*Research article*

# Evaluating Water Purification Capacity of *Patinopecten yessoensis* Shells Inoculated with Effective Microorganisms

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**Abstract** In Japan, the installation of biotopes, defined as aquatic systems aiming to recreate a natural environment, has been popular since the 20th century. Due to the closed character of many biotopes and high nutrient inputs, one of the main issues related to water quality in a biotope is the occurrence of blue-green algae. In this environment, reduction and control of the blue-green algae cannot be done by chemicals. Water quality must be maintained by other non-chemical means. Therefore, this study aims to investigate the effectiveness of *patinopecten yessoensis* shells inoculated with effective microorganisms as a method for nutrient and water quality control in a biotope environment. In Japan, huge numbers of *patinopecten yessoensis* shells are left out in the open because they cannot be properly handled. At the same time, *patinopecten yessoensis* shells are porous and have been studied for water purification. Accordingly, this research has focused on the water purification capacity of *patinopecten yessoensis* shells inoculated with effective microorganisms. In the study experiment, *patinopecten yessoensis* shells inoculated with *lactobacillus, bacillus subtilis var. natto,* and *saccharomyces cerevisiae* were used to purify water samples taken from a pond in Yatoyama Park in Kanagawa Prefecture, Japan. To compare nitrogen removal capacity, the experiments consisted of 8 treatments in total. In the first group of four treatments, *patinopecten yessoensis* shells were burned at 600 degrees to increase pore size and distribution, and were then inoculated with the aforementioned microorganisms. In the second group of four treatments, *patinopecten yessoensis* shells were not burned, and were only inoculated with the microorganisms. Uninoculated burned and unburned shells were used as control treatments. The experimental results showed total nitrogen removal rates of 42% for unburned and uninoculated shells, 0% for *lactobacillus*-inoculated unburned shells, 45% for *bacillus subtilis var. natto*-inoculated unburned shells, and 58% for *saccharomyces cerevisiae*-inoculated unburned shells. Regarding the burned shells, results showed 51% nitrogen reduction at the uninoculated shells treatment. *Lactobacillus*-inoculated burned shells reduced nitrogen by 47%, *bacillus subtilis var. natto*-inoculated burned shells reduced by 34% and *saccharomyces cerevisiae*-inoculated burned shells reduced the nitrogen in the water sample by 69%. Since this was a short-term experiment, a long-term experiment as well as field-applicable methods need to be discussed in the future.

**Keywords** water purification, microorganism, *patinopecten yessoensis, lactobacillus, bacillus subtilis var. natto, saccharomyces cerevisiae*

## INTRODUCTION

Biotope is a term used to describe a biological space; biotopes attracted attention in Germany in the 1970s when environmental problems arose. In Japan, the expansion of urban areas due to rapid population growth since the period of high economic growth has led to a decrease in farmland and forest land. Accordingly, the habitat and growth environment for living organisms has decreased, and biodiversity has been damaged due to environmental changes, invasions, and pollution. For these reasons, biotopes have begun to be created in many places since the end of the 20th century. However, since biotopes are closed water systems with high nutrient inflow, the occurrence of blue-green algae has become one of the problems. The use of chemicals is an effective means to suppress and control the occurrence of blue-green algae. However, the use of chemicals in biotopes with biodiversity can lead to the destruction of biodiversity. Hence, it is necessary to maintain water quality by means other than chemicals.

In Japan, about 300,000 to 600,000 tons of *patinopecten yessoensis* are caught annually, and it is said that "about half of the catch is shells of *patinopecten yessoensis*" (Fishery Waste Disposal Guidelines Revised, Ministry of the Environment, 2020). The *patinopecten yessoensis* shells would normally be properly disposed of at a processing plant, but the huge number of shells is causing a shortage at the plant. As a result, many shells are left out in the open without being properly handled. However, *patinopecten yessoensis* shells are porous and have been studied for water purification. Accordingly, research attention has been focused on the water purification potential of *patinopecten yessoensis* shells inoculated with effective microorganisms.

## OBJECTIVE

The purpose of this study is to investigate the effectiveness of *patinopecten yessoensis* shells inoculated with effective microorganisms as a method of nutrient removal and water quality management in a biotope environment. There has been little discussion of water purification using *patinopecten yessoensis* shells inoculated with effective microorganisms.

The objectives of this study were to investigate the water quality in the biotope, to evaluate the inoculation capacity of microorganisms in *patinopecten yessoensis* shells, and to evaluate and compare the water purification capacity of *patinopecten yessoensis* shells inoculated with effective microorganisms.

