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Contents

Activity for SDGs and SciLets at Mie University

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Abstract: In the present work, we show the activity of Sustainable Development Goals (SDGs) and scientific, local and environmental 'talented staff'; in short, SciLets with the University Impact Rankings supplied by The Times Higher Education and the United Nations Academic Impact (UNAI). For SDG 12: responsible consumption and production, the world and Japan ranking of Mie University was 31th and top on 2019.

Key words: SDGs, University Impact Rankings, SciLets, United Nations Academic Impact (UNAI)

1. Sustainable Development Goals (SDGs)

In adopting the 2030 Agenda for Sustainable Development, world leaders resolved to free humanity from poverty, secure a healthy planet for future generations, and build peaceful, inclusive societies as a foundation for ensuring lives of dignity for all. This collective journey has at its heart a promise to leave no one behind. The 2030 Agenda is deliberately ambitious and transformational, with a set of 17 integrated and indivisible Sustainable Development Goals and targets to guide us. Crucially, it is a universal agenda, applying to all countries; even the richest have yet to fully ensure women's rights, conquer inequality or safeguard the environment. The 17 sustainable development goals to transform our world:

Goal 1: No poverty
Goal 2: Zero hunger
Goal 3: Good health and well-being
Goal 4: Quality education
Goal 5: Gender equality
Goal 6: Clean water and sanitation
Goal 7: Affordable and clean energy
Goal 8: Decent work and economic growth
Goal 9: Industry, innovation and infrastructure
Goal 10: Reduced inequality
Goal 11: Sustainable cities and communities
Goal 12: Responsible consumption and production
Goal 13: Climate action
Goal 14: Life below water
Goal 15: Life on land
Goal 16: Peace and justice strong institutions
Goal 17: Partnerships to achieve the goal

2. University Impact Rankings 2019

The Times Higher Education University Impact Rankings are the only global performance tables that assess universities against the United Nations' Sustainable Development Goals (SDGs). The Times Higher Education uses carefully calibrated indicators to provide comprehensive and balanced comparisons across three broad areas: research, outreach, and stewardship.

Which SDGs are included? There are 17 UN SDGs and the Times Higher Education are evaluating university performance on 11 of them in our first edition of the ranking (click on a category below to view its specific methodology): SDG 3 – Good health and well-being, SDG 4 – Quality education, SDG 5 – Gender equality, SDG 8 – Decent work and economic growth, SDG 9 – Industry, innovation, and infrastructure, SDG 10 – Reduced inequalities, SDG 11 – Sustainable cities and communities, SDG 12 – Responsible consumption and production, SDG 13 – Climate action, SDG 16 – Peace, justice and strong institutions, SDG 17 – Partnerships for the goals. Universities can submit data on as many of these SDGs as they are able. Each SDG has a series of metrics that are used to evaluate the performance of the university in that SDG. Any university that provides data on SDG 17 and at least three other SDGs is included in the overall ranking.

Ranking of Mie University

SDG 3: Good health and well-being
Score 44.5-58.2
World Ranking 201-300
Japan Ranking 19 (in 40 universities)

SDG 4: Quality education
Score 39.3-47.9
World Ranking 201-300
Japan Ranking 5 (in 39 universities)

SDG 5: Gender equality
Score 26.6-42.0
World Ranking 201-300
Japan Ranking 6 (in 28 universities)

SDG 8: Decent work and economic growth
Score 34.6-53.8
World Ranking 101-200
Japan Ranking 7 (in 28 universities)

SDG 9: Industry, innovation and infrastructure
Score 30.3-50.6
World Ranking 101-200
Japan Ranking 8 (in 36 universities)

SDG 10: Reduced inequality
Score 31.1-44.5
World Ranking 101-200
Japan Ranking 2 (in 27 universities)

SDG 11: Sustainable cities and communities
Score 41.1-59.5
World Ranking 101-200
Japan Ranking 4 (in 30 universities)

SDG 12: Responsible consumption and production
Score 70.3
World Ranking 31
Japan Ranking 1 (in 24 universities)

SDG 13: Climate action

Score	60.1
World Ranking	73
Japan Ranking	5 (in 25 universities)

SDG 16: Peace and justice strong institutions

Score	40.8-61.3
World Ranking	101-200
Japan Ranking	9 (in 28 universities)

SDG 17: Partnerships to achieve the goal

Score	1.4-35.8
World Ranking	301+
Japan Ranking	25 (in 43 universities)

3. Scientific, Local and Environmental 'Talented Staff'

Our Mie university has fabricated the new program of human resource development (program name: scientific, local and environmental 'talented staff'; in short, SciLets). The Mie university is required to conserve the regional environment from an academic point of view and contribute to regional vitalization. From April 2017, Mie university started in earnest the operation of the program for the development of Scientific, Local and Environmental 'Talented Staff'. This program aims to promote sustainable regional development by utilizing the experience and structure of environmental work that have been accumulated throughout the whole university, and which have attracted frequent positive external evaluations, as well as promoting the reform and maintenance of education, research, and the social cooperation system. Scientific, Local and Environmental 'Talented Staff' (SciLets) program, which aims to develop, refers to staff who qualifies on the designated course, "regional environmental science field", acquiring skills to engage in specialist environmental jobs in corporations and administrative agencies.

4. United Nations Academic Impact (UNAI)

The United Nations Academic Impact (UNAI) is an initiative that aligns institutions of higher education with the United Nations in supporting and contributing to the realization of United Nations goals and mandates, including the promotion and protection of human rights, access to education, sustainability and conflict resolution. Since 2010, UNAI has created a vibrant and diverse network of students, academics, scientists, researchers, think tanks, institutions of higher education, continuing education and educational associations. There are over 1300 member institutions in more than 130 countries that reach millions of people in the education and research sectors around the world. Since its inception some thirty international networks of universities and other institutes of higher education and research have endorsed UNAI and encouraged their members to join, representing a global diversity of regions and a thematic wealth of disciplines. The work of these institutions is vital to achieving the Sustainable Development Goals as they serve as incubators of new ideas, inventions and solutions to the many global challenges the people face. United Nations Academic Impact provides the integral link to these stakeholders to ensure that the international community harnesses the energy and innovation of young people and the research community in service to humanity. Every subject and discipline can have a UN imprint. UN wants relevant institutions to recognize this link and, often without additional effort or expense, undertake activities that can directly support United Nations mandates and objectives. UNAI assists stakeholders in this regard by disseminating information on UN initiatives and activities, providing ideas on how these activities can be applied at the local level on college campuses, in classrooms and in communities, and by providing a platform where university students, academics and researchers can connect and share ideas, research and resources to further the Sustainable Development Goals and other UN mandates. Mie University could been recognized as UNAI on Jan. 2019.

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Proposal for Sustainable Hotel Accommodation Targeting Millennial Generation

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Abstract: The number of tourists visiting Japan increases each year, and as a consequence, there is a strong demand for hotels. However, tourists may decrease after the Tokyo Olympics in 2020 and the Osaka Expo in 2025, and the possibility of an oversupply of hotels may become an issue. Thus, it would be necessary for hotels to seek new targets as alternative guests that could replace current tourists in Japan. The present study considers domestic residents as new targets for the hotels. In order to motivate domestic millennial generation to stay at the hotels, a new concept of hotel for the potential guests is proposed. This new concept is signified as "Capsule Hotel Open to the Outdoors", and the design of the hotel is based on the concept. Each room of this new type of hotel has a window and will provide workplaces, stores, meeting places for communication, while still ensuring personal privacy. The new hotels would attract young domestic residents, meaning they would not have to rely on international tourists for their business, sustainably contributing to the regional and/or national economy. Also, the scheme would be applicable for related issues in China and other countries.

Key words: inbound, Millennial Generation, hotel, capsule

1. Introduction

Regarding the infrastructures for accommodations, there is currently an increasing demand in Japan because the number of tourists visiting Japan has increased every year, and Japan hosts the Tokyo Olympics in 2020 and the Osaka EXPO in 2025, which would attract even more tourists. The inbound tourists are currently the main guests for the hotels, which greatly contribute to the regional economy. On the other hand, the number of inbound tourists in Japan may decline after the end of the Olympics and other events, although the Japanese government expects it to continue to increase. Considering the number of inbound tourists to countries that hosted past Olympics, the number declined in Australia after Sydney Olympics in 2000, it increased in Greece after Athens Olympics in 2004, it declined in China after Beijing Olympics in 2008, and it increased in England after London Olympics in 2012 (Japan Tourism Agency, 2012). This data shows that the possibility of declining inbound tourists in Japan following the Tokyo Olympics still remains. Consequently, the following oversupply of hotels could occur and negatively impact the economy.

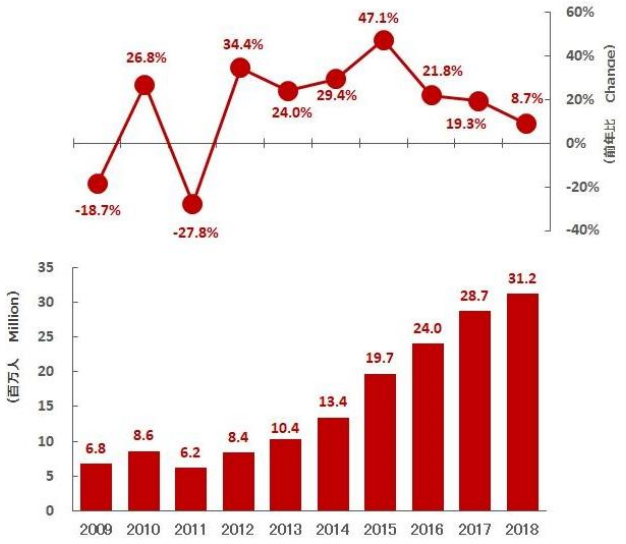

Fig. 1. Visitor Arrivals to Japan for last 10 years (Japan National Tourism Organization. 2018)

While the Japanese government is trying to expand the number of inbound customers, they should alternatively consider the potential of their domestic population as future guests. In fact, the government is also focusing on increasing the number of domestic travelers. Considering the viewpoint of architects, in order to motivate domestic people to stay at a hotel, a new and innovative concept is required, which may drastically change the traditional image of a hotel.

2. Capsule Hotels

2.1 Structure of capsule hotels

The "Capsule Hotel" is a unique type of accommodation developed in Japan. It is called a "capsule" hotel since a guest's personal space is similar to a capsule which contains a bed and typically a small TV attached to the ceiling. The size of an individual capsule is small, just large enough to sleep inside. Usually, two capsules are loaded vertically and they are placed in a row, as shown in the below figures. The open end entrance of a capsule can be closed with a curtain or a screen for privacy, but cannot be locked. Since capsules are unlocked, usually separate lockers located at one location such as the front desk are provided for luggage.

Fig. 2. Capsules

Fig. 3. Inside a capsule

The above are the pictures of the first capsule hotel designed by Kisho Kurokawa, built in Osaka in 1979. Since then, capsule hotels have spread to other cities in Japan. The main guests of capsule hotels have been most likely office workers who have missed the last train or travelers who wish to keep their accommodation cost as low as possible (Lifull Home's Press, 2017).

2.2 Revolutions in capsule hotels

Many traditional capsule hotels are exclusively for men, and thus, women have had limited options finding accommodations at affordable prices. Also, although there are some capsule hotels that allow female guests, women have tended to avoid staying there for the security reason and the unstylish image. Recently, there have been some attempts such as building a new type of capsule hotel whose design is stylish, thus attracting more female guests. For example, some hotels offer the option of "First Cabin", a luxurious capsule based on the image of a first class flight, which is garnering attention (First Cabin Inc., n.d.). Although compact, the ceiling is higher than the conventional capsules, and space is plenty. The price and size of the First Cabin capsules are set between those of business hotels and conventional capsule hotels. Because of this innovative concept, the percentage of female customers is higher than in the other capsule hotels (Nigiwai Kuukan Lab, 2016).

Fig. 4. Capsules of "First Cabin Hotel"

There is another innovative capsule hotel named "9 Hours". They have positioned their service as a "transit service", which is distinct from conventional hotels that function as a base for guests who seek to enjoy sightseeing for a certain period, while also enjoying their experience at the accommodation. 9 Hours hotels are typically located near an airport or a terminal station (9hours Inc., n.d.). They allow guests to rest for a short time, even for only 1 hour, during the guests' transit time. Their capsules are built with the image of a spaceship, and they focused on the basic functions to allow guests to rest and refresh, cut out all the extra facilities and improved the quality of amenities and bedding (Bizpow, 2018).

Fig. 5. Capsules and amenities of "9 Hours Hotel"

The above examples of new styles of capsule hotels such as First Cabin and 9 Hours show that the capsule hotel industry attempts to obtain new types of guests such as women and business people who need a short rest during their work. The current project is proposed along this line of revolutionary design in capsule hotels.

3. Project Design

3.1 Project site

The designed hotel is planned to be located in "Amerika-mura" or *America-Village* in Osaka, Japan. This is an area just to the west of the Shinsaibashi shopping district that has been a well-known center of Japanese youth culture for over 40 years. Within this small area, there are over 2,000 stores selling local and imported fashion items, cafes, restaurants, bars, and clubs. They are centered around Sankaku Park, a triangular concrete plaza and popular local hang-out space, where young people gather to show off their unique styles and discover the latest trends (Osaka Station Guide, n.d.).

3.2 Design outline

Specifically, the new concept is signified as a "Capsule Hotel Open to the Outdoors". While conventional capsule hotels do not provide windows and are dark, with each room being segregated from other spaces, the new hotel in our plan is designed to face the outdoors, receiving sunlight through windows. Also, the hotel is multi-functional in a way that it contains a large space for comfortable communicative, shops, and working spaces for the store staff. In addition, the hotel will be offered as a place for domestic people to tentatively stay or take a nap for a break from work.

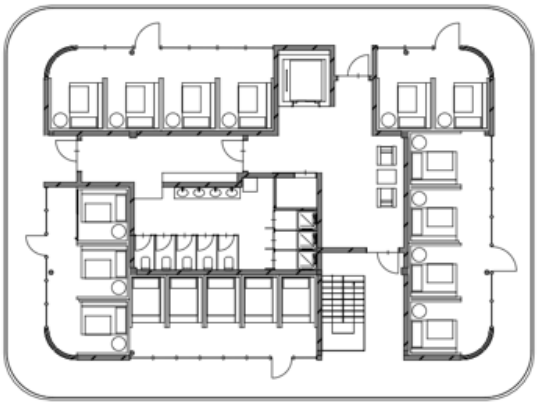

Fig. 6. Floor plan

As this hotel is located in *Amerika-mura*, a center of youth culture, the hotel has a fashion store and a café in it. The store and the co-working space of fashion designers is attached to the café, and the café users can look at the work through the glass wall. As an epicenter of fashion, with café users viewing the process of making clothes with their own eyes, their purchasing motivation would be increased. In addition, providing the fashion store with a space for making clothes at a relatively low price also helps young designers develop their businesses.

