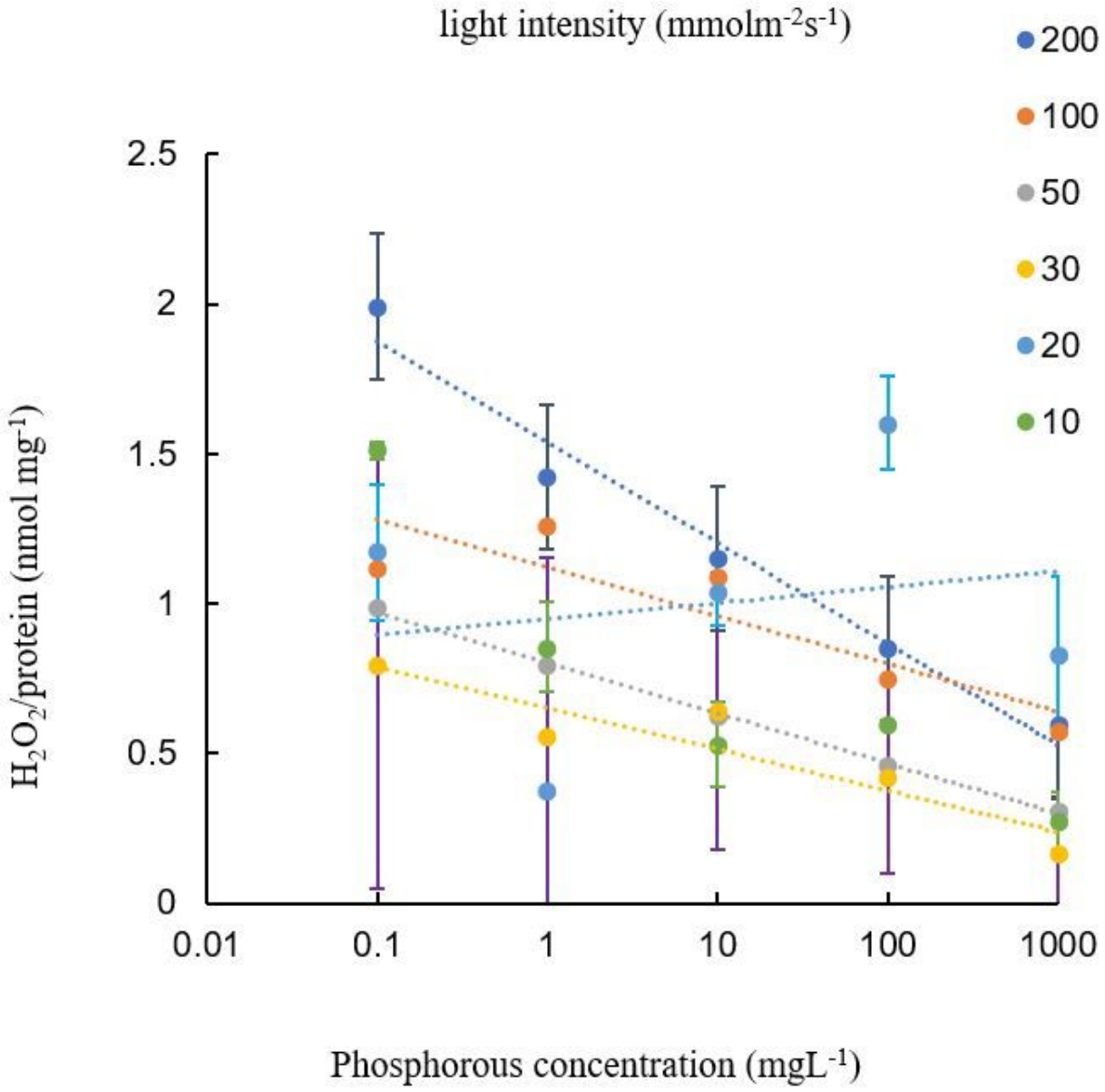

Figure 4

H2O2 content per protein for different phosphorus concentration level (mgL-1) and for each PAR intensity level (µmolm-2s-1). Vertical bars indicate standard deviation. Dotted lines show the approximate relation for each light intensity.