## METHODOLOGY

### Field Survey

This study was conducted at 3 biotopes in Yatoyama Park, Zama City, Kanagawa Prefecture, Japan (Site 1 - Site 3). Yatoyama Park has been developed as a natural ecology observation park in a place that retains a satoyama atmosphere. It is a place being rich in biodiversity with fireflies in the summer and waterflow in the fall and winter. However, according to the manager of Yatoyama Park, eutrophication of the biotope and the occurrence of blue-green algae have been confirmed. There are concerns about the impact on people who visit the park and the negative effects on the surrounding environment. Water samples were collected approximately once a month from June 2021 to February 2022, and total nitrogen (TN), total phosphorus (TP), potential hydrogen (pH), electric conductivity (EC), and suspended solid (SS) were measured. The water quality level in the biotope was determined based on the environmental standards listed by the Ministry of the Environment.

### Water Purification Experiment Using *Patinopecten yessoensis* Shells Inoculated with Effective Microorganisms

In the study experiment, discarded *patinopecten yessoensis* shells from Aomori Prefecture, *lactobacillus*, *bacillus subtilis var. natto*, and *saccharomyces cerevisiae* were used. These microorganisms were used because they have been used in water purification and are relatively easy to obtain.

Discarded *patinopecten yessoensis* shells were crushed with a hammer and then divided into two groups, with one group being unburned (U) and the second group being burned at 600 degrees to increase pore size and distribution (B). In addition, the burned shells were rinsed once in water to remove the calcium hydroxide generated after burning.

The unburned and burned shells were inoculated with the above-mentioned microorganisms for 3 days. Uninoculated burned and unburned shells were used as control treatments, in total the experiments consisted of 8 treatments.

After inoculation, the shells were rinsed under running water to wash away the easily detached microorganisms. The inoculation capacity of *patinopecten yessoensis* shells was evaluated by performing a colony count using a mixed culture method for *lactobacillus* and a plate dilution method for *bacillus subtilis var. natto*, and *saccharomyces cerevisiae.*

In addition, to compare the nitrogen removal capacity, *patinopecten yessoensis* shells were immersed in 1 L of water from the biotope at Yatoyama Park for a water purification experiment for 3 days. The ultraviolet absorption spectrophotometric method was used to measure total nitrogen. The details of the 8 treatments are as follows.

**Table 1 Combination of shell processing and microorganisms**

| | | | |
|---|---|---|---|
| Unburned and uninoculated shells | UC | Burned and uninoculated shells | BC |
| *Lactobacillus*-inoculated unburned shells | UL | *Lactobacillus*-inoculated burned shells | BL |
| *Bacillus subtilis var. natto*-inoculated unburned shells | UB | *Bacillus subtilis var. natto*-inoculated burned shells | BB |
| *Saccharomyces cerevisiae*-inoculated unburned shells | US | *Saccharomyces cerevisiae*-inoculated unburned shells | BS |