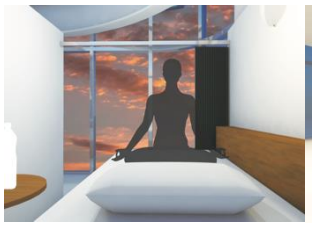

Fig. 7. Inside a capsule

Fig. 8. Working space

There is also a unique trick in the capsule. In a conventional capsule hotel, luggage is usually stored in a locker installed separately from the capsule, but in our plan, a storage space is placed under the bed so that a guest does not have to go back and forth to the separate floor to take out his/her luggage.

An amazing feature of this capsule hotel is that the bed reclines, which makes possible two effects. First, while the sound alarm cannot be used in many traditional capsule hotels because of concern for other guests, the bed can move up with the reclining function at a specified time. Such a method has already been proven to be effective as it is used by the driver of a first train of the day that cannot be late. Second, by reclining the bed, the space created can be efficiently used. By reclining, it provides a space for one to stand and change clothes. As a result, a guest will be able to complete the preparation for going out within their own capsule. From the perspective of the hotel, it does not have to use much space for lockers or changing rooms.

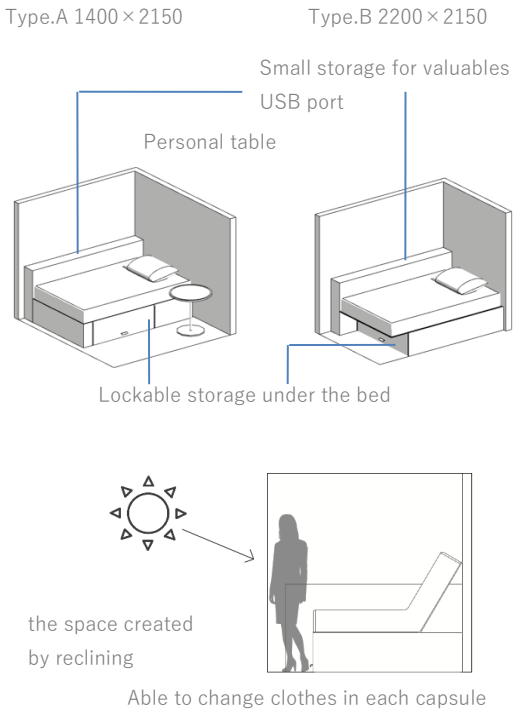

Fig. 9. Capsule types

3.3. Target guests

The current project suggests quite a new and innovative style of capsule hotel. Because this is far from traditional capsule hotels, it is expected that the hotel would be accepted by the people who are flexible in adjusting to something new. For example, young generations called “Millennial Generations” could be targeted as the guest population of the present proposal. The Millennial Generation refers to the people that became adults in the 2000s. They are said to be individualistic, but accepting of the diversity of individuals. In addition, their values regarding consumption are greatly different from previous generations (MarkeZine, 2016).

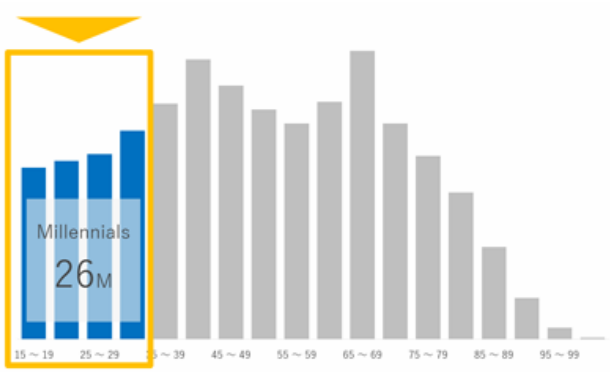

Fig. 10. Population and Household of Japan 2015 (Statistics Bureau of Japan (SBJ), 2015)

People in the Millennial Generation emphasize their private time, and many of them prefer flexible styles of working, such as teleworking. It is not important for Millennials that they get together and work face-to-face in the same place. In contrast, from the viewpoint of the Millennial generation, working space does not have to be the fixed place where all the colleagues stay if the work content can be completed by himself/herself. It seems more valuable for them that each one individually works at a convenient place to him/her without being disturbed by colleagues. Also, communications between the workers would be electrically completed through web meetings or online chats, which could be more efficient as a working style (Find Model, 2019).

As mentioned above, the Millennial Generation accept new values and show interest in new experiences, which implies that they would accept the innovative concept of our project design, which is distinct from that of existing hotels.

4. Conclusions

The new hotel proposed in this project that is based on an innovative concept would attract young domestic residents, by which hotels would not have to rely on tourists for their business, making it possible to sustainably contribute to the regional and/or national economy. Moreover, the new concept would aid in the stable management of hotels. Also, the scheme would be applicable for related issues in China and in other countries.

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Proposal of Facility to Increase Demand for Traditional Arts and Handicrafts Essential to Realize the Recycling Economic Society

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Abstract: This study proposes a facility which encourages people to realize the high quality of traditional arts and handicrafts. The objective of this project is to promote the increased use of environment-friendly materials that are used for traditional art and handicraft works. In order to effectively expose traditional arts and handicrafts to an increased number of people, a multi-purpose building containing a theater, a gallery, and other facilities is proposed. In the building, people would touch the artwork, listen to traditional music, see performance such as dancing arts, and watch drama and movies. Also, this building would be located in the region of Nara, an ancient capital of Japan, for which reason there are many traditional arts and handicrafts already in the region. This region is also a famous sightseeing spot for both Japanese and foreign visitors because it preserves traditional culture. With the presence of this traditional-culture center, it is expected that people would enjoy and appreciate traditional arts and handicrafts, which would consequently promote more use of the environment-friendly materials that are used as traditional materials. This project is proposed as an essential method to encourage the increased use of environment-friendly materials in the world.

Key words: traditional arts and handicrafts, Japanese culture, facility design, sightseeing, sustainability

1. Introduction

The current study proposes an architectural project to spread environment-friendly industry, which accordingly would contribute to the preservation of the environment.

One example of global environmental problems is that of marine pollution. Waste materials such as fishing nets, Styrofoam chips, and empty cans and bottles are in the sea. Among them, plastic amounts to 70-80 percent (Derraik, 2002). Plastic has been produced since 1930, and today its world production is approximately 348 million tons. The problem is that approximately 40% of the products this annual production represents are thrown away, negatively impacting the environment (Hopewell, et al., 2009; Plastics Europe, 2013). Toward a solution to the pollution problem, there has been research that suggests changes of the material and development of biodegradable material (Iwata, 2015).

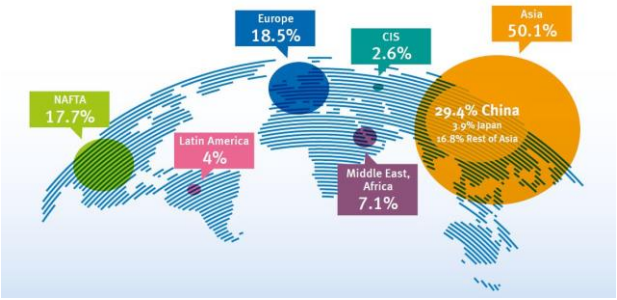

Fig. 1. Distribution of global plastics production (Plastic Europe, 2018)

As shown in the above argument, the preservation of the environment is an important issue in today's world. In this study, the raw natural materials of Japan are focused upon, which are, for example, used in the traditional arts and handicrafts of Japan. The materials are reusable, and they dispose little industrial wastes in the process of production and consumption. They are also highly durable. The environment-friendly traditional arts and handicrafts are a representative industry in the current trend that pursues the preservation of the environment, in order to "live together with nature, use high-quality products for a long time, and stay spiritually rich" (Ministry of Economy, Trade and Industry, 2000, pp.2-3). However, there are problems for the traditional industry in the field of arts and handicrafts, namely the decrease of demand for such traditional products. The severe situation may reflect the increase of new cheaper materials, which accordingly has resulted in fewer craftsmen/craftswomen, i.e., few successors of the techniques or skills. Therefore, there needs to be a plan for traditional works to attract more people.

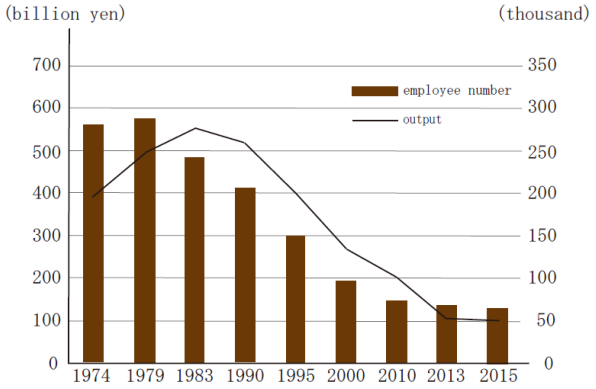

Fig. 2. The transition of employee numbers and output of traditional industry (Shiki no bi, 2019)

In this proposal, a design of a facility is proposed, which would spread the attractiveness of Japanese traditional arts and handicrafts to the world. Specifically, a multi-purpose building is designed, which has a theater, a gallery, and other facilities. In the building, people would look at and touch traditional artwork made of environment-friendly materials. In addition, in order for this building to serve as a cultural center, it offers traditional music, performance such as dancing arts, dramas, and movies. This facility would attract people to traditional arts and handicrafts, which would consequently promote the increased use of environment-friendly traditional materials in the world. Following this Introduction section that describes the general environmental problem and environment-friendly traditional industry, the next chapter explains more about Japanese traditional arts and handicrafts, followed by a review of the case in Europe, namely in Italy. Then the discussion will proceed to the project design that the present study proposes.

2. The Present Situation of Traditional Arts and Handicrafts of Japan

2.1 Attractiveness of the Japanese culture

The recent pop culture including animation and comic books is well known to the world and is very popular among younger

generations. However, there are also a number of traditional Japanese arts and handicrafts that have charmed the world for a number of decades, if not centuries. These representatives of Japanese culture are, for example, *The Tale of Genji* written by Shikibu Murasaki said to be the worlds oldest full -length novel, *Thirty-six Views of Mt. Fuji* drawn by Hokusai Katsushika, and the Arita ware that Sanpei Ri is considered to be the founder of. Moreover, other examples of Japanese culture attracting foreigners include the theatrical arts of Kabuki and Noh, Bunraku, Nihon Buyo dance, and Rakugo comic storytelling. Art accomplishments such as tea ceremony and calligraphy, as well as Shinto shrines, Buddhist temples, gardens, biwa and koto music, and others are also world renowned.

2.2 Animation movie influence on traditional arts and handicrafts

The products that combine animated cartoons known to the world as a new Japanese culture together with traditional arts and handicrafts of the historical proud-hearted Japanese culture have appeared recently. A good example of this is the traditional braided cords of the Iga Region which gathered great attention of young generations. This was because the braided cords appeared in a very popular animation movie released in 2016, *Kimi-no Nawa* or “Your Name" in English translation.

2.3 Overseas advance of Japanese traditional arts and handicrafts

One of the products which recently attracted attention in Japan and the world is "Nanbu Ironware". This is a traditional art and handicraft of the Iwate Prefecture in the Tohoku District. Nanbu colorful teapots gained popularity in Europe and America with 30 colors of red, green, orange, white and others and became a stylish hobby of Westerners to enjoy (Iwashimizu, 2017). Nanbu Ironware is called "Iwachu" as a term of endearment.

Fig. 3. Nanbu colorful teapot

2.4 Materials of the traditional arts and handicrafts

The traditional arts and handicrafts are developed with the principal objective of using natural material to its utmost. This is the main motivation of the current project that would spread this environment-friendly traditional industry to the world. However, it should be noted that, as in the cases of the ivory and tortoise shell (raw material from shell of hawksbill turtle, a kind of sea turtle), the issue of dramatic decrease in population of wild animals presents an alarm that all traditional arts and handicrafts are not necessarily good for the preservation of the environment.

3. Actions for the Italian Traditional Arts and Handicrafts: The case in Italy

3.1 The current state of Italian traditional arts and handicrafts

One of the characteristics of the Italian industry structure is the presence of small and medium sized enterprises. Their products are known for their beautiful design, high quality, and branding, and they play an important role in the Italian export industry. However, they failed to maintain the international competitiveness from 2009 to 2012. In particular, light industrial products such as furniture, bags and clothes drastically decreased in their exports (Miyagi, 2013).

In this situation, the value of the traditional arts and handicrafts that had been inherited for a long time and have supported the people and culture of Italy was re-recognized (Maruya, 2016).

3.2 Arts tourism

An action taken in Italy utilizing traditional arts is art tourism. For example, a trade fair of "Art & Tourism" was held in Florence in 2012. Also, in the Lazio Province (Provincia), which contains Rome, a famous sightseeing city, a total of 36 sightseeing routes have been organized to energize the region. Importantly, the routes focus on their traditional arts and handicrafts as a source to show off the province (Maruya, 2016). In addition to the presence of Rome, Lazio is a place where many tourists gather for its history of Etruria and the Roman Empire, as well as its status as the center of the Catholic Church (Maruya, 2016).

Fig. 4. Distribution of traditional industrial sightseeing routes in Lazio (Maruya, 2016)

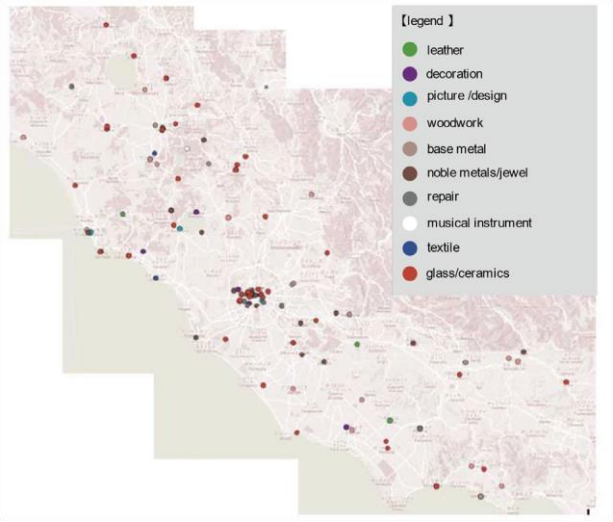

Fig. 5. Locations of the artistic traditional industrial companies (Maruya, 2016)

Regarding the Lazio sightseeing routes, the length of a route is designed to be approximately 40 kilometers in average so that a tourist can go through it in 1 day by car and/or by walking. Each route has a specific theme of culture or nature, and each route consists of several particular places that a tourist is recommended to stop by. The places include historical sites, beautiful nature spots, and traditional-art or handicraft stores.

The 36 routes are arranged for a tourist to cover 6 provinces and the city of Rome (Maruya, 2016).

