Evaluation of biological treatment for decreasing water hardness

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ABSTRACT

Water in which the hardness is too high causes scale buildup in household appliances, and is known to decrease the efficiency of soap. This study investigates the effectiveness of biological treatment for water softening as opposed to the conventional method with chemical reagents. The water softening effect of biological treatment, which was prepared by circulating well water in a plastic container containing water tank pebbles for 3 months, was evaluated by introducing sample water containing a high hardness under various light irradiation conditions (continuous light irradiation, continuous shading, and intermittent light irradiation). Water hardness was decreased by 82% following 72 h of light exposure. Additionally, when exposed to intermittent light irradiation, water hardness was decreased markedly only during the light irradiation. The grown algae in the biological treatment tank were identified as *Aphanochaete magnum*, which inhabit widespread freshwater areas, by using 18S rRNA sequencing. Our data demonstrate that raw water can be softened using a biological treatment under various lightning conditions. It shows that the photosynthesis of naturally occurring algae plays a major role in the water softening effect. Our data suggest that biological treatment could be a novel method to soften water rather than the conventional chemical treatment.

Key words | algae, hardness, softening

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INTRODUCTION

Hard water is water that contains large amounts of calcium and magnesium ions. The WHO classifies water based on the amount of dissolved calcium carbonate it contains. Water containing up to 60 mg/L is defined as soft water, 60–120 mg/L is moderately hard water, 120–180 mg/L is hard water, and 180 mg/L and above is termed very hard water. In addition, the recommended human dietary calcium intake is approximately 1,000 mg/day (WHO 2011).

Water in which the hardness is too high causes scale on household appliances and is also known to decrease the effectiveness of detergents and soaps (Sepehr *et al.* 2014). In industrial operations, the use of very hard water causes calcium cations to react, resulting in the precipitation of $CaCO_3$ along inner pipe walls, which is grounds for concern (Chaussemier *et al.* 2015). Moreover, the ingestion of very hard water induces diarrhea (Sengupta 2013).

In regions where the hardness of the raw water is high, a chemical treatment is used to soften the water by adding NaOH (de Moel *et al.* 2006) or by adding $Ca(OH)_2$ and NaOH (Mahvi *et al.* 2005). The bicarbonate in the raw water reacts, resulting in the precipitation of calcium carbonate. As shown in formula (1), as a chemical reaction, the $CaCO_3$ that is precipitated is then collected, resulting in an overall decrease in raw water hardness:

$$Ca(HCO_3)_2 + NaOH \rightarrow CaCO_3\downarrow + NaHCO_3 + H_2O \quad (1)$$

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Although this method is commonly used as a treatment to soften raw water, reagents such as sodium hydroxide and sulfuric acid must be added to the raw water.

This study aims at using a biological approach, based on algae, to decrease water hardness in place of a chemical treatment. This biological treatment is a procedure that uses the photosynthesis of algae grown in a treatment tank to precipitate the components of raw water that are responsible for its hardness. To date, previous authors have prepared a simple biological filtration apparatus using a 2 L PET bottle. When a sample of hard water was passed through the bottle, the water hardness was decreased (Miyahira *et al.* 2014). In the present study, the potential of using a biological treatment method as the treatment for softening raw water in purification plants was considered. Using a flat container, a biological treatment tank was prepared to investigate the effect on reducing water hardness.

METHODS

Preparation of a biological treatment tank

A plastic container (inner dimensions: 320 mm × 233 mm × 128 mm) and water tank pebbles (each of approximately 5 mm diameter) were used to prepare a biological treatment tank. The container was packed with the pebbles to a height of 6 cm and 5.5 L of well water, collected in Tokyo, Japan. Then the well water circulated at a speed of 0.75 L/min for approximately 3 months (Figure 1). To promote growth of algae in the tank, a water tank heater was installed to maintain a temperature of 25 °C and, moreover, a white LED (maximum of 11,890 lx) was placed on top of the container.