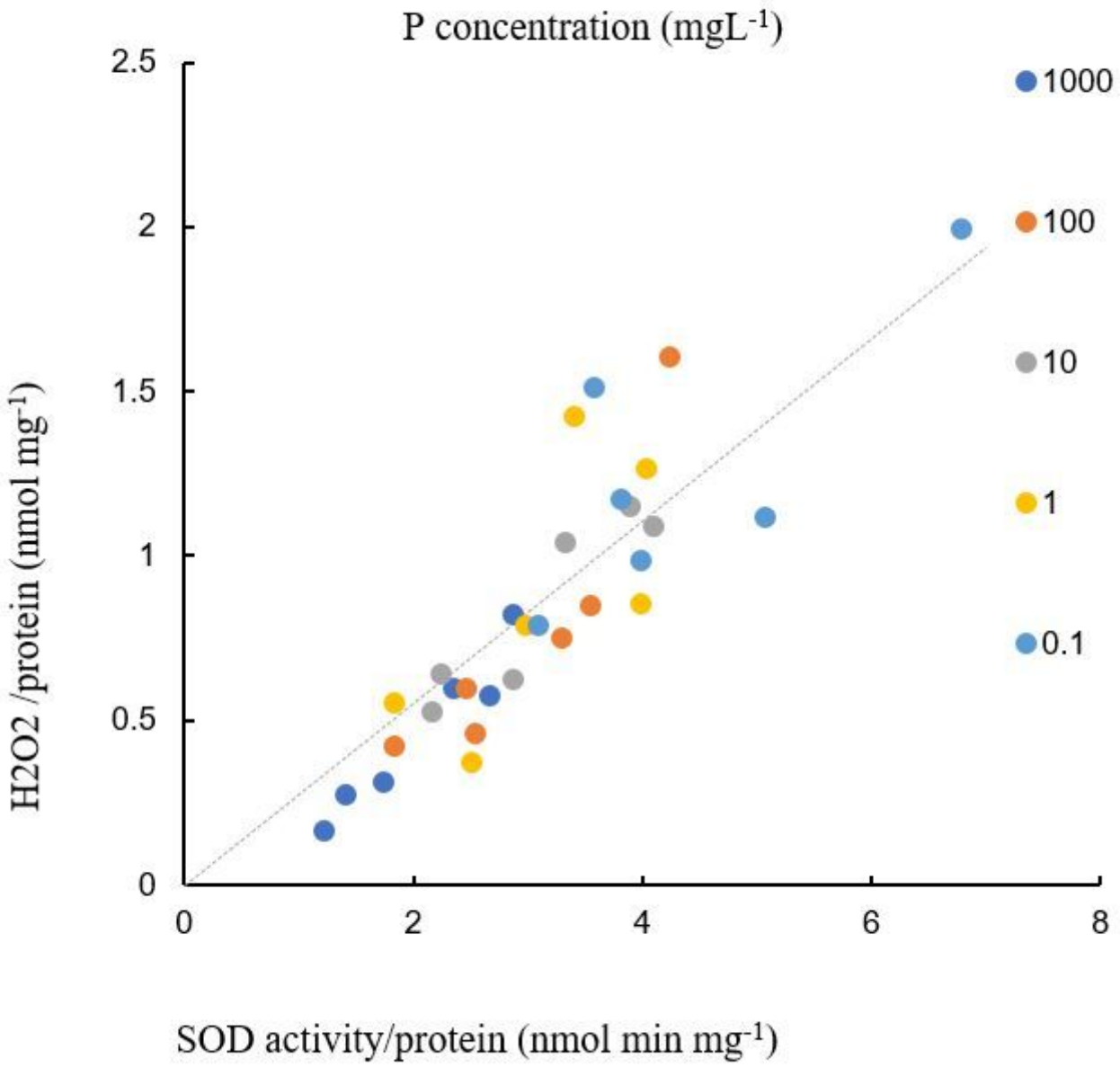

Figure 5

SOD activity per protein for different phosphorus concentration level (mgL-1) and for each PAR intensity level (µmolm-2s-1). The approximate relation is shown by the diagonal line.