4. Project Design

4.1 Action in Japan: Current project

The case in Italy shown above is an example of actions that utilize traditional arts and handicrafts for sightseeing. Turning to the current project, Japanese traditional arts and handicrafts are utilized to promote the uses of environment-friendly materials. This project is essentially cultural and architectural, but it may consequently contribute to the preservation of the environment.

4.2 Project site

The project site is located next to Kintetsu Railway Nara Station that tourists frequently use. In this Nara Region, there are famous tourist attractions including Nara Park and Todai-ji Temple, and many tourists visit here every day. Nara City was the capital of Japan from 710 to 784, and the Emperor lived in this city until the capital was moved to Kyoto. In this ancient capital, there are many Shinto Shrines and Buddhist Temples, and the city has more UNESCO World Heritage sites than any other prefecture in Japan. In recent years, the number of foreigners visiting Nara increased rapidly, and the purpose of tourists is mostly to experience the Japanese history and culture.

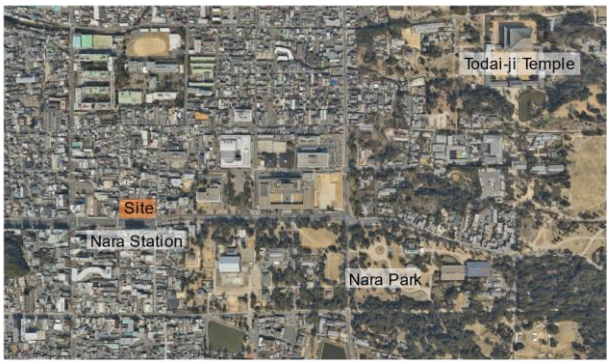

Fig. 6. Project site around Nara Park

4.3 Project concept

In Japan, there is a variety of art culture such as music, drama, dancing, film, animated cartoons, and comic books. Traditional arts and handicrafts of Japan were imported from China or influenced by Chinese culture and were then developed in Japan for a long time . As mentioned, the traditional arts and handicrafts have been developed with the principal objective of using natural material. In order to increase the demand for traditional arts and spread the environment-friendly principle, it is necessary to attract the people of the world to Japanese traditional arts and handicrafts. Because many of these cultural arts remain in Nara, Nara is set as the center of the current project.

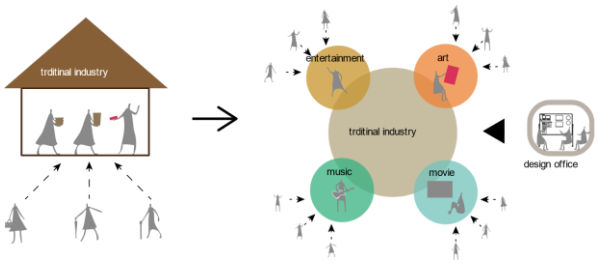

Fig. 7. Concept diagram

4.4 Structure of facilities

This project is planned to establish a cultural center where various facilities are developed within. Facilities consist of a movie theater, a gallery, a hall, a live music club and a bookstore. The shape of the building is based on a Japanese temple-motif, and the characteristic of wooden grids continues from the exterior to the interior. The 1st floor welcomes visitors with the aisle and space where they feel as though being submerged under the latticed ceiling, causing a spiritual uplift from expectations of a new experience to come. As visitors ascend, various Japanese arts are exhibited to entertain them.

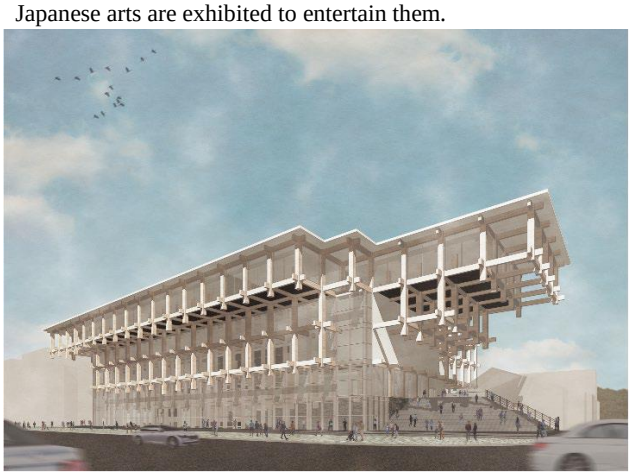

Fig. 8. Image perspective of the building

Fig. 9. Image perspective of interior space

Fig. 10. Image perspective of interior space

4.5 Contents of facilities

In the movie theater, different films from classic ones to the newest ones are shown every day. The gallery exhibits traditional works including those combined with animated comics. Also, this gallery is used for individual or group exhibitions and the product announcement of various fields. All

these traditional arts and handicrafts in the gallery contribute to the people's awareness of the beauty and quality of products made of natural materials. In addition, in the hall, visitors enjoy Japanese traditional entertainments such as Kabuki and Noh drama, Bunraku, Nihon Buyo dance, the court music of Japan and others. The stage can be transformed to Hira-doma, which provides a flat floor in the entire hall, and the rollback type of movable seats allows various types of seating arrangements for many types of performances. At the concert hall, visitors enjoy live music. At the bookstore, it is allowed for visitors to read books at the café space within the bookstore.

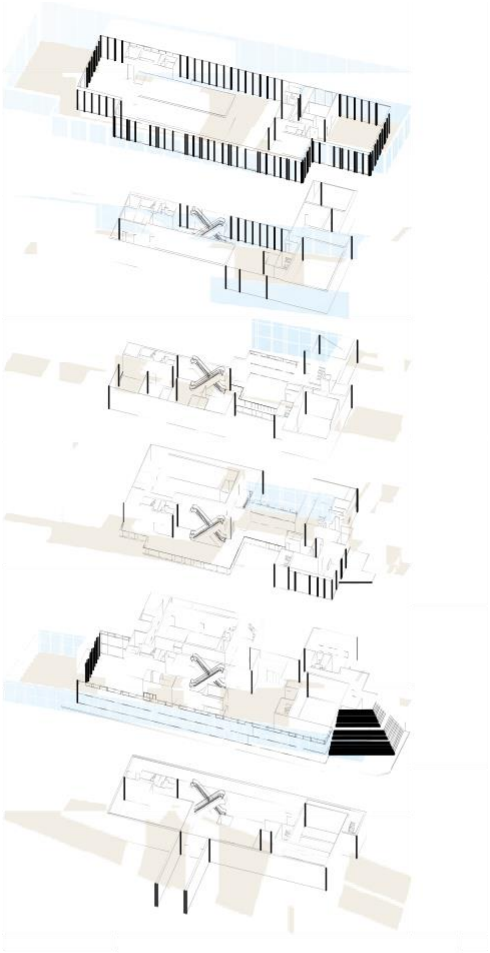

Fig. 11. Axonometry of project design

Fig.12. Image perspective of hall

5. Conclusion

This project suggests an approach that aims to gain people's attention to Japanese traditional arts and handicrafts, and focusing especially on their environmental friendliness.

The aim is accomplished by establishing the traditional-culture center that attracts visitors, which then may promote the increased use of environment-friendly materials. This project may not be a direct contribution to environmental preservation, but it is an architectural proposal, though indirect, for the encouragement of the increased use of natural materials.

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Architectural Proposals for Improving Sleep Deprivation of Business People

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Abstract: In Japan today, one-fifth of working people are suffering from sleep deprivation. The insufficient sleep that is followed by inefficient work results in approximately 135 billion dollars of economic loss in Japan. Sleep is an important factor to maintain mental and physical health, and thus the resolution of sleep deprivation would lead to the improvement of labor productivity and work efficiency. This is quite significant in the current Japanese society with the ongoing decrease in the labor population. In this project, a sleep research laboratory is designed and proposed as a facility to resolve sleep deprivation. This facility is mainly composed of a research building and an accommodation space. The facility is where patients take short-term training sessions, and where physician researchers provide diagnoses and treatments. This architectural proposal would improve people's sleep situation , and accordingly the productivity in the business field would also be improved. Overall, this project is expected to contribute to people's health as well as to society regarding its productivity.

Key words: facility design, healthy, sleep deprivation, sleep research laboratory, accommodation

1. Introduction

Today, it is often claimed in various countries that people work too much, and thus suffer from different medical problems, which would be negatively impacting the society. The objective of the present study is to decrease one of the medical problems, sleep deprivation, and accordingly to increase the productivity of working people. In particular, this project is proposed in the framework of architectural development.

As the aging problem is ongoing in the society of Japan, the number of young and laboring population is decreasing. Accordingly, many working people are experiencing long-time working, and one out of five of them are suffering from sleep deprivation (Ministry of Health, Labour and Welfare, 2014).

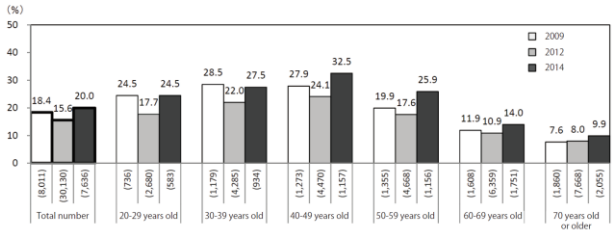

Fig. 1. Japanese sleep deprivation data (Ministry of Health, Labour and Welfare, 2014)

Sleep is closely related to the maintenance of physical health, such as fatigue recovery, obesity prevention, stress relief, and memory consolidation (Nishino, 2017). On the other hand, insufficient sleep increases the risk of obesity, depression, anxiety disorders, and lifestyle-related diseases (Tsutsui, 2008). Also, sleep deprivation accumulatively may cause "micro sleep", which is a momentary doze (Nishino, 2017, pp. 42-45). The micro sleep occurs to a person while the person is unaware that he/she is sleeping, a situation which would be highly problematic if it occurs during an important business meeting, or when driving, as a particularly risky case.

According to previous research, sleep deprivation results in the decrease of productivity, which leads to the economic loss of approximately 135 billion dollars in Japan (RAND Corporation, 2016). Also, an existing study argues that, if labor productivity is increased by 4%, the required manpower is expected to be reduced by approximately 3 million people by 2030 (Persol Research Institute & Chuo University, 2019).

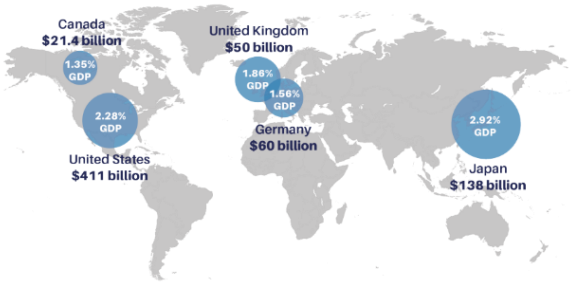

Fig. 2. Economic loss due to insufficient sleep (RAND corporation, 2016)

The above research implies that the resolution of the problem of sleep deprivation would contribute to the increase of productivity. However, while a number of companies promote work-style reformations and recommend naps to workers, few people know how to effectively sleep. Sleep-related problems have been addressed in many Asian countries such as Indonesia, Korea, and Malaysia. This may cause great loss on national scale if these problems are not properly dealt with. As a response to this problem, it is important to increase treatment facilities, to strengthen official institutions, and to train human resources (Okawa, 2012).

In the current study, an architectural approach is proposed in order to decrease sleep deprivation and improve productivity. Specifically, a design for a sleep research laboratory is proposed, which consists of a research space and an accommodation space. In the facility, patients who suffer from sleep deprivation take short-term training sessions, and physician researchers provide diagnoses and treatments. For the treatment, brain waves and apnea are examined when trainees are sleeping. Based on the examinations, advice on sleeping methods and optimal sleeping time-length are suggested to patients. Also, in the accommodation space, an appropriate sleeping environment is provided based on the researchers' instructions. This architectural proposal would improve people's sleep situation, and accordingly the productivity of the business field would also be improved.

2. Purposes of Sleep

The main purposes of sleeping consist of five points, to give rest to the brain and body, to organize and localize memories, to adjust the hormonal balance, to raise immunity to avoid illness, and to remove brain waste (Nishino, 2017, pp. 68-76). Among the above, the most important purpose of sleeping is to give rest to the brain and body.

In the human body, autonomic nerves are always moving unconsciously. The autonomic nerves maintain body temperature, move the heart, facilitate breathing and digestion, and regulate hormones and metabolism. Also, the autonomic nerves include sympathetic and parasympathetic nerves. The sympathetic nerves stimulate the brain and increase its intensity to activate the body. On the other hand, parasympathetic nerves play a role of moderating the movement and respiration of the heart and lungs (Nishino, 2017, pp. 69-71).

Both of these autonomic nerves have important roles, but the problem with busy business people is that they are too frequently in the state in which sympathetic nerves are dominant. Whenever the brain is under tension, fatigue and stress accumulates. If a person's nerves' state does not smoothly switch from the sympathetic to the parasympathetic dominant state at night, he/she will have a problem in falling asleep and the sleep will be shallow. If the autonomic nervous system is unbalanced, there is a risk that basic body functions such as controlling body temperature and intestinal function may be disrupted（Nishino 2017, pp.68-71）.

3. Mechanism of Sleep

While sleeping over a night, one is not always sleeping the same way. A sleep is constituted by different elements such as breathing, brain waves, body positions and postures, etc. that keep changing during a sleep. There are two types of sleep: REM sleep (in which the body is sleeping but the brain is awake) and non-REM sleep (in which both the brain and the body are asleep), and they appear repeatedly during a sleep. REM sleep is the abbreviation of Rapid Eye Movement, in which sleep is shallow. As the dawn approaches, the span of REM sleep continues longer, and the brain gets closer to be awake and the body relaxes. In non-REM sleep, the body moves and one can even turn over although it is a deep sleep. Non-REM sleep gets shallower and shorter as it approaches the morning. Also, growth hormone and other hormones are secreted at this time (Watanabe, & Watanabe, 2002).

As shown in Figure 3 (Watanabe, & Watanabe, 2002), REM sleep and non-REM sleep appear in turn, and it is a general sleep rhythm that non-REM sleep appears deeper and longer at first, and shallower and shorter REM sleep increases before awakening. The appearances of REM and non-REM sleep are repeated about every 90 minutes. When the appearances are repeated 4 to 5 times, one is likely to obtain an appropriate amount and quality of sleep and to comfortably wake up.