Evaluation of the biological treatment tank for reducing water hardness

An additional 5.5 L of commercial mineral water with high hardness (a water hardness of 304 mg/L displayed on the label) was added to the biological treatment tank prepared in the previous section as the sample water and was made to circulate at a speed of 0.75 L/min. The efficacy in decreasing the water hardness was examined. The experiment was performed under the following conditions.

Subject to continuous light irradiation and continuous shading

A white LED (11,890 lx), attached to the top of the biological treatment tank, provided 72 h of continuous illumination and is defined as the 'continuous light irradiation' condition. Additionally, when the LED was switched off, the biological treatment tank was shaded by wrapping with aluminum foil for 72 hours, which is defined as the 'continuous shading' condition.

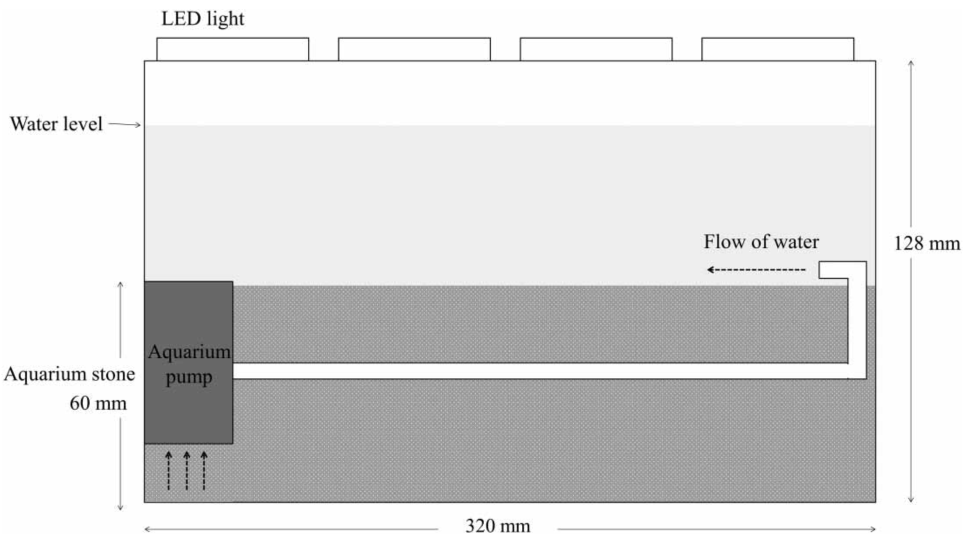

Figure 1 | Overview of the biological treatment tank.

Subject to intermittent light irradiation

For 48 hours the white LED light was switched on and off periodically at 12 h intervals, which is defined as the 'intermittent light irradiation' condition.

During the study, water sampling from the treatment tank was performed every 12 h to measure the hardness and the pH. Since it was aimed at measuring total hardness of the sample water rather than each concentration of ions related to water hardness, chelatometric titration was used in the present study. The water hardness was measured by chelatometric titration using a 0.01 mol/L EDTA standard solution, and the pH was measured using a pH meter (HORIBA, Ltd, Japan).

Identification of the species of algae that grew in the biological treatment tank

The algae species that grew in the biological treatment tank were identified using a sequence in the 18S/16S rRNA region. To extract the template DNA, the sampled algae (1 g) were suspended in a Tris-EDTA buffer and dissolved in distilled water, after which they were washed in isopropanol and 70% ethanol. This was amplified by a PCR using a 18S/16S rRNA as the universal primer (Wang *et al.* 2014) (Table 1) and an Ex *Taq* (TaKaRa-Bio Inc., Japan). Subsequently, a fluorescent-labeled standard reaction was performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific K.K., MA), and the base sequence of the template DNA was analyzed using an Applied Biosystems® Genetic Analyzer (Thermo Fisher Scientific K.K., MA). Homologous analysis by Basic Local Alignment Search Tool (BLAST) using the DNA Data Bank of Japan (DDBJ) was performed on the acquired base sequence. Among the species matched by the BLAST search, the one having the highest homology and the closest morphology was used to represent the identified species.