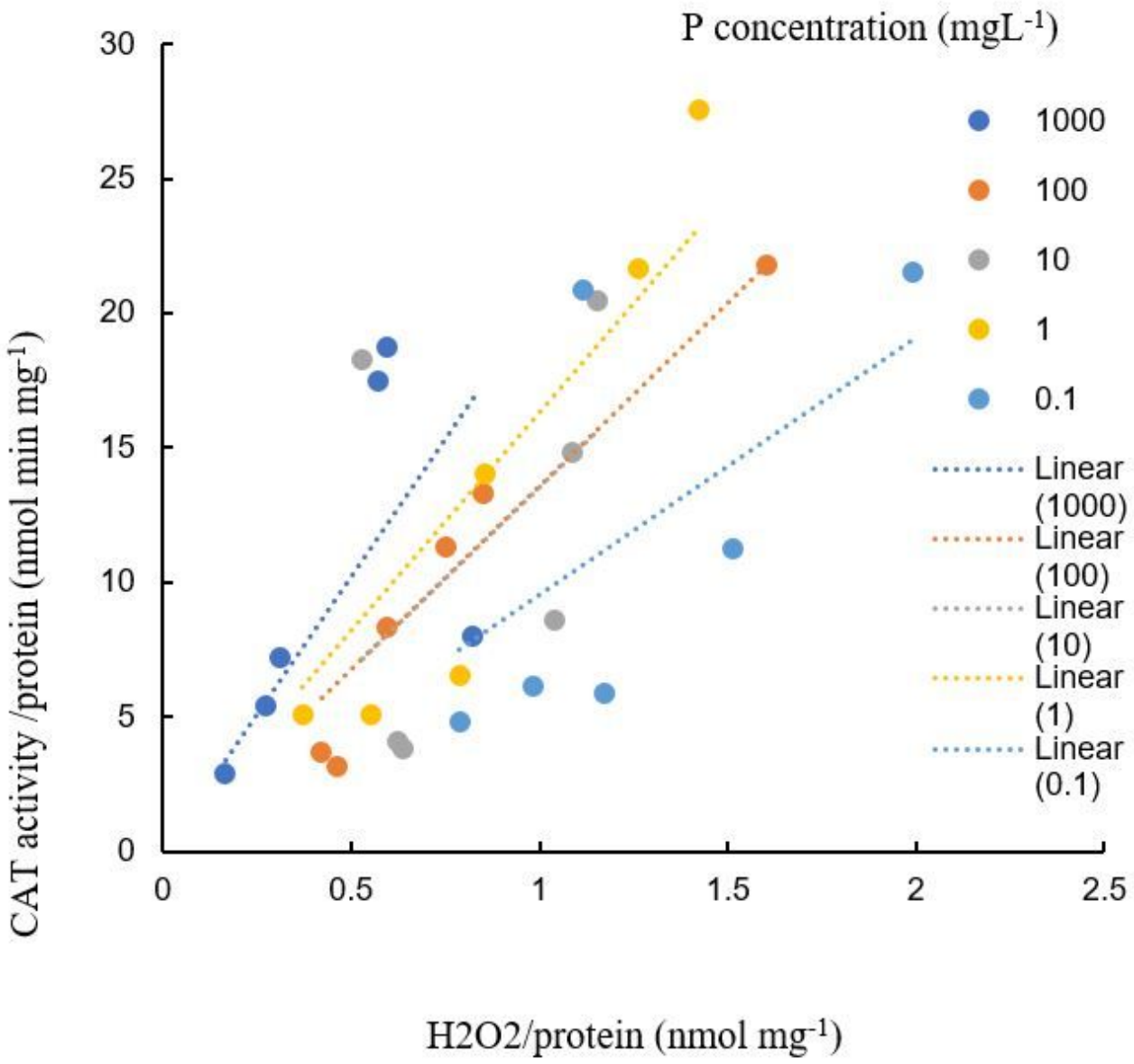

Figure 6

CAT activity per protein for different phosphorus concentration levels (mgL-1) and for each PAR intensity level (µmolm-2s-1). Dotted lines indicate the approximate lines for each phosphorus concentration.