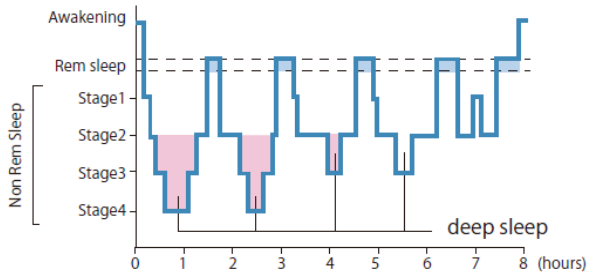

Fig. 3 One night sleep (Watanabe, & Watanabe, 2002)

4. Importance of the First 90 Minutes of Sleep

What needs to be realized in maintaining a good sleep is that it is important to make the first non-REM sleep deep. As shown in the figure 3, if one can gain a deep first non-REM sleep, he/she will be able to gain the smooth cycle in the rest of the sleep, and the autonomic nerves and hormones function well, which leads to his/her good performance the next day. In addition, growth hormone is most abundantly secreted during the first non-REM sleep, and it promotes the growth and normal metabolism of adult cells (Nishino, 2017, pp. 9-11）.

Moreover, it has been confirmed that, while awakening for a long time increases the sleep desire, much of the desire is released/satisfied by the first non-REM sleep (Nishino, 2017, pp. 53-56）. If the quality of this first sleep is enhanced, one will get up with a fresh mind, daytime sleepiness will disappear, and work efficiency will be improved.

In summary, it is important for parasympathetic nerves to be dominant in order to rest the brain and body firmly in the first 90 minutes of a sleep, which is when the deepest non-REM sleep appears.

5. Cognitive Behavioral Therapy

It is important for patients of sleep deprivation to gain sleep knowledge. This is because knowledge about sleep can help serious sleep disorders (Nishino, 2017, pp. 66-68). In Japan and in the United States, about 20-30% of people suffer from chronic insomnia, and sleep medications are the common treatment for such insomnia. Currently there are medicines with few side effects, but the problem is that the dose may gradually increase and a patient might not be able to stop the medication (Nishino, 2017, pp. 66-68). On the other hand, insomnia has a high "placebo effect" (Nishino, 2017, pp. 66-68). For example, a patient may easily fall asleep when the doctor prescribes a tablet made of wheat flour and says to the patient, "this is a very strong sleep inducer." The above fact indicates that sleep is closely related to the brain. Therefore, we have the method called "cognitive behavioral therapy", which treats insomnia without using drugs. The features of cognitive behavioral therapy are as follows (Nishino, 2017, pp. 66-68);

1 Acquire correct knowledge and deep understanding (cognition).
2 Make habits that improve the quality and performance of activities of the next day (behavior).

Without accurate cognitive understanding, people often take wrong behavior. For example, some people drink a lot and take a large amount of alcohol before going to bed in order to release stress and sleep well, but these are typically wrong cognition and behavior. Actually, a large amount of alcohol will make your sleep shallow and deteriorate one's sleep quality. In contrast, with right behavior based on correct cognitive understanding, sleeplessness due to stress will be resolved. It has also been pointed out that it is effective that sleep physician researchers first explain the patient's sleep physiology and then switch to cognitive behavioral therapy (Honda, 2007).

6. What is the Best Sleep Space

Regarding the optimal sleeping environment (bedroom environment), it is important to prepare the most suitable conditions in terms of "light, sound, smell, temperature, and humidity" (Japan Institute of Sleep Science, n.d.). First, the lighting activates one's brain and affects the biological clock. Therefore, when one gets up in the morning and is exposed to the sunlight, the circadian rhythm in the body is adjusted and one can start the day comfortably with a clear mind (Japan Institute of Sleep Science, n.d.). In addition, certain types of scents have an effect of making the parasympathetic nerve

dominant in the body. Among the scents, the cedrol component that is contained in cypress and cedar trees has a relaxing effect. For example, in an experiment, subject people were given a scent of cedrol for a total of 4 hours, that is 2 hours before and 2 hours after bedtime. A finding that is particularly noteworthy in this experiment is that the smell of cedrol shortened the time taken to fall asleep by 45% after entering the bed, as compared to when there was no smell. This is as effective as sleeping pills. Furthermore, it is also mentioned that the frequency of temporary awakening during a night was decreased (Amano, 2010).

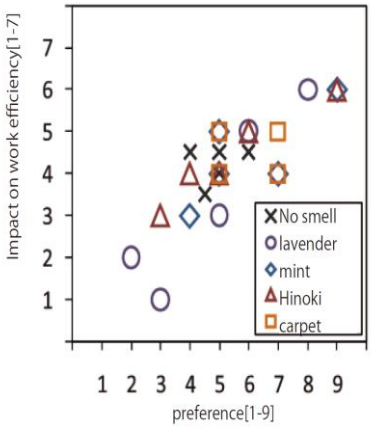

Fig. 4 Preference of Scents and Influence on Work Efficiency (Amano, 2010)

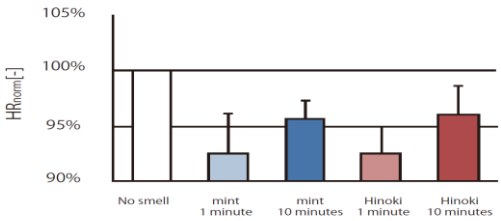

Fig. 5 Electrocardiogram Analysis Result (Heart Rate) by Odor Exposure (Amano, 2010)

Furthermore, sleep is closely related to worker safety, fatigue recovery and health maintenance. Improving sleep deprivation also leads to improved productivity (Takahashi 2014). Thus, the aroma of cedar and cypress that has a relaxing effect appears to be an important element for improving work efficiency (Tsunetsugu, 2016).

7. Sleep Deprivation Treatments in Japan

Research on treatments for the symptoms of sleep problems of different countries allows us to know and compare the awareness of sleep disorders in these countries. According to the international comparison (conducted in 2002) of insomnia patients' behaviors, in Spain, Germany, Brazil, and Belgium, 40-50% of the patients visited medical institutions to receive treatments and used sleep medicines frequently, as shown in Fig. 6 below. In China, 25% received medical treatments, and 35% used sleep medicines. However, in Japan, the rate of those who received medical treatment is less than 10%, and those who used sleep medicine is less than 20%. Instead, 30% drank alcohol to deal with insomnia, which is higher than in other countries. This data indicates that, while more Japanese people suffer from insufficient sleep, there is little awareness for medical treatment and consultation in Japan, compared to in other countries (Okawa, 2012).

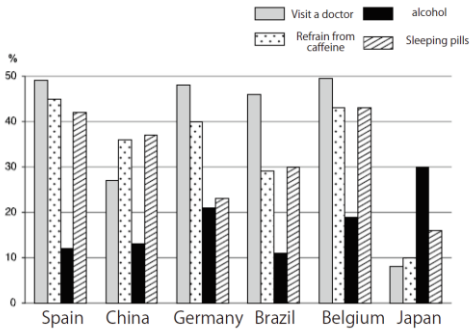

Fig. 6 International Comparison of Behaviors of Insomnia Patients (Okawa, 2012)

8. Project Design

8.1 Project Site

As mentioned in the Introduction section, the objective of this present study is to propose an architectural project in order to resolve the problem of sleep deprivation. Specifically, a facility for deterring or treating workers' sleep deprivation is designed in this study.

The facility is being planned in Shima City, Mie Prefecture. This site is on a complex rias coastline, where the waves are calm, oyster farming is carried out, and Ama, female shell divers, are still present. Shima is also known as the place where the Ise-Shima Summit (the conference by political leaders of seven developed countries and a league of nations, EU) was held in 2016. For these reasons, the tourist industry of Shima is thriving. There is also a general amusement park ("Shima Spain Village") nearby. This area is also used as a sightseeing spot while at the same time being on the Kintetsu Train Line allowing it good access from the metropolises of Osaka and Nagoya. Hotels are also used astraining sites for companies. In cooperation with such companies, the planned facilities will be used for working people who are suffering from sleep deprivation, and training and treatment can be conducted while experiencing the local nature and culture. The above mentioned features are integrated scientifically within facility programs.

7.2 Design Outline

This proposed facility accommodates workers of nearby companies who suffer from trouble sleeping. In the facility, workers receive training for their usual jobs, but during the training, brain waves are measured and data on the workers' brain activations are collected. At the same time, physician researchers create and provide treatments for the workers. In summary, this is the facility where patients can both work and receive treatments.

This facility mainly consists of a research building and an accommodation building. For the arrangement of the whole facility group, the research building was placed near the distribution of natural sound, and at the location one can feel the wind and the natural sound while moving toward the accommodation building. The accommodation wing was placed eastward for a view of the sunrise all year round. As the *Asahi*, the rising sun, works to adjust the circadian rhythm of the body, sun bathing can be carried out immediately after getting up.

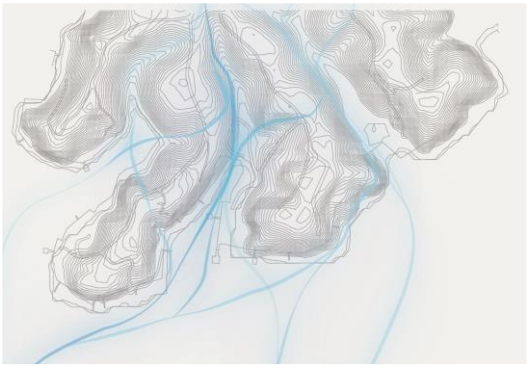

Fig.7 Distribution of wind in the Shima Peninsula

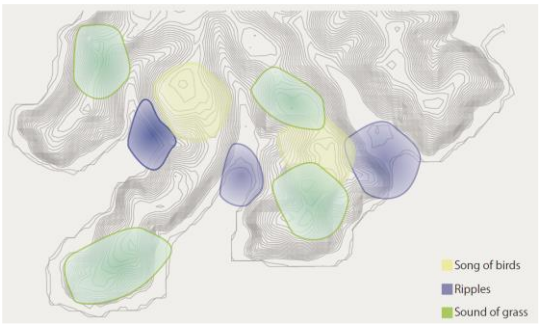

Fig. 8 Distribution of natural sounds in the Shima Peninsula

The research building consists of laboratories for sleep research practices and temporary offices of the trainees. A diagnosis of brain waves and apnea are conducted during the sleep of the trainee, and advice about how to sleep and optimal sleep time are given. On the basis of the advice, through the training and office work during the day, training for relaxation of the sympathetic nervous and work efficiency is carried out. The sleeping environment in the accommodation building is kept appropriate to make the parasympathetic nerve dominant. In addition, the entire accommodation building is made of wood, and the smell of cedrol is there for a more comfortable sleep. Also, stucco or diatomaceous earth and tatami mats are used in the bedrooms. These materials function to control humidity; they absorb moisture in the air when the humidity is high, and exude moisture when it is low. With this mechanism, a comfortable sleeping environment is created.

All the above systems and environments would raise people's awareness of sleep, allow them to learn about sleep, and encourage them to improve their sleep habits. As a result, the facility will decrease the sleepiness of business people and improve their productivity.

9. Conclusion

This paper focused on the problem of sleep deprivation and the consequential economic loss and productivity decline. As a countermeasure, the current project was designed. Since the number of working people suffering from sleep deprivation is increasing while the total population is decreasing, solving sleep deprivation is becoming an important matter. With the help of the development of the facilities such as the one proposed in this study, it is expected that people will be able to work more healthily and efficiently without sleep deprivation and thus productivity is improved.

Grade: 4th year Undergraduate
Major: Architectual planning and design
Field of research: Facility Design for research laboratory and healthy

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The Museum of KAOCHI Valley Proposal

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Abstract: This thesis study explores the relationship between museums and tourism by focusing on Nabari, a small Japanese city with a well-preserved natural environment. This museum proposal aims to attract more tourists to visit Nabari as well as to prevent residents from moving from the city. Nabari city is a small city in Mie Prefecture with a population of approximately 80,000 (Nabari 2019). Since 2000, the city population has been declining and becoming an aging society. While the number of workers in primary and secondary industries is decreasing rapidly, employees in tertiary industries are increasing each year and occupy a large percentage of the city. Tourism starts to play a key role in maintaining and developing Nabari's economy and culture. However, there are not enough cultural, historical and other entertainment-related tourism resources other than nature in the city. This project proposes a design of a contemporary museum in Nabari, located along the Shorenji River in the Kaochi Valley, which will exhibit the history of the Kaochi Valley natural history, local Nabari city culture, and modern arts. In addition to providing a cultural resource for the city, this concept will also provide a free space for visitors and staff as well as spaces for meetings and research. The Museum of Kaochi valley will welcome visitors throughout the year to explore its nature, learn about local culture, and raise awareness for Nabari tourism.

Key words: Museum proposal, tourism, population, nature.

1. Introduction

With many developed countries faced with the problems of population decline and an aging society, tourism is seen as one of important factors to maintaining economic development and population stability. However, typical tourism industries depend on many related factors which are representative of the countries, such as cultural heritage or a significant nature resource, that a general rural area doesn't usually have.

Studies have found that museums are important for tourism industries, since most museums have certain potential to attract visitors, including tourists as well as the ability to accommodate them. Museums offer a unique opportunity for consuming and experiencing cultural heritage without damaging the resource. The key to success is to develop a practical relationship, beneficial to both sectors, based on a solid theoretical ground and understanding. Tourism must develop an awareness of museum concepts and practices, while museums should seek to understand tourism and how it functions. Communication is important since both sectors have much to mediate between each other regarding the theoretical approach as well as the practical solutions. Museum audiences have a strong link to tourism, since tourists are a part of the audiences and for some museums even a large part of the total number of visitors. Therefore, it is important for museums to consider what kind of audience the tourists are and the different relationships they will have with the museum in question [(1)].

In order to make it more feasible to demonstrate the relation between museums and tourism, this thesis proposes a design of a contemporary museum in Nabari, located in Mie Prefecture in Japan, which will focus specifically on audiences who are mostly interested in 'nature' and 'art'.

This study approaches the concept of tourism from the smallest unit viewpoint as possible in order to be able to know what the target audience is. The study focuses on small tourist areas that have natural tourist resources that citizens of nearby big cities frequently visit. In other words, instead of political capitals or famous tourist attractions representing countries, this study focuses on a general small city having a population of 10,000 people located near a big city.

2. Study area

Nabari, located in Mie Prefecture of Japan, is a small city with a population of 78,555 (Nabari 2019) and located between 2 large cities, Osaka and Nagoya. Like many other cities in Japan, Nabari is also facing problems of population decline and an aging society. Moreover, its population is decreasing faster than the Japanese average. Since 2000, the number of people moving out of the city has been increasing significantly and 70% to 80% of them are in their 20s. The number of workers in primary industries (Agriculture, forestry, commercial fishing) and secondary industries (mining, construction, manufacturing, etc.) is decreasing each year. However, the number of employees in tertiary industries such as education, medical and welfare, entertainment, tourism, etc. are increasing significantly. In 2010, the number of employees working in tertiary industries occupied approximately 64.7% of workers in Nabari[(2)]. Thus, tourism can be considered as one of the most important factors in developing Nabari's economy.