Table 1 | Primer used for identification of algal sample

Primer	Sequence
1422F	5′-ATAACAGGTCTGTGATGCC-3′
1642R	5′-CGGGCGGTGTGTACAAAGG-3′

Statistical analysis

All the experiments measuring water hardness and pH were performed in triplicate ($n = 3$). The results were shown as mean value ± standard error. In the results, significant differences were analyzed by Tukey's test using Microsoft Excel.

RESULTS

Decrease in the water hardness by the biological treatment

Changes in the water hardness and pH of the sample water under the 72 h of continuous light irradiation condition and continuous shading condition are shown in Figure 2. Under the continuous light irradiation condition, the water hardness decreased with time, and decreased in total by 82% compared with that at 0 h. On the other hand, under the condition of continuous shading, the water hardness decreased by a maximum of 25% from the water hardness at 0 h. The reduction level of water hardness under continuous light irradiation was significantly higher than that of the continuous shading condition ($p < 0.01$) (Figure 2(a)). The pH difference of the sample water increased to 1.7 at 72 h under the continuous light irradiation condition. On the other hand, there was no significant difference between the pH values at 0 h and 72 h under continuous shading (Figure 2(b)).

Changes in water hardness and pH of the sample water under the intermittent light irradiation condition, where stages of irradiation and shading alternated at 12 h intervals (light irradiation conditions during 0–12 h and 24–36 h, and shading conditions during 12–24 h and 36–48 h) are shown in Figure 3. Under the continuous light irradiation condition, the water hardness at 12 h decreased by 39% from that at 0 h, and by 27% at 24 h compared with that at 12 h. Then at the end of the study period (48 h), the water hardness had decreased by 40% compared with that at 24 h. While under the condition of intermittent light irradiation, the water hardness did not decrease with significant differences during the shading periods (12–24 h, and 36–48 h). At 24 h and 48 h of treatment, the water hardness under the continuous light irradiation condition was significantly lower than that under the intermittent light irradiation condition

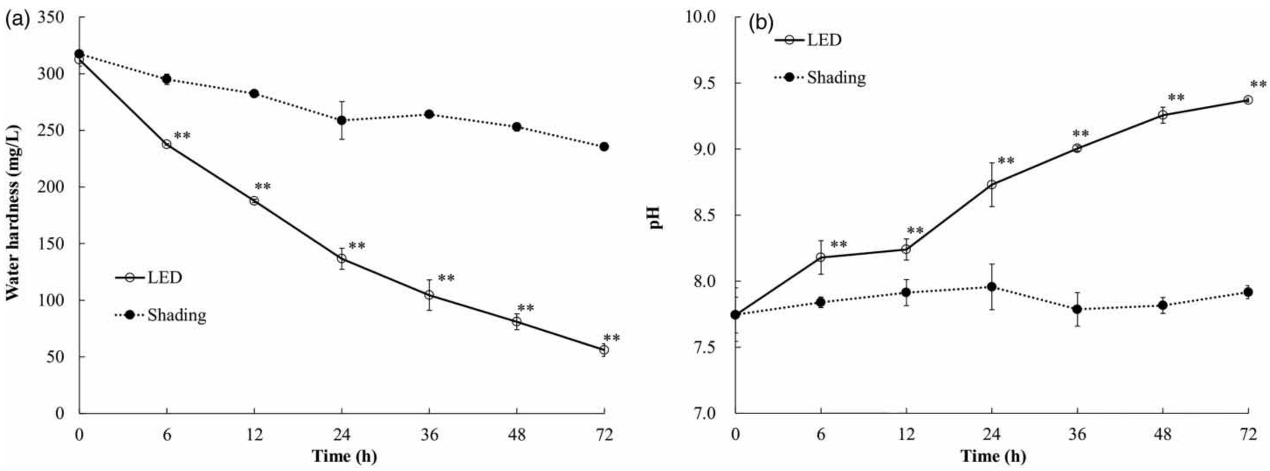

Figure 2 | (a) Changes in the hardness of the sample water under continuous light irradiation for 72 h (○) and continuous shading for 72 h (●); (b) changes in the pH of the sample water under continuous light irradiation for 72 h (○) and continuous shading for 72 h (●). Values indicated by double asterisks showed a *P*-value of <0.01, which were compared between the continuous light irradiation condition and continuous shading condition.