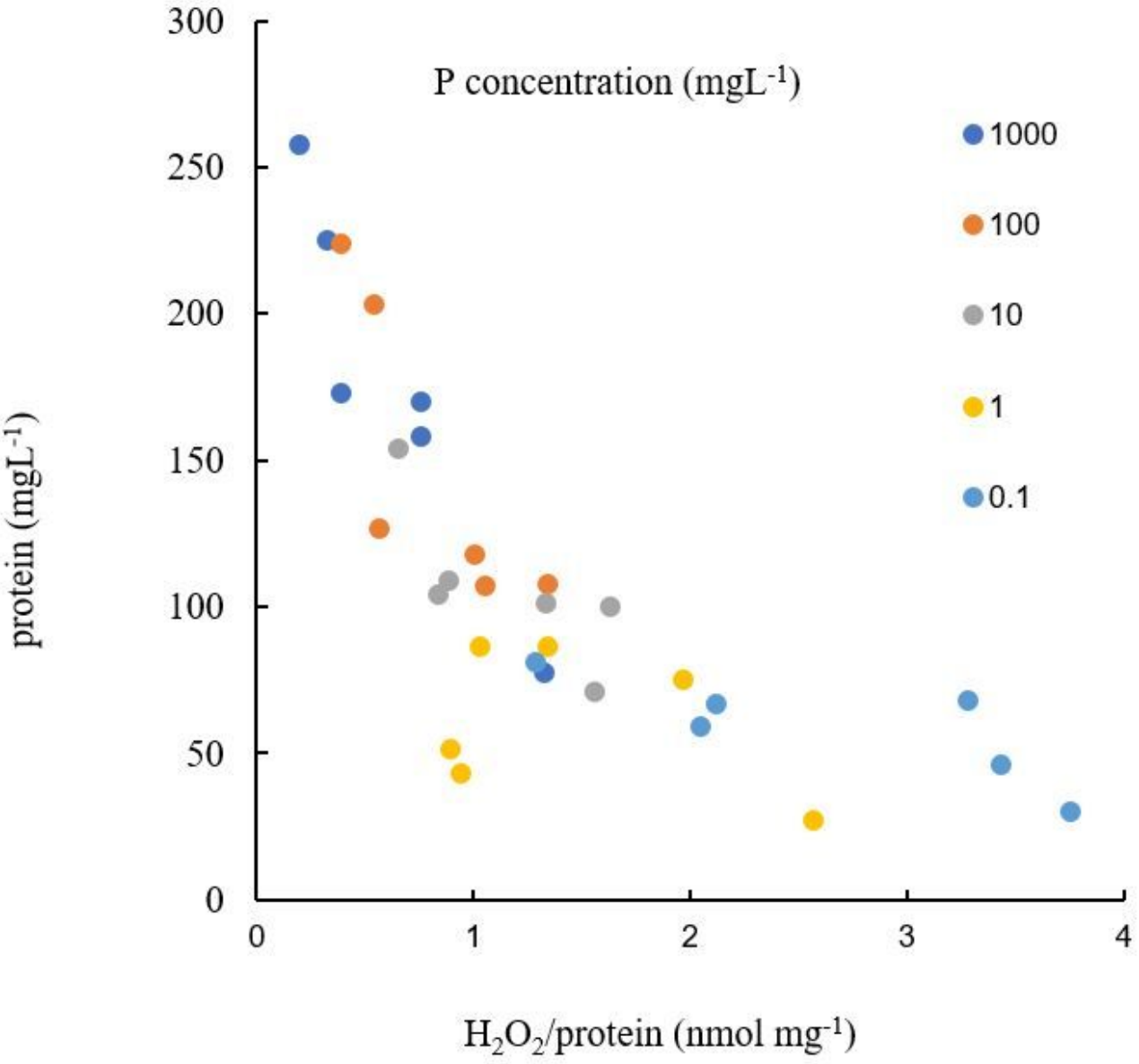

Figure 7

Protein content in water for different phosphorus concentration levels (mg/L) and for each PAR intensity level (µmolm-2s-1)