Nabari tourism mainly depends on its natural landscape, specifically, the Akame Shijuuhachi waterfalls and the Kaochi Valley, which have been selected as one of the hundred best waterfalls in Japan and best forests in Japan for forest bathing [(3)]. In 2014, approximately 400,000 visitors came to Nabari to see natural sites such as the Akame Shijuuhachi waterfalls, the Kaochi Valley and Lake Shorennji. However, most of visitors come to view the autumn leaves around the end of November and the beginning of December and almost all of the travel activity is hiking. In particular, Kaochi valley visitors decrease significantly in summer. Although visitors are satisfied with the natural landscape, they are less satisfied with regards to activities and services during their trips. There are not enough cultural, historical and other entertainment-related tourist resources in addition to the nature. Moreover, there are competing destinations near Nabari, which offer scenic sites and tourist activities similar to those of Nabari. It turned out that destination uniqueness is not statistically significant. Compared to other areas, nevertheless, the city lacked in uniqueness that makes it stand out from its competitors. This means, in turn, that the city desperately requires other attractions other than nature to enhance the satisfaction of

visitors [4].

3. Site

3.1. Site description

The site for this thesis project is located in Shorenji-cho, Nabari, Mie prefecture, Japan.

This site was chosen for several reasons. First, it is located between Lake Shorenji and the Kaochi Valley along the Shorenji River, so it will be an attractive place for tourists to visit between these two spots. Second, the site has convenient access to public transportation. Third, the site has a high degree of visibility and access due to its location. Fourth, the site is an underutilized area, where a museum can be built without cutting down trees or destroying the natural landscape. Fifth, it is near cherry blossom parks, a restaurant, and a tennis court.

3.2. Kaochi Valley

The Kaochi Valley is an ancient ravine located along the Shorenji River, a tributary of the Nabari River. It's an eight kilometer stretch of incredible rock formations, sheer cliffs and walls of stone columns all set among some of the most beautiful natural forest and blue water river scenery. The walls of rock, much of them created by lava flows through fissures in the earth millions of years ago, make for some spectacular sightseeing. The gateway to this remarkable spot is across a bright red bridge that arches across the still blue of the Shorenji Lake, created in 1970 when the Shorenji River was dammed [5].

3.3. Transportation and Access

The design site is located in between Lake Shorenji and the Kaochi Valley, which are connected by Nabarisonisen Road about 10 km from the Nabari Kintetsu Line train station. There is a bus service that runs close to the site from the train station along the Shorenji River to the Kaochi Bridge, taking 30 minutes to arrive at the site. There is also another route for hikers and bikers which starts from Lake Shorenji and connects with the Nabaris Hot Springs by the Shorenjiko Bridge, leading directly to the design site.

4. Precedent Analysis

4.1. Precedent 1: Benesse House Museum

The Benesse House Museum, located on Naoshima island, is an interesting precedent to consider and analyze with respect to this thesis project, as both are similarly located in rich natural landscapes. It is a good example that shows how the museum transformed the remote island into a tourist attraction.

The first museum of the Naoshima island, the Benesse House Museum, was finished in 1992 with Tadao Ando in charge of the architectural direction[6]. In order to protect the environment, part of the building and its garden were set underground. Contemporary art is displayed not only within the Museum galleries, but also in all parts of the buildings and surrounding area. People can explore art, nature, architecture, and their own thoughts in a multi-layered and synergistic fashion.

The Benesse House Museum, I believe, is a valid and useful precedent for this thesis. It is a successful example of how a museum can transform an area to become unique, more attractive, and raise the awareness of the site among both national and international tourists. It is also an example of how architecture can coexist with nature peacefully.

4.2. Precedent 2: Japon Louvre Sculpture Museum

The Japon Louvre Sculpture Museum is the only sister museum to the French Louvre museum in the world. This museum exhibits approximately 1300 sculptures which are direct replicas of the actual sculptures in the Louvre in France [7]. This museum's architecture was designed by the influential Japanese architect Kisho Kurokawa, who was one of the leading figures of the Metabolist movement in the 1960s and 70s. Like many modern buildings designed by Kisho Kurokawa, it was built with exposed concrete walls and glass. The placement and presence of the enormous sculptures in front of the building allows it to stand out from other attractions in the area. It opened in 1987 and enjoys approximately 100,000 visitors per year [8].

The Japon Louvre Sculpture Museum is an interesting precedent to analyze with respect to this thesis project. Firstly, both are located in Mie Prefecture and both occupy natural sites. Second, both areas are well known already by tourists for their hot springs and beautiful mountain scenery. Third, it is located only 39 km away from this thesis design site. The Japon Louvre sculpture museum is a successful example of how a contemporary art museum can invite tourists who are fundamentally interested in art to come to an unknown natural rural area and appreciate its surrounding nature.

4.3. Precedent 3: Adachi Museum of Art

This museum is also very interesting as a precedent for analysis in this thesis project, as many of the design elements are similar. The Japanese architect Saichi Kojima designed this museum in Shimane Prefecture, Japan. It houses a collection of about 1300 Japanese modern art works [9]. This museum was designed to showcase differing perspectives of the landscape during each of the seasons. The Japanese gardens surrounding the building were designed to connect with the natural mountain landscape. Through this method, it functions as an infinity garden with a varying landscape throughout the year. This makes the surrounding nature a part of the garden of the museum. Inversely, the museum itself blends in and becomes one part of the surrounding nature. These design concepts will be carefully considered during this thesis museum design process.

5. Design approach

The Museum of Kaochi Valley will raise awareness of the Kaochi Valley, and the city of Nabari and make it stand out from other natural landscape sites near the city.

5.1. Conceptual Design Strategies

Underground

One of the primary design objectives of this thesis project was to create a successful architecture coexisting with nature. The site is located on a mountain approximately 70 m above the road and surrounded by forest. In order to protect the natural landscape, the museum height will be designed to be as low as possible yet still provide a free and open space. To achieve this concept, part of the building will be buried underground. This allows us to design the above ground floor ceiling higher. The public space will be separated by only column and glass walls where visitors can move freely from space to space.

Site context

As above mentioned, the design site is in the Kaochi Valley which is well known for its rock formations, sheer cliffs, and walls of stone columns. The choice of using exposed concrete as the primary exterior finish and cladding of the building is an attempt to relate the museum to the site context. The above ground face of the building will be simply a lining up of concrete columns inspired by the walls of stone column in Kaochi Valley.

Shakei (Borrowed-scenery) method

Considered known as a Chinese and Japanese gardening method which uses the surrounding landscape as a part of the garden, as described in Precedent Analysis 3.

Similarly, in this museum design proposal, the surrounding landscape will be considered as a garden and care will be taken in the design process. The museum will be designed with various viewpoints to showcase an array of imagery throughout the four seasons.

5.2. Program

This thesis proposes a design for a contemporary museum, located in the city of Nabari, along the Shorenji River. The museum's program will contain spaces for temporary and permanent exhibitions, collections and research. The museum would exhibit the rhyolitic tuff, which shapes the Kaochi Valley's unique landscape. Moreover, it would also exhibit local Nabari culture and modern arts. Additionally, this design would incorporate a space for Lake Shorenji lake and Kaochi Valley visitors and eco-tour programs, conducted by local residents and an NPO. In addition to providing a cultural resource for the city, this museum would also provide a public indoor space for meetings and research.

5.3. Modern Arts Exhibition Collection

Nabari local artists are welcomed to propose exhibitions to attract visitors as well as to educate them in local culture .

Moreover, this project will propose both indoor and outdoor western sculpture exhibitions, a concept borrowed from The Japon Louvre Museum to make the Kaochi Valley Museum become a various collection exhibitions area and to raise awareness of Koachi Valley as well as Nabari to both Japanese and foreign tourists.

5.4. Museum Layout Tabulation

The museum's floor areas are response to the usages as shown in Table 1.

Table 1. The museum's floor area allocation

Function	Area(m^2)
Entrance hall	274
Counter	18.4
Office	80
Locker	16.8
Director office	30
Reception room	30
Library	40
Research room	80
Flexible space	150
Restaurant	170
Shop	49
Rest room	105
Unpacking space	65
Exhibition preparation room	65
Nabari local culture exhibition	117
Storage	132.5
Permanent exhibition	500.5
Temporary exhibition	230
Natural history exhibition	264
Other	696.4
Total	3113.68

6. Final Plan and Perspective Images

The following images illustrate the final design drawings for The Museum of Kaochi Valley. It includes floor plans, sections, elevations and perspective images.

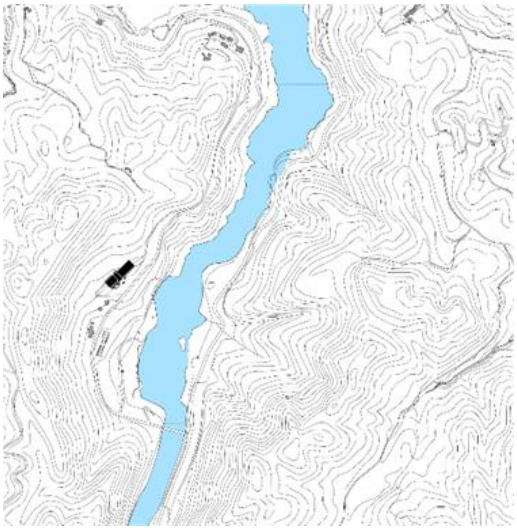


Fig.1. Site Plan

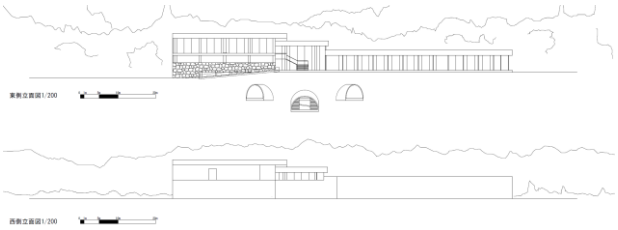


Fig.2. East and West Elevation Plan

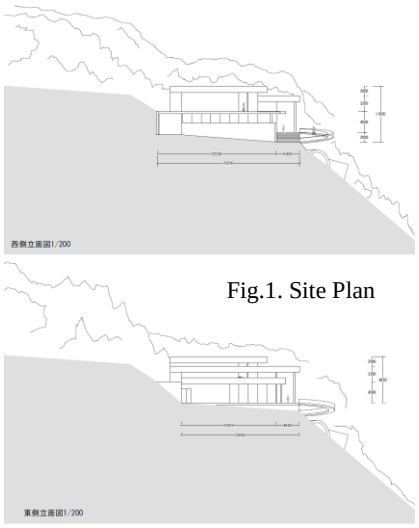
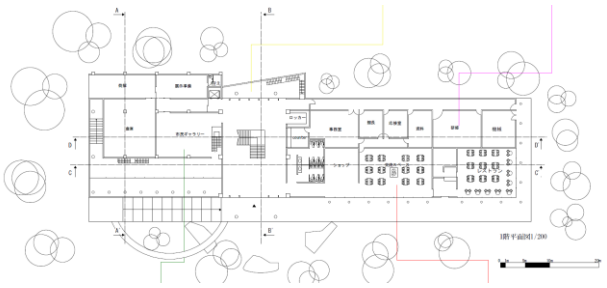


Fig.1. Site Plan

Fig3. North and South Elevation Plan



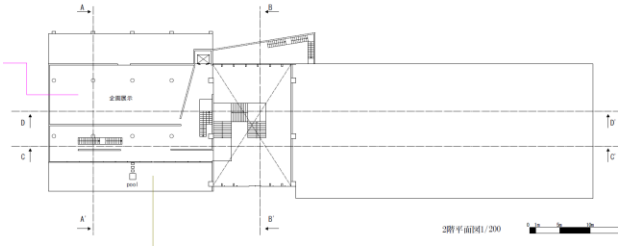


Fig.4. First Floor Plan

Fig.5. Second Floor Plan

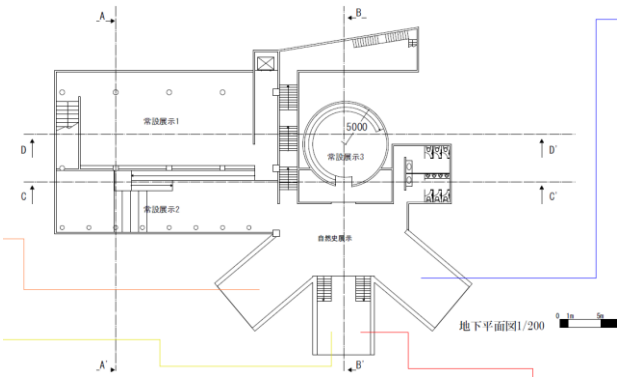


Fig.6. Underground Floor Plan

Fig.7. Perspective image of Kaochi Valley Museum

Fig.9. Perspective of Flexible Space

Fig.8. Perspective of Natural Exhibition Space

6.1. Summary

The final thesis design takes into consideration the many site-related, programmatic aspects of the design challenge.
The concept of building part of the museum underground will protect the natural landscape. The flexible space will provide a public space for Lake Shorenji and Kaochi Valley visitors to relax and communicate with local people after hiking or biking. Moreover, it would also incorporate a workshop and culture experience space for tourists. Nabari local cultural exhibitions will provide a way to exhibit local residents' paintings and crafts, allowing visitors to learn about and experience local culture. The natural history exhibition, which is designed according to the various exterior landscape views of the seasons, will attract nature loving visitors all year round. The temporary exhibition space for contemporary art and sculpture will be the main target for art loving tourists.

7. Conclusion

The design proposal for the Museum of Kaochi Valley will be a valid example of how the museum and tourism industries correspond with each other. By focusing on what kind of visitor comes to each area, the museum programs and spaces can be planned and organized. In addition to the existing natural tourist resources, museums can play an important role in making tourist attractions more significant and unique from other similar destination areas. In this sense, museums can be considered as important draws for tourists as well as a potential solution to solve the problem of city depopulation.

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A Low-Cost Home Energy Saving System Based on ARM Cortex M4

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Abstract: This paper presents the development of a low-cost home energy-saving system. Power saving is encouraged due to the scarcity of energy sources throughout the world. Usually, people leave their homes hurriedly for work in the morning forgetting to turn off some electric appliances, only realizing they left their lights on when they return home in the evening. This energy wastage can be minimized by automating the operations of home appliances. Energy-saving home automation is a term commonly known nowadays. Although there are a number of energy-saving home automation systems available on the market, the biggest problem is their high price. The proposed system is designed to avoid such energy-wasting, for an extremely low price. An Advanced RISC Machine (ARM) Cortex M4 microcontroller as the main control unit that senses and controls electric appliances connected to it is proposed. This system minimizes the electric wastage in busy homes and provides user convenience for handling home appliances with a price 65% lower than the existing system in the market.