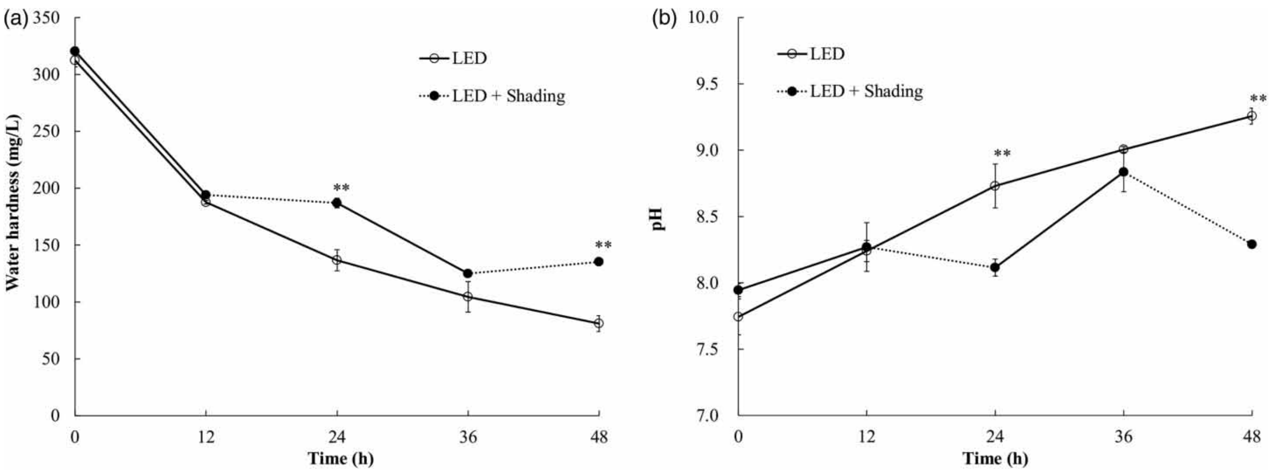

Figure 3 | (a) Changes in the hardness of the sample water under continuous light irradiation for 48 h (○) and intermittent light irradiation for 48 h (●); (b) changes in the pH of the sample water under continuous light irradiation for 48 h (○) and intermittent light irradiation for 48 h (●). Values indicated by double asterisks showed a *P*-value of <0.01, which were compared between the continuous light irradiation condition and intermittent light irradiation condition.

($p < 0.01$) (Figure 3(a)). The pH value of the sample water decreased during 12–24 h and 36–48 h (darkness periods) under intermittent light irradiation (Figure 3(b)).

Species identification of the algae that grew in the biological treatment tank

The algae that grew in the biological treatment tank was sampled and analyzed using the 18S rRNA sequence. As a result, '*Aphanochaete magnum*' was identified with 91% homology.