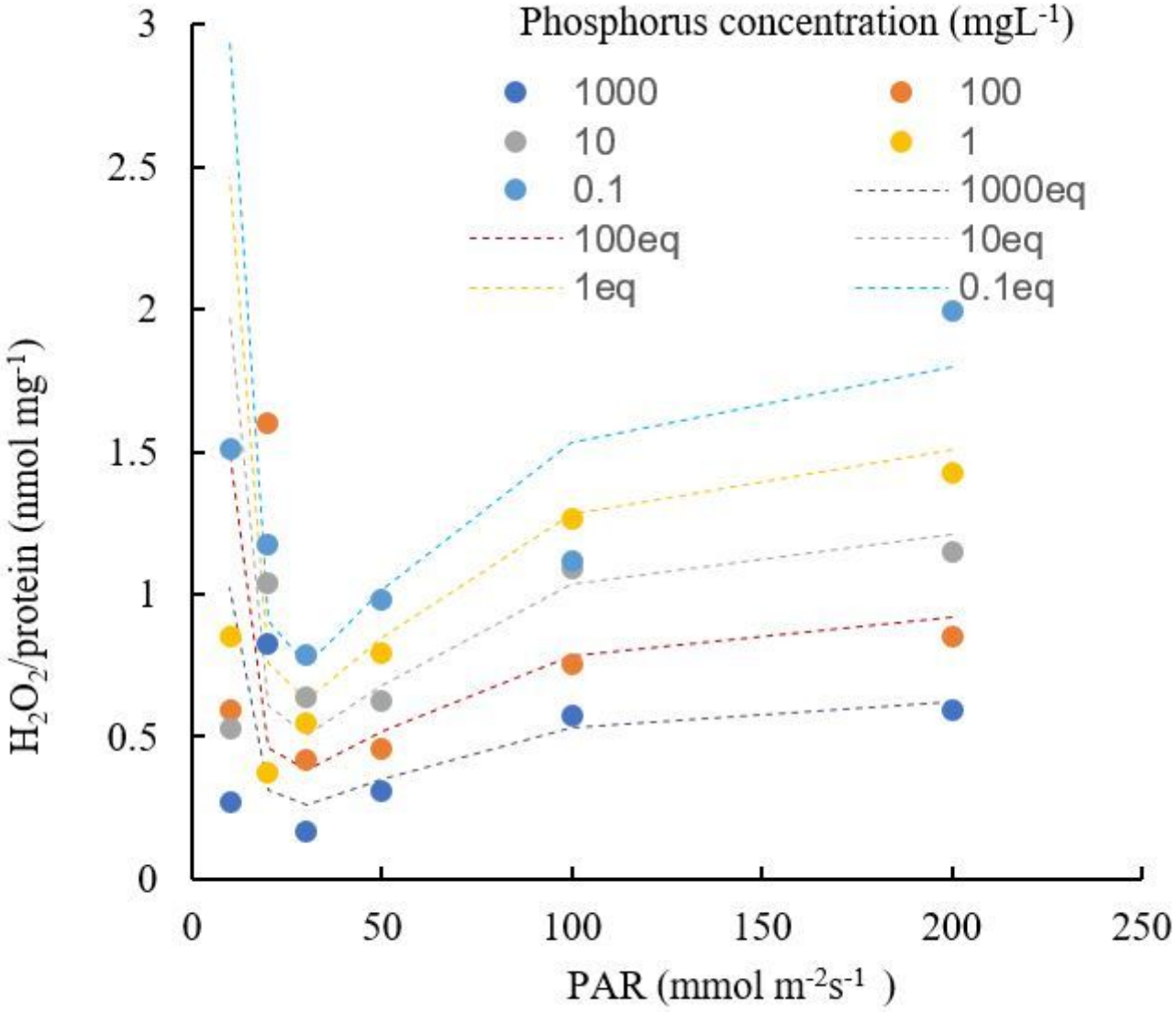

Figure 8

The simulated results of H2O2/protein by equation (1) compared with experimental results.