Keywords: Energy saving, Home Automation, ARM cortex m4, Wi-Fi, ESP 8266

1. Introduction

Energy efficiency, including that of residential, industrial and municipal applications, is central to achieving the energy and climate goals of countries around the world. But despite the enormous potential for savings, energy efficiency continues to be underdeveloped because of policy, technical, and financial barriers. Intrinsically electricity wastage takes place in the home environment due to forgetfulness to turn off home appliances. This is one of the biggest problems in people's busy lives. Figure 1 shows the average electric energy usage of a home in the USA, which demonstrates how different home appliances use electric energy. Energy wastage could result from any appliance forgotten to be turned off when not necessary. This energy wastage can be minimized by automating the operations of home appliances. (Jiang et al2014) This is called home automation, meaning that home appliances can be controlled from anywhere. Home automation technology has made great strides in recent years. We are now at the point where full home automation is now achievable for anyone. Although there are a number of energy-saving home automation systems available on the market, they are often expensive.

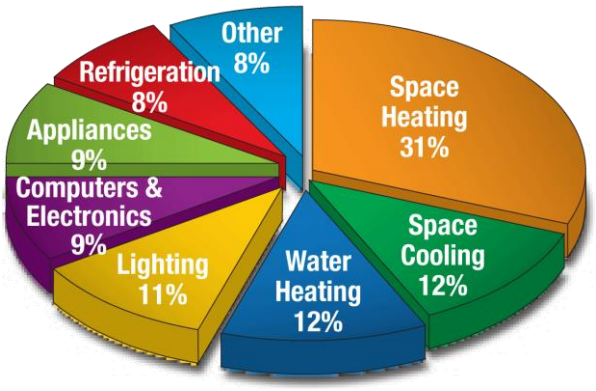

Figure 1: Average energy usage of a home in the USA in 2007(Anon 2019)

The current paper describes a low-cost solution to monitor and control electric appliances of a home remotely, thereby eliminating electric wastage caused by a failure to of turn off equipment.

Existing energy-saving home automation systems can be purchased in the range of 291 to 1559 USD. According to the bill of materials, we can introduce a system priced at 100 USD, a cost 65% lower than the next cheapest system on the market. Features and benefits of home automation systems in recent years, wireless systems like WLAN have become more and more common in home networking. Deploying a wireless network is especially advantageous when, due to new or changed requirements, extensions of the network are necessary. Some home automation systems using wireless Bluetooth technology (Gunge et al.2016) is to ensure that safety must be of the prime concern at home.

This system consists of an ARM Cortex M4 microcontroller as the main controlling unit, a Wi-Fi module for the web interface, and different sensors and actuators. An ARM Cortex M4 microcontroller is one example of a low-cost and high-performance board. In this research using such hardware, we present a solution that can be easily installed at an extremely low-cost, providing user convenience while reducing electric wastage. To demonstrate the feasibility and effectiveness of this system, devices such as light switches and power plugs, have been integrated into the main control system. This project is a basic model of a home automation system implementing an energy-saving system.

2. Methodology

An ARM Cortex M4 microcontroller is used as the main control unit. This control unit performs three major functions. First, it controls the appliances that interface with the system. Second, it reads sensors attached to the system, and third, it establishes a communication link with the Wi-Fi module. The Wi-Fi module, ESP8266, is interfaced with the main control unit using the UART protocol. The web interface developed using PHP server-side scripting language provides the remote access to the system. Further control commands can be sent to the system through the web interface. A Custom Printed Circuit Board (PCB) is designed to connect all sensors and appliances via relays. A basic model is designed to demonstrate the energy-saving

system of home automation.

A block diagram of the proposed system is shown in Figure 2. ARM Keil uVision software is used for programming the microcontroller and the Arduino IDE is used for programming the Wi-Fi module.

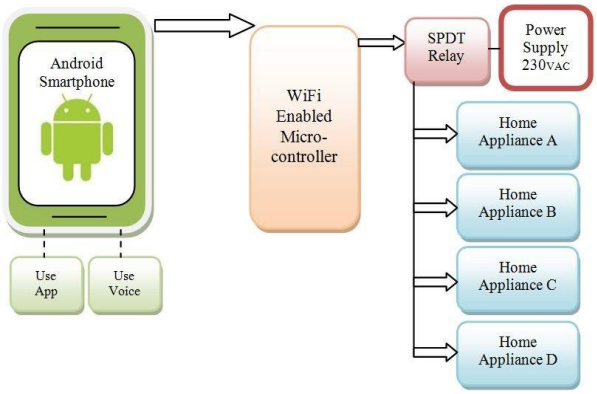

Figure 2: Block diagram of the Home Energy Saving System

3. Results and Discussion

The operation of the system is straightforward. The web interface can be used to monitor the status of home appliances and send commands to the system. The web interface has two tabs: one is for controlling appliances (Figure 3) and the other is for showing their status (Figure 4). The appliances, such as lights, fans, air conditioners, water pumps, etc can be controlled by the user with control web interface. The status of any equipment, whether it is turned on or off, is displayed on the status tab, enabling the user to turn off unnecessary appliances from anywhere through the Internet.

Status	Turn On	Turn Off	Lamp Name	Description
ON	ON	OFF	Living Room	Near the book shelf
ON	ON	OFF	Dining Room	Near the Table
ON	ON	OFF	Bed Room	Near the bed
ON	ON	OFF	Kitchen	Near the kitchen door
ON	ON	OFF	5W Bulb	5W Bulb
ON	ON	OFF	Garden Lights	Garden Lights
ON	ON	OFF	Fan	Fan in living room
ON	ON	OFF	Water Pump	Water Pump
ON	ON	OFF	Buzzer	Buzzer

Figure 3: Web interface that controls the entire system

The web server gives sensor information in different places within the house and the security state of the house. It also gives the status of the various sensors reading as 1 and 0. As shown in Figure 4, the sensor reading display also has three columns with sensor name, description and sensor status.

Status	Sensor name	Description
0	Temperature Sensor	Temperature in home
0	Gas Sensor	Gas leakage
0	Wet Sensor	It is raining
0	Security sensor home front	Security sensor home front
0	Security Sensor home inside	Security Sensor home inside
0	Door Lock	Door Lock
0	LDR Circuit	LDR Circuit

Figure 4: Sensor readings display

The system can be expanded to include various other options which include security features like alarms or siren alarms for emergency situations. This kind of system with respective changes can be implemented in hospitals for disabled people as reported in the literature (Bernheim et al.2011).

The total cost of the proposed system is about 100 USD. Compared to the systems on the market, the proposed system offers comparable features at a lower cost.

5. Conclusion

The goal of this study was to develop a low-cost energy-saving system using an ARM Cortex M4 for busy households. We believe that the system proposed here has the potential to add convenience for the user while curtailing the electric wastage at home. Hence, it can be concluded that the required goals and objectives of the low-cost energy-saving system using an ARM Cortex M4 have been achieved. The ability to monitor and control home appliances remotely adds benefits for the user. The system proposed in this paper provides the features to reduce electric wastage due to the forgetfulness of busy life into the entire society while adding convenience to use home appliances.

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An Analytical Research of the Landscape Characteristics of Ama-Villages in Ijika and Kuzaki Districts in Toba City

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Abstract: Toba, in Mie Prefecture is the region with the highest number of Ama in Japan. Since Ama fishing according to a specific rules and contribute to environmental protection by not to depleting the resources of the sea, their ways attract attention. The landscape of an Ama village is worthy of being preserved as a historical and cultural landscape, which necessitates the development of a landscape plan of Toba. This study clarified the landscape characteristics of an Ama village as the first step of the project. The characteristics of the terrain, land use, residences and lifestyle of residents were investigated in two villages where more than 50 Ama currently live. The result clearly revealed that the landscape characteristics of the Ama village had been formed around elements unique to the Ama on the basis of the fishing village's landscape. Furthermore, the results of surveys collected in three villages and analyses on the landscape of the Ama villages will be presented. Lastly, the landscape plan based on these results will be formulated, for the landscape of Ama village as tangible assets to be inherited along with Ama culture as intangible assets for the future.
Key words: landscape act, landscape plan, landscape resource, Ama, Ama village

1. Introduction

Today, there are limits placed on landscapes in various countries of the world to maintain and create attractiveness of the region by creating cities that emphasize landscape conservation. Especially in European countries, such regulations have already been widely established, and the traditional, unified and beautiful cityscapes are preserved.

Following these countries, Japan also enacted the Landscape Act in 2005. This is a law that aims to promote the maintenance and preservation of good landscapes as a common property of the residents. This law demands that architecture in a city observes a series of regulations and follows the general plan in order to form a beautiful landscape, thereby creating a comfortable development of community. Until now, for example, the landscape planning has been advanced in several cities known for their historical monuments and architecture. The landscape of a city is formed by the interaction of various factors such as the climate in the region, the history of the development of the city, and the cultural and economic activities of the population. Landscape planning is the best opportunity to bring out the local character and revitalize.

In recent years, it has also been decided to formulate a landscape plan for the city of Toba, in Mie Prefecture, which is known for having the highest number of Ama in Japan. An Ama is a female diver, a profession peculiar to Japan and Korea. Ama have contributed to preventing the depletion of ocean resources by determining the fishing season and the size of the available shellfish. In this way, they achieve sustainable fishing (Fig.1). Various cultural research has already been conducted with regard to Ama, and the "Ama Research Center" was established at Mie University. However, no study has been conducted on an Ama village from an architectural point of view. This study has investigated the characteristics of the landscape of an Ama village as the first step to develop a landscape plan based on the Ama culture.

The landscape of a village can be grasped from the following four elements: (1) terrain, (2) land use and sea surface use, (3) buildings and structures (residences, temples, Ama sheds, etc.) and (4) residents' lifestyle and customs. In this study, the survey on these four elements were conducted in two village where Ama are currently living and working.

Fig. 1. Photograph of the Ama doing fishing

2. Survey outline

2.1 Survey area

Toba is located at the northeastern end of the Shima Peninsula in Mie Prefecture, and forms the entrance of Ise Bay, an important point of maritime traffic since ancient times. In Toba, the survey target was an Ama village on the main island where more than 50 Ama were actually in practice in 2017 according to a survey by the Toba Sea-Folk Museum. The applicable districts are the 3 districts of Ijika, Kuzaki and Ousatsu. Considering scale, the districts of Ijika and Kuzaki were selected as they were comprised of only 200 to 250 residences each (Fig.2).

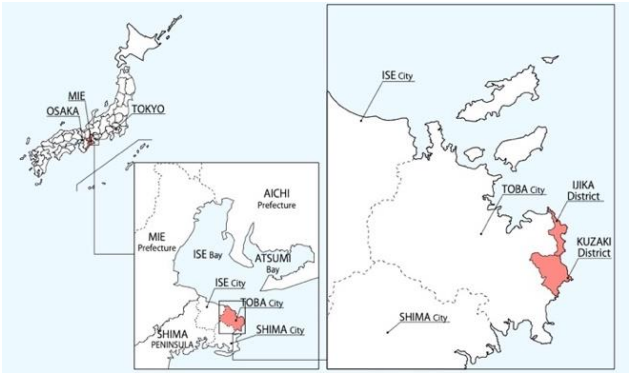

Fig. 2. Map of the survey districts

The survey was begun by understanding the range of residences. There were 255 residences in Ijika District and 212 residences in Kuzaki District. The area of the survey was determined along the topographic boundaries and contours that cover all surveyed residences. (Fig.3)

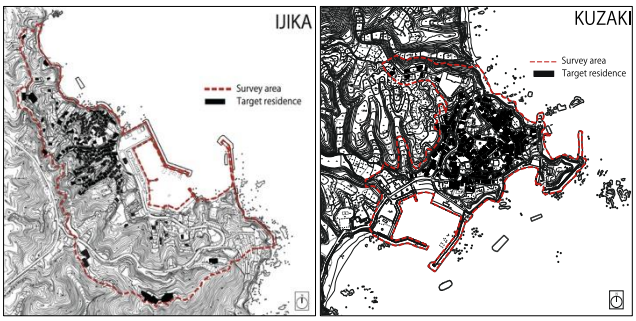

Fig. 3. Map of the survey area

2.2 Literature data survey

(a) In order to understand the basic information regarding the history, culture and society of the Ama villages of Ijika and Kuzaki Districts, a literature survey was conducted at the Toba Public Library, Toba Sea-Folk Museum, Mie Prefectural Library, and Mie Prefectural Museum.

(b) To grasp the transition and current status of land use and sea surface use of Ama fishing in Ijika and Kuzaki Districts, a survey was conducted to identify the location of farmland and fishing grounds. Farmland was surveyed using aerial photographs taken in 1975 and 2012 to 2013, where the existence of the ground surface was confirmed and compared. On the other hand, as for the fishing ground, that in Ijika District was determined based on data provided by the city of Toba, and that in Kuzaki District was determined based on a previous survey ("Traditional fishing techniques by Toba and Shima's Ama", 2016).

2.3 Landscape survey

To better understand the current status of buildings and structures, a landscape survey was conducted locally within the area shown in Figure 3 in Ijika and Kuzaki Districts on site.

(a) For the survey of residences, shown in Fig.4, recording and photographing of each item were performed visually from the street for all residences.

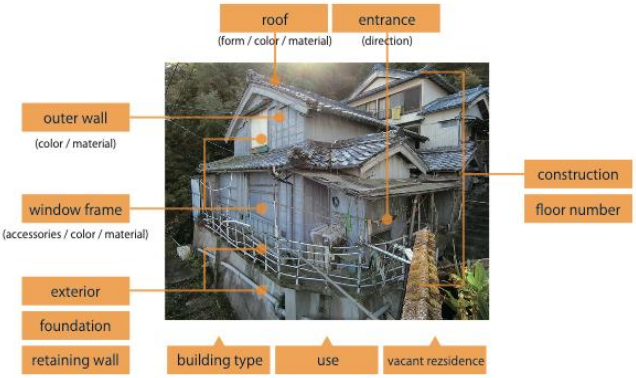

Fig. 4. Survey item of residence

(b) For the survey of temples, shrines and monuments, that were found in the map and literature, confirming and photographing were performed.

(c) For the survey of Ama-sheds, confirmation and photographing were performed on foot. Some sheds were photographed inside as well with the permission of the user.

(d) For farmland and fishing grounds, partial photographing as performed because as overall imaging was not possible.

3. Results of the survey

3.1 Comparative analysis

A comparison study was conducted using the results of the surveys in the Ijika and Kuzaki Districts, and the landscape characteristics of the Ama villages were clarified based on the differences and similarities. The results shown below were summarized for each of the fields: (1) terrain, (2) land use, sea surface use, (3) buildings and structures (residences, temples, Ama sheds, etc.) and (4) residents' lifestyle and customs.