DISCUSSION

Calcium ions, which are the contributing components to water hardness, can be removed as they precipitate as calcium carbonate, as raw water is alkaline, and in the presence of an oxygen supply (Lu *et al.* 2017). The reaction of calcium ions in raw water, which is triggered by the biological treatment, is shown by the formula below. Algae photosynthesis is accompanied by a consumption of CO_2 on the right side of formula (2). Therefore, the equilibrium shifts to the right, enabling the calcium ions to precipitate as calcium carbonate (Usui & Azuma 1992).

$$Ca^{2+} + 2HCO_3^- \rightleftharpoons CaCO_3 + CO_2 + H_2O \quad (2)$$

In the biological treatment tank used in this study, the hardness of the sample water decreased markedly in the presence of light as compared with shading (Figure 2(a)). Moreover, the pH increased in association with a decrease of water hardness (Figure 2(b)). Although the hardness slightly decreased under the shading condition, the decrease rate of water hardness was smaller than under continuous light irradiation. The reason why the pH value did not increase under continuous shading was because the algae did not photosynthesize and so the CO_2 in the water was not absorbed by the algae. And we considered the reason that the water hardness slightly decreased under continuous shading to be due to the absorption pebbles.

After all the experiments, green algae grown in the biological treatment tank was recovered and weighed after dehydration by centrifuging at 4,000 rpm for 10 min. The wet weight of the algae was 18.2 g. Additionally, the precipitate was analyzed by X-ray diffraction and determined to be calcite ($CaCO_3$) (data not shown). As the algae grown in the tank was not determined to be calcareous algae, it could be considered that calcium ions in the sample water were precipitated as calcite as described in formula (2). From these findings, it could be presumed that the water softening effect of biological treatment was not only due to the absorption of pebbles, but mainly due to the activity of the algae in the biological treatment tank.

Subsequently, under the condition of intermittent light irradiation, the decrease rate of water hardness and the increase rate in the pH of the sample water were obviously low during the shading period (Figure 3(a)). This result demonstrates that under intermittent light irradiation (and even continuous light exposure), the biological treatment decreases the water hardness and increases the pH of raw water (Figure 3(b)). Both Figures 2(a) and 3(a) show that pH increases with a decrease in sample water hardness. These phenomena are consistent with our consideration that photosynthesis of algae could be a primary factor of water softening as mentioned above. The reason why the pH of sample water was lowered during the dark periods was thought to be the discharge of CO_2 by the breathing of green algae due to the stopping of CO_2 absorption from the photosynthesis reaction of the algae.

When the 16S/18S rRNA universal primer was used to identify the algae species that grew in the biological treatment tank, *Aphanochaete magnum* was identified at 91% homology. The genus *Aphanochaete* belongs to green algae that inhabit widespread freshwater areas (Godward 1934). In order to verify the dominant group of the flora in the biological treatment tank, algae grown in the tank were analyzed by next-generation sequencing. As a result, *Chlorophyta* was the dominant group at a 94.7% abundance ratio (data not shown). From these data it was confirmed that phytoplanktonic species dominated in the biological treatment tank. It implies that the water softening effect by algae in the biological treatment could be obtained with specific common green algae inhabiting the extensive freshwater regions.

Previously it has been reported that some phytoplankton species are able to grow in alkaline pH environments (at an average of pH 9.52) (López-Archilla *et al.* 2004). *Aphanochaete magnum* was found in an alkaline pH environment; shallow ponds at pH of 6.7 to 8.5 (Godward 1934). Therefore, it is considered that the water hardness of the sample water decreased continuously since *Aphanochaete magnum* can grow at alkaline pH.

It has been reported that algae precipitate dissolved calcium in environmental water as $CaCO_3$ (Stabel 1986; Santomauro *et al.* 2012). These reports showed that calcite was precipitated on the bottom of the lake, and the pH was increased by the photosynthesis of algae. The obtained data in this study were consistent with that of these reports. However, there have been no reports regarding applying this phenomenon for water softening of drinking water. This is the first report to suggest that biological treatment can be a novel method to soften drinking water.

CONCLUSIONS

This study demonstrates that a simply prepared biological treatment tank can be used to soften raw water. The data obtained highlight that the water softening effect was mainly due to the photosynthesis of green algae that grew naturally in the biological treatment tank. Although it has been reported that calcite precipitates in environmental water by this reaction which can be called a natural phenomenon, the present study showed it can be used for water softening of drinking water. It suggests that this biological treatment is a cost-effective and highly useful water softening method.

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