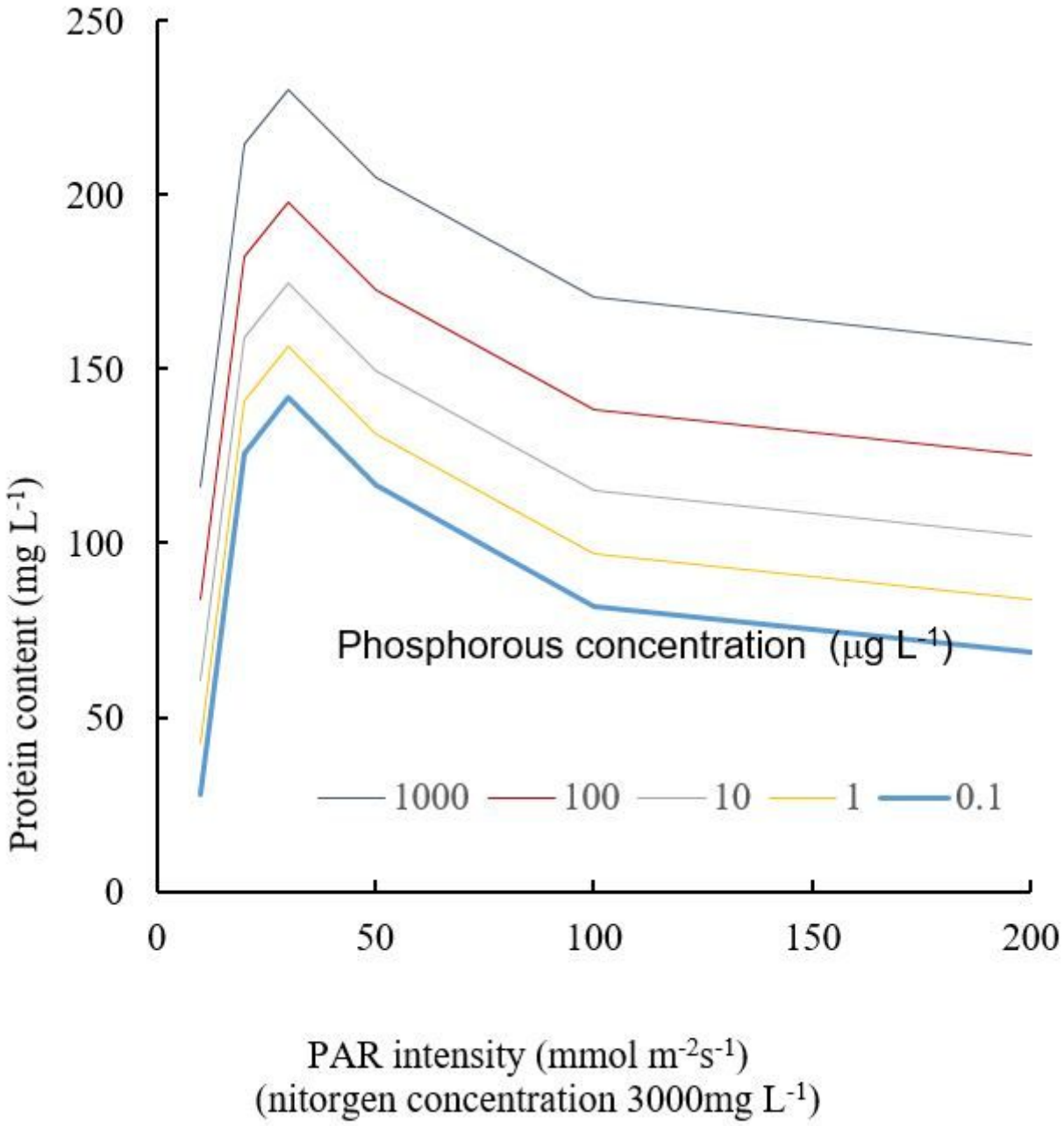

Figure 9

The simulated results of protein content by equation (2)