3.2 Terrain

Ijika District is a settlement located on the northeastern end of the Shima Peninsula, and the Kuzaki District is on the tip sticking out and exposing itself to the Pacific Ocean. There are five terrain classifications in Toba, but the Ijika and Kuzaki Districts have different terrain classifications. Many of the coastline of the area, including that of the Ijika District, is of the ria coast type. This is where sea erosion cliffs, reefs and shattered sand are deposited to create a small sand beach, which enhances the Ama fishery. The terrain of the area including the Kuzaki District was formed due to repeated water separation and submersion, giving it has many flat grounds and making it more favorable for agriculture than in Ijika District.

A common feature of both areas is that they are exposed to the sea, so unlike the other areas that form the bay, they are absolutely unsuitable for aquaculture. The inability to switch to aquaculture is an important factor in the continuance of Ama Fishing and is the basis of the natural landscape of the Ama Village. (Fig.5)

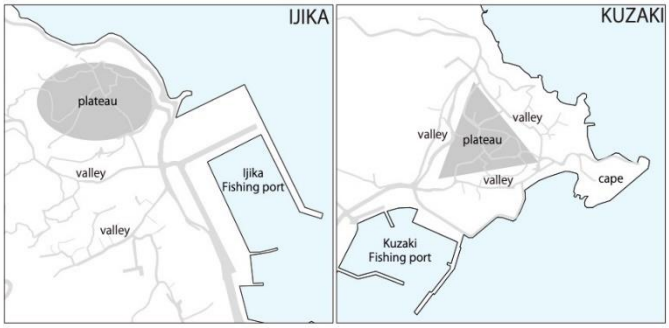

Fig. 5. Terrain of Ijika and Kuzaki Districts

3.3 Land use and sea surface use

Ijika District has limited farmland because residents create farmland on hilly terrain. In addition, fishing grounds are located south of the fishing port. According to the residents, they are also located to the north of the village, and it is thought that they have spread to the north and south as well. More detailed surveys will be conducted in the future (Fig.6). On the other hand, Kuzaki District has a wide area of farmland because it has much level ground, but in recent years deserted land has increased. In addition, fishing ground can be confirmed to be around the cape and fishing port. (Fig.7)

From the above comparison, it can be considered that there is a difference in the degree of importance of agriculture in both areas, and that Ijika District can be judged as a "main fishing sub agricultural" village and Kuzaki District as a "half fishing half agricultural" village.

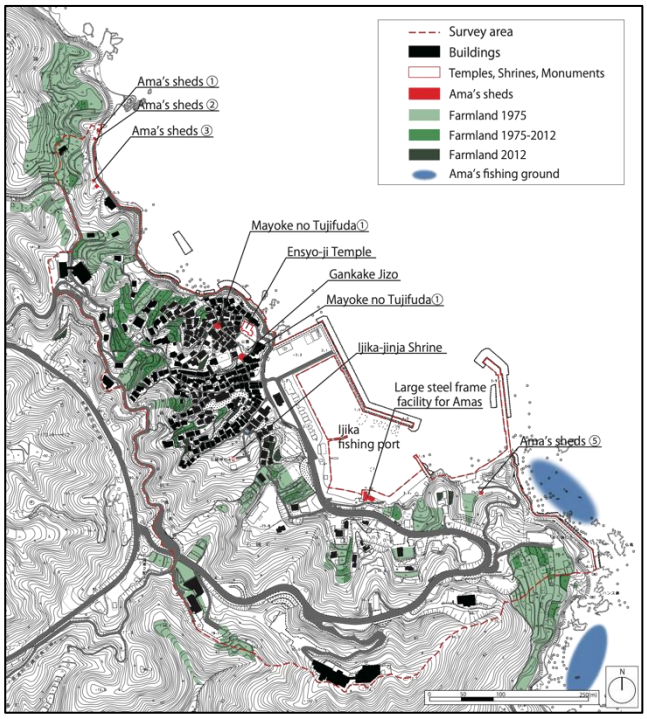

Fig. 6. Distribution of landscape resources (Ijika District)

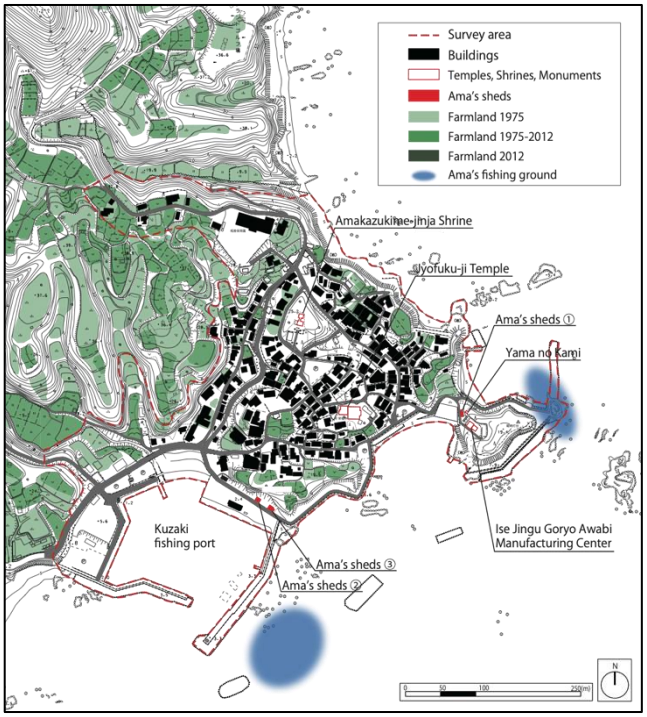

Fig. 7. Distribution of landscape resources (Kuzaki District)

3.4 Buildings and structures

(a) Residences

Table 1 shows the main points of the survey results for residences. The residences are characterized by topographical and climatic conditions and are built in high density. It was revealed that the characteristics of the residences of the Ama village were the same as the characteristics of the residences of other general fishing villages. For example, a colorful exterior, which may be painted the same color as one's ship, is a feature of fishing villages.

Table.1. Point of survey result

Ijika district	item	Kuzaki district
"Main house"occupies the majority.	building tipe	The ratio of "Main house" and "Sub house" is 7 to 1.
"Residence"occupies the majority.	use	The ratio of"Residence" and "Commercial" is 6 to1 .
"Vacant residence"occupies 20%.	vacant residence	"Vacant residence"occupies 20%.
"Wooden"occupies 70%.	construction	"Wooden"occupies 55%.
"First floor" and "Tsushi second floor" ※1 occupy 40% together. "Third floor"and above 5%.	floor number	"First floor" and "Tsushi second floor" occupy 45% together. "Third floor"and above 6%.
The ratio of"Tsumairi" ※2 and "Hirairi"※3 is 3 to 4 .	entrance	The ratio of"Tsumairi" ※2 and "Hirairi"※3 is 2 to 5 .
The ratio of "Gable" and "Hip gable" is equal.	roof (form)	The ratio of "Gable" and "Hip gable" is equal.
"Gray"occupies the majority.	roof (color)	"Gray"occupies the majority.
"Japanese tile" occupies 60%.	roof (mateial)	"Japanese tile"occupies 40%.
A few "Kannuki" ※4 is seen.	window (accessories)	A few "Kannuki" is seen.
The ratio of"Brown" and "Silver" is 6 to4 .	window frame(color)	The ratio of"Brown" and "Silver" is 5 to4 .
"Wooden"occupies 10%.	window frame(mateial)	"Wooden"occupies 20%.
"White" and "Broun" occupies the majority.	exterior(color)	"White" and "Broun" occupies the majority.
Higher ratio in the order of "Wooden","Concrete","Siding".	exterior(mateial)	Higher ratio in the order of "Concrete","Wooden","Siding".
"Stone foundation"occupies 15%.	foundation	Stone foundationoccupies 10%.

※1 The second floor with a low ceiling that is used for storage in the old form of a Japanese residence
※2 Enters from the vertical wall to the ridge beam.
※3 Enters from the horizontal wall to the ridge beam.
※4 Crossbars for closing doors and windows .

Fig. 8. "Tsushi Second floor"

Fig. 9. "Hip gable"

Fig. 10. "Japanese tile"

Fig. 11. "Kannuki"

Fig. 12. Exterior color

Fig. 13. "Stone fundation"

(b) Temples, shrines and monuments

There are *Ijika Shrine* (Fig.14) and *Ensho Temple* in Ijika District, and *Amakazukime Shrine* (Fig.17) and *Jyofuku Temple* in Kuzaki District. Ijika District also has *Butai*, *Gankake Jizo*, and Kuzaki District has the *Ise Jingu Goryo Awabi (Abalone) Manufacturing Center* (Fig.18), and *Yama no Kami* (Fig.19). It turned out that all temples and shrines are closely related to Ama. For example, *Ijika Shrine* has *Kodakara Ishi* (Fig.15) where folklore about Ama is transmitted. Also, *Amakazukime Shrine* is a shrine where a legendary Ama is enshrined.

Besides these, in Ijika District, there is a bill called *Mayoke no Tsujihud*a (Fig.16) at several street intersections.

Fig. 14. Ijika-jinja Shrine (Ijika)

Fig. 15. Kodakara Ishi (Ijika)

Fig. 16. Mayoke no Tsujihuda (Ijika)

Fig. 17. Amakazukime-jinja Shrine (Kuzaki)

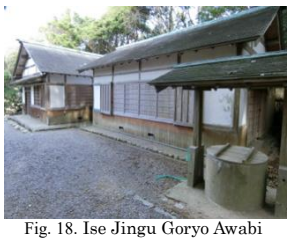

Fig. 18. Ise Jingu Goryo Awabi Manufacturing Center (Kuzaki)

Fig. 19. Yama no Kam (Kuzaki)

(c) Ama-sheds

Ama-sheds are a places where Ama prepare for fishing, change wet clothes after fishing, and warm up their bodies at a fire. Ijika District has a large steel frame facility for Ama to the south of the fishing port (Fig.20). There are also four sheds to the north of the village (Fig.21) and one to the south of the village. In Kuzaki District there are two sheds to the south of the fishing port (Fig.22), and three other sheds between the the fishing coop and the entrance of the cape (Fig.23). The distribution is shown superimposed in Fig.6.

Ijika and Kuzaki Districts Ama sheds are completely different in appearance. Those of Ijika District are constructed with different materials and colors, including for example corrugated steel sheet and wood, but those of Kuzaki District are made with materials and colors similar to each other. As for the scale and specifications, it was found that there was no standard, and the materials and colors were the result of the voluntary selection of the Ama group, and individuality appeared in different regions.

Fig. 20. large steel frame facility for Amas (Ijika)

Fig. 21. Ama's sheds in the north (Ijika)

Fig. 22. Ama's sheds in the north (Kuzaki)

Fig. 23. Ama's sheds from the fishing coop to the cape entrance (Kuzaki)

3.5 Residents' lifestyle and customs

The landscape of the village is established by adding the lives of the residents based on the terrain, farmland, fishing ground and buildings mentioned above. It is possible to say that the sights of Ama fishing, working at the fishing port, and working in the garden or at the front door form a landscape of an Ama Village.

Unusual annual events also show the essence of an Ama village. Among the major annual events for both Ijika and Kuzaki Districts, many are related to Ama and the fishery (Table 2, ○ marks pertaining to the Ama). In addition to the events held on the beach in both districts, Ijika District has events that touch on folk components that (an old dike, stone pillars, Jizo, etc.) dot the fishing port.

Table. 2. Annual event of Ijika district and Kuzaki district

Ijika district	month	Kuzaki distrist
Yumihiki Jinji	Jan	Yumihiki
		○Notto Syogatsu
○Kazukiori	Feb	Jusai・Kinensai
○Iso Oriawase	Apr	Hana Matsuri
-	Jun	○Nosiawabi Dukuri
○Nakasangu	jul	○Amakazukime-jinja Reidaisai
Tenosai		Tenosai
Bon Gyoji	Aug	Bon Gyoji
-	Nov	Nifune Matsuri
Otine Jinji	Dec	Toshikosi Matsuri

4. Conclusion

This study revealed that the landscape characteristics of the Ama village had been formed by combining the elements unique to the Ama on the basis of the fishing village's landscape. Ama manage a sustainable fishing system, and it is thought that the landscape characteristics of Ama villages have some points in common with the characteristics of Ama fishing. That is, it was not confirmed whether or not the fact of being an Ama affects the floor plan of a residences, or if constructing a sea shed that would control the landscape of a fishing port. This is similar to the way the Ama fishing does not overuse the limited resources of the sea; the landscape resources that create the Ama village's likeness are by no means dominant within the fishing villages, but they are coordinated and integrated. However, this is limited to fixed and ordinary landscapes, and it can be said that the sea woman's cultural character is prominently displayed in a fluid and unusual landscape such as an annual events.

Furthermore, the results of surveys collected in the three villages and analyses on the landscape of Ama village will be presented. In the future, the landscape plan based on these results will be formulated, so that the tangible assets of the Ama village landscape may be inherited along with Ama culture as intangible assets in future.

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Fundamental Study on Application of Geopolymer Paste as a Crack Injection Material

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Abstract: Geopolymer (GP) is a new and environment-friendly inorganic material that can be hardened without emitting CO_2 in the manufacturing process. Therefore, it has recently attracted attention as a cement substitute material. The purpose of this study is to examine whether GP could be used as a crack injection material. Fly ash (FA) and ground granulated blast furnace slag (BFS) with three different specific surface area were used as the main powders for the GP in this study. Fluidity, setting time and compressive strength tests were conducted to investigate the fresh and strength properties of GP with different powder ratios. Then, injection tests were carried out to examine the penetrating performance into cracks. The result showed that a decrease of the fluidity generally shortened setting time and the injection speed became faster in GP with BFSs having larger specific surface areas. However, the compressive strength was not clearly influenced by the specific surface area when the substitution rate of BFS was 40%. GP has a good potential to be used as a crack injection material, when designed in appropriate mixtures. Future studies will propose the range of mixture that can be applied as the crack injection material for actual sites.

Key words: Geopolymer, Crack injection material, Specific surface area, Injection behavior

1. Introduction

Concrete is the most widely used construction material in the world. However, CO_2 emissions from the binding agent in the manufacturing process of cement account for 4% of the total CO_2 emissions in Japan [1-2]. Therefore, to prevent the progress of global warming, developing new cement-free materials used in construction work has been required.

Geopolymer (GP) has attracted attention as a cement alternative and environmentally-friendly material due to its potential to without using cement. Also, it has the advantage of using industrial byproducts such as fly ash (FA) and ground granulated blast furnace slag (BFS), etc.

However, there are some problems in facilitating GP production. For instance, the hardening mechanism of GP differs from cement which hardens by hydration reaction. Moreover, GP's properties largely change with the materials. Therefore, the method of material utilization is still in the investigation phase, since the reaction mechanism of GP has not been precisely understood [3].