## RESULTS AND DISCUSSION

### Water Quality In Biotope

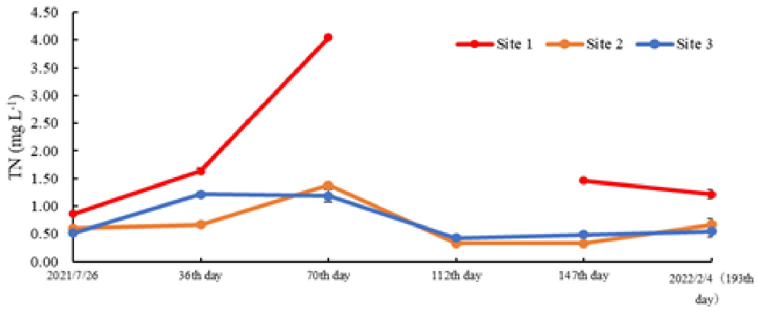


**Fig. 1 Changes in total nitrogen (TN) in the biotope (26 July 2021 to 4 February 2022)**

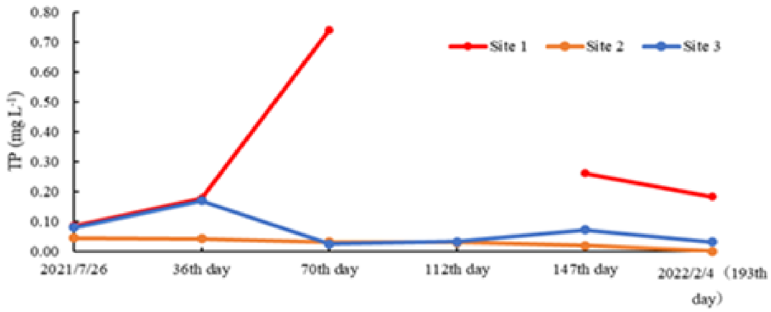


**Fig. 2 Changes in total phosphrus (TP) in the biotope (26 July 2021 to 4 February 2022)**

Figure 1 indicates the change in total nitrogen. The average value of total nitrogen measured at each water sampling site was 1.84 mg $L^{-1}$ at Site 1, 0.66 mg $L^{-1}$ at Site 2, and 0.73 mg $L^{-1}$ at Site 3. The data tended to be higher in summer and fall, which seems to be due to precipitation, fallen leaves, and waterfowl. Figure 2 indicates the change in total phosphorus. The average total phosphorus measurement data for each water sampling site was 0.29 mg $L^{-1}$ for Site 1, 0.03 mg $L^{-1}$ for Site 2 and 0.07 mg $L^{-1}$ for Site 3. As with the total nitrogen data, the data tended to be higher in the summer and fall, which seems to be due to precipitation, fallen leaves, and waterfowl. Also, the reason there is no data for Site 1 on the 112$^{th}$ day is that the water source was buried by sediment, and water sampling was impossible.

In addition, both total nitrogen and total phosphorus tended to be higher at Site 1. This is because Site 1 is closer to the inflow point than other sampling sites, resulting in a greater inflow of sediment. The biotope at Site 1 has been found to have poor water quality compared to the environmental standards set by the Japanese Ministry of the Environment (Table 2). Therefore, there is a need for water purification. The source of nitrogen inflows and their relationship to the surrounding environment, such as waterfowl and vegetation, also need to be analyzed in detail.

**Table 2 Environmental standards for total nitrogen (TN) and phosphorus (TP) in lakes**

| Item type | Standard value | |
|---|---|---|
| | Total nitrogen | Total phosphorus |
| I | 0.1 mg/L or less | 0.005 mg/L or less |
| II | 0.2 mg/L or less | 0.010 mg/L or less |
| III | 0.4 mg/L or less | 0.030 mg/L or less |
| IV | 0.6 mg/L or less | 0.050 mg/L or less |
| V | 1.0 mg/L or less | 0.100 mg/L or less |

*Note: Ministry of the Environment, 2022*

### Microorganism Inoculation Capacity of *Patinopecten yessoensis* Shells

Figure 3 shows the number of microorganisms inoculated on *patinopecten yessoensis* shells. The number of microorganisms inoculated with *lactobacillus* on unburned shells was 2.8.E+05 cfu $g^{-1}$, that with *bacillus subtilis var. natto* on unburned shells was 6.0.E+05 cfu $g^{-1}$, and that with *Saccharmyces cerevisiae* on unburned shells was 2.3.E+07 cfu $g^{-1}$. Also, the number of microorganisms inoculated into the burned shells with *lactobacillus* was 1.9.E+07 cfu $g^{-1}$, that into the burned shells with *bacillus subtilis var. natto* was 2.6.E+05 cfu $g^{-1}$, and that into the burned shells with *Saccharmyces cerevisiae* was 1.6.E+07 cfu $g^{-1}$.

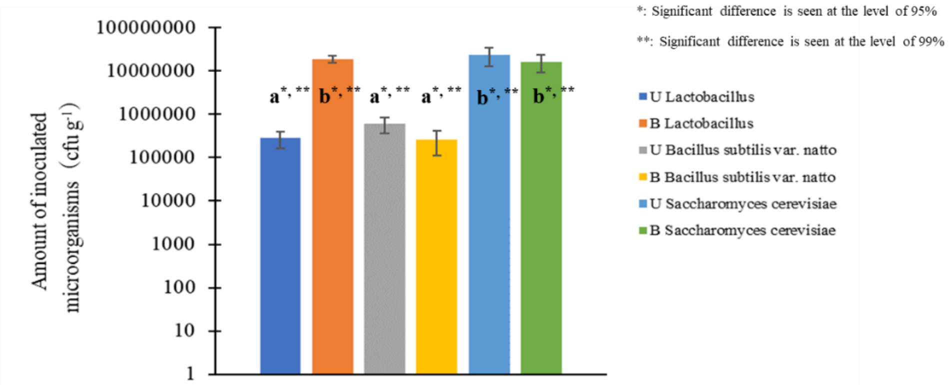


**Fig. 3 Amount of microorganisms inoculated in each shell**

The amount of inoculated *lactobacillus* was increased by burning the shells at 600 degrees. The amount of *bacillus subtilis var. natto* and *Saccharmyces cerevisiae* inoculated was not significantly changed by burned shells. The change in the amount of inoculum seems to be caused by changes in the pore size, specific surface area, and organic matter content of the *patinopecten yessoensis* shells. A more detailed analysis of pore size, distribution, and specific surface area will be necessary to determine the relation between the pore and the number of microorganisms inoculated.

### Nitrogen Removal Capacity of *Patinopecten yessoensis* Shells Inoculated with Effective Microorganisms

The experimental result according to the Fig. 4 showed total nitrogen removal rates of 42% for unburned and uninoculated shells, 0% for *lactobacillus*-inoculated unburned shells, 45% for *bacillus subtilis var. natto*-inoculated unburned shells, 58% for *saccharomyces cerevisiae*-inoculated unburned shells. Regarding the burned shells, results showed 51% nitrogen reduction at the uninoculated shells treatment. *Lactobacillus*-inoculated burned shells reduced nitrogen by 47%, *Bacillus subtilis var. natto*-inoculated burned shells reduced by 34% and *saccharomyces cerevisiae*-inoculated burned shells reduced the nitrogen in the water sample by 69%. The highest total nitrogen removal rate was indicated by the treatment of burned shells inoculated with *saccharomyces cerevisiae*. Watabe et al. (2014) mentioned that *saccharomyces cerevisiae* is ableto incorporate nitrogen into the fungus itself. Hence, its removal capacity seems to be significantly different from that of other microorganisms. The reason that the inoculation of burned shells with *lactobacillus* did not remove total nitrogen seems to be that the rinsing process after the inoculation was not performed properly.

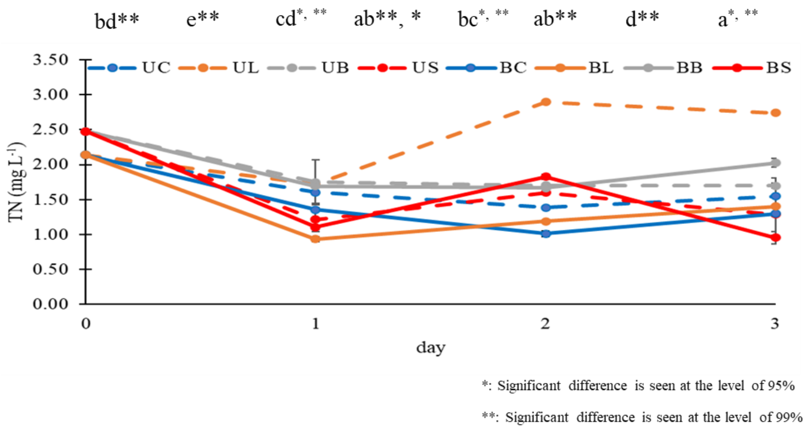


**Fig. 4 Changes in total nitrogen (TN) in water purification experiments**

## CONCLUSION

In this study, the water quality in the biotope was determined, and at the same time, the microorganism inoculation capacity of *patinopecten yessoensis* shells and the water purification ability of *patinopecten yessoensis* shells inoculated with effective microorganisms were evaluated.

The water quality of the biotope at the subject site was found to be inadequate and in need of water purification. It is necessary to conduct a more detailed survey because of the influence of the surrounding environment, including fallen leaves, precipitation, waterfowl, and vegetation.

The microorganism inoculation capacity of *patinopecten yessoensis* shells was evaluated to a certain capacity for all microorganisms. Among them, *Saccharmyces cerevisiae* was found to have a high inoculation capacity. It is assumed that the size of the *Saccharmyces cerevisiae* was suitable with the internal pore size and specific surface area of the *patinopecten yessoensis* shells. In the future, it is necessary to study the relationship between microorganism inoculation capacity and pore size, and specific surface area.

The water purification ability of *patinopecten yessoensis* shells inoculated with effective microorganisms was evaluated for total nitrogen removal except for *lactobacillus*-inoculated unburned shells. The highest removal capacity was observed in burned shells inoculated with *Saccharmyces cerevisiae*. This was a short-term and small-scale experiment. Future experiments should be conducted to consider long-term and field applicability for the reuse of *patinopecten yessoensis* shells.

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