Regardless of the above challenges, there are studies making important contributions to GP's practical use. For example, Ichikawa [4-5] found that BFS greatly contributes to the increase in strength of FA-BFS based GP. Furthermore, it was shown that the setting time becomes shorter with increasing BFS substitution. Moreover, the relationship between elapsed time and the fluidity of GP showed stability when a mixture of water glass No.2 (WG2) and sodium hydroxide solution (NH) is used as an alkali solution. In addition, it was also confirmed that GP is excellent in adhesion and sulfuric acid resistance, allowing for effective as a repair material for concrete structures [3,6]. Also, according to Sato's research, the fineness of the particle size is related to the injection depth [7].

Based on the above findings, this study aims to utilize GP as a crack injection material, by using BFS with a large specific surface area. Although the fundamental study on GP properties as a crack injection material has been conducted [8], no actual injection experiments have been carried out. Thus, it is necessary to investigate not only the basic characteristics of GP using BFS with large specific surface areas but also the possibility of GP as a crack injection material.

2. Outline of experiment

2.1 Materials and mix proportion

Materials used in the experiment are shown in Table 1. To consider the effect of particle size, three types of BFS differing in specific surface area are used. Figure 1 shows the particle size distribution of the powders. It can be confirmed that the larger the specific surface area, the finer the particles that are included.

Table 2 shows the experimental factors and details. Using FA and BFS with 3 specific surface areas, the BFS substitution rate was set to three levels of 0, 20 and 40%. In this experiment, the mass ratio of the solution and the powder (W/P) was 0.5, and the solution ratio set constant at Water glass No.2 (WG2) : Sodium hydroxide solution (NH) = 2 : 1. The test was also performed for W/P=0.7 only in the case of the BFS substitution rate of 40%.

2.2 Specimens preparation

GP paste was produced by the following steps. Firstly, FA and BFS were put into in a basket and blended together by a hand scoop. Then the alkali solution which is prepared by mixing WG2 and NH for more than 24 hours was added and mixed again for 60 seconds. Finally, after scratching off powders for 30 seconds, the mixing was continued for 120 seconds.

The fresh GP paste was subjected to a flow table test and setting time test. After that, it was molded using a plastic cylinder for conducting a compressive strength test.

After casting, we covered the top of the formwork with a vinyl sheet and secured it with a rubber band. Then, curing was performed under the conditions of 20℃ and 60% relative

Table 2 Experiment factors and details

FA	BFS		W/P	Solution ratio WG2 : NH
Powder ratio (%)		Specific surface area		
80	20	4000	0.5	2 : 1
		6000		
		8000		
60	40	4000	0.5 0.7*1	
		6000		
		8000		

*1: Expect for setting time test

humidity for 28 days.

After curing, the loading surfaces of specimens were smoothed with a grinder and a compressive strength test was conducted.

2.3 Experiment method

(a) Basic characteristics

The flow test was performed following the JIS R 5201. The setting time was measured through a proctor penetration test following the JIS A 1147. The compressive strength test was performed according to JIS A 1108.

(b) Injection test

Fig.2 shows the overview of the apparatus. Fig.3 shows a cross-sectional view. A cracked specimen was prepared by referring to [8]. The crack width was produced by-inserting 0.2 mm Teflon sheets between 5 mm thick-vinyl chloride boards and firmly tightening with bolts. After that, the surrounding area of the crack specimen was fixed with clips at the specified crack width, especially checking the area of the inlet, using a scale loupe. Then, sealing was performed to prevent the leakage of the injection material. The injection pressure was set at 0.2 MPa by using rubber and the injection test was conducted under a temperature of about 8 ℃ to 13 ℃. The injection test on each GP paste investigated whether GP can penetrate into the crack, measuring reached depth and its time.

3. Results and discussion

3.1 Flow value

Fig.3 (a) shows the results of 15-drops flow table test at W/P=0.5. The graph shows that the influence of the specific surface area of BFS on the flow value was not seen in the range of 20 to 40 % of the BFS substitution rate. In addition, as the specific surface area increases, the flow value tends to decrease. This is considered to be due to the increase in the contact area with the solution. Fig.3 (b) shows the results of 0-drop flow test of FA60BFS40. The flow value increased as the W/P increased. It is considered that the increase in fluidity is due to the increase in the amount of solution.

Table 1 Materials

	Code	Material	Specific surface area (cm^2/g)	Density (g/cm^3)
Powder (P)	FA	Fly ash class1 JIS	5600	2.29
	BFS	Ground granulated Blast furnace slag	4000	2.91
			6000	
			8000	
Solution (W)	WG2	Water glass No.2	-	1.45
	NH	Sodium hydroxide solution (10mol/L)	-	1.35

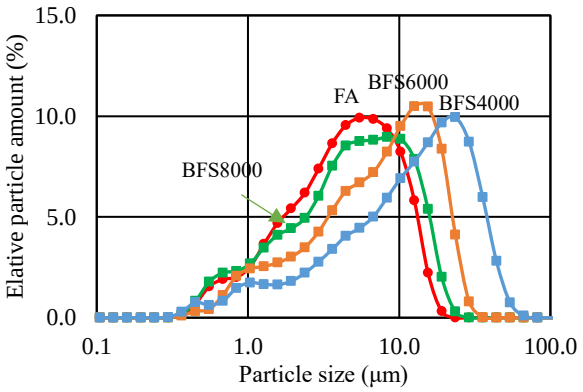


Figure 1 Particle size distribution

3.2 Setting time

Table 3 shows the results of the initial setting time and final setting time of each GP paste. The relationship of penetration resistance value and elapsed time is shown in Fig.5. The setting time is shorter as the BFS substitution rate and the specific surface area of BFS become larger. It can be considered that, as the specific surface area of BFS becomes larger, the contact area with the solution is increased, so that the development of strength becomes faster.

3.3Compressive strength

Fig.6 shows the results of the compressive strength. It was confirmed that as the BFS substitution rate increases, the

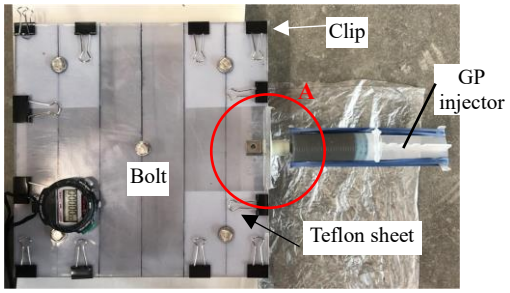
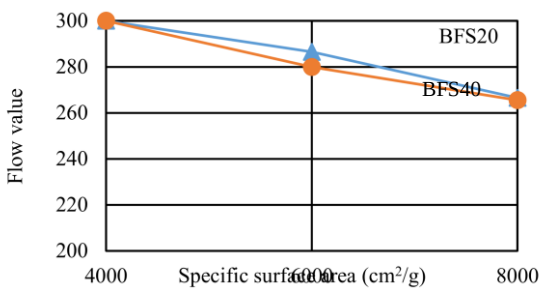
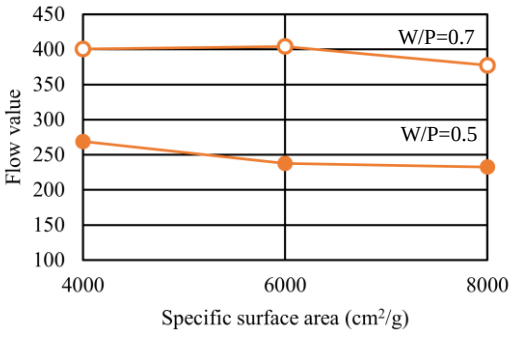


Figure 2 Overview of apparatus

Figure 3 Cross-sectional view of inlet point A



(a) W/P=0.5 (15 drops)



(b) FA60BS40 (0 drop)

Fig.4 Results of flow test

Table 3 Setting time test

Setting time	BFS20			BFS40		
	BFS4000	BFS6000	BFS8000	BFS4000	BFS6000	BFS8000
initial	3 h 59 min	2 h 17 min	1 h 32 min	2 h 56 min	1 h 25 min	42 min
final	10 h 13 min	5 h 4 min	3 h 12 min	4 h 10 min	2 h 14 min	58 min

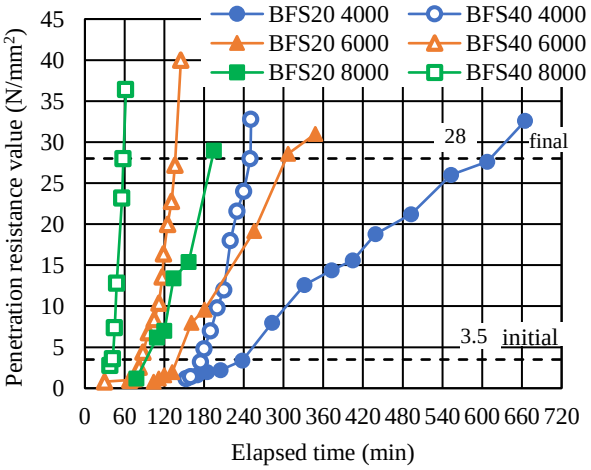


Figure 5 Time history of penetration resistance value

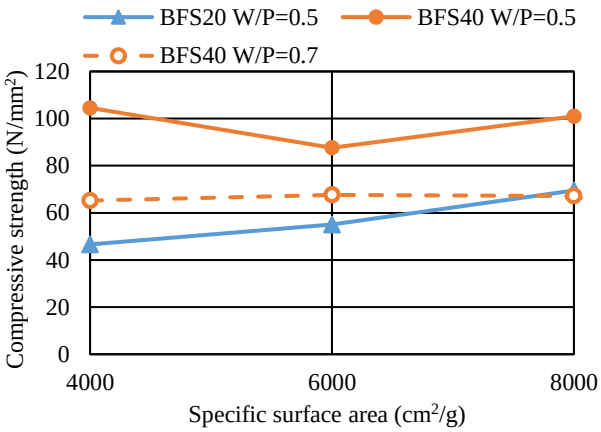


Figure 6 Compressive strength

Table 4 Injection depth test results

	BFS20 (%)			BFS40 (%)					
	BFS4000	BFS6000	BFS8000	BFS4000	BFS6000	BFS8000	BFS4000	BFS6000	BFS8000
	W/P=0.5						W/P=0.7		
Injection depth	5 cm	×	5 cm	5 cm	10 cm	5 cm	30 cm	30 cm	30 cm

compressive strength increases. When the BFS substitution rate was 20%, the compressive strength increased as the specific surface area of BFS became larger. However, when the BFS substitution rate was 40%, the effect of the specific surface area of BFS on the compressive strength was not confirmed.

Furthermore, while compressive strength decreased as the W/P became larger, it was confirmed that the strength (30 N/mm^2) which is considered sufficient in practicability can be obtained in all conditions.

3.4 Injection test

Table 4 shows the results of the injection depth of each test. Fig.7 shows the injection test result of W/P=0.5 and Fig.8 the result of W/P=0.7. The red line shows the final depth reached. From these results, it can be confirmed that all GP paste used in this experiment could be injected into the 0.2 mm crack width. The injectable depth was approximately 5 cm to 10 cm when W/P was 0.5, and 30 cm or more when W/P was 0.7. In the case of W/P=0.5, the result was considered due to high viscosity and decrease of working-time. On the other hand, the viscosity decreased because the amount of solution increased when W/P was 0.7, and it is considered that injection could be performed more deeply.

The relationship between the elapsed time and the injection depth is shown in Fig.9. The time required to reach the same injection depth became shorter with the increase in the specific surface area of BFS.

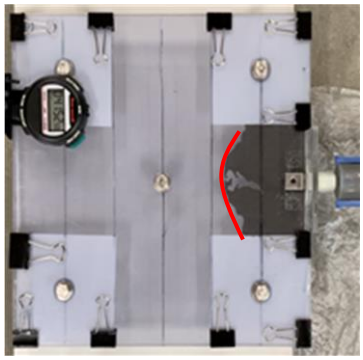


Figure 7 Final state of injection test of FA80BFS20 (specific surface area 4000)

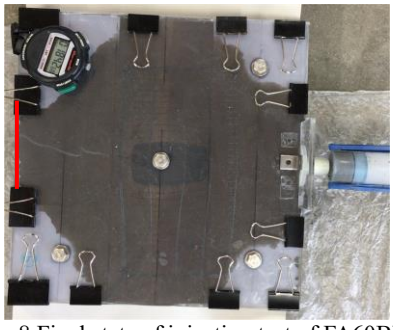


Figure 8 Final state of injection test of FA60BFS40 (specific surface area 4000)

4. Conclusion

This study conducted experiments to investigate the fluidity, setting time, compressive strength, and injection performance of

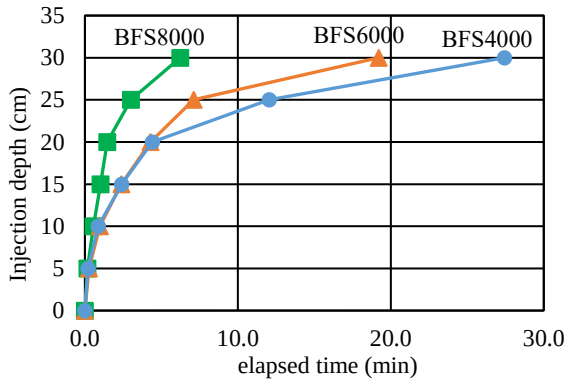


Figure 9 Relationship between injection depth and elapsed time

geopolymer paste using fly ash (class 1 FA, JIS 6201) and three types of ground granulated blast furnace slag (BFS) with different specific surface areas. The obtained results are shown below.

1) The fluidity decreases as the specific surface area of BFS becomes larger, due to the increase in the contact area with the alkali solution.

2) The setting time becomes shorter as the specific surface area of BFS and its BFS substitution rate increase.

3) The compressive strength increases with the increase in the specific surface area of BFS in the small substitution rate. However, in the large BFS substitution rate, the effect of the specific surface area of BFS on compressive strength was not confirmed.

4) As a result of the simple crack injection experiment, the injection depth becomes deeper by increasing the value of alkali solution to powder ratio in all cases.

5) The time required to reach the same injection depth became shorter as the specific surface area of BFS increased.

Finally, the findings obtained in this study will be expected to contribute to the utilization of GP as a crack injection material in the construction field. Moreover, this research will not only conduce development of technology in the construction field but also will lead to the effective use of industrial byproducts and the reduction of CO_2 emissions, a major agent of global warming.

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Study on Influence of Transportation Time on Properties of Porous Concrete

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Abstract: Porous Concrete (POC) has a continuous void structure, whose constituting materials are coarse aggregate and cement paste. The on-site construction POC goes through the process of transportation after manufacture, compaction, and curing. Each process should be performed appropriately, as it has a great impact on the quality of POC. The purpose of the present study is to quantitatively assess the impact of each process to guarantee the quality of POC. The effect of elapsed time between mixing and placement on the compressive strength of POC is to be examined. In this study, we carried out a series of an experiment by varying the transportation time of POC. The fresh properties and the compressive strength of POC were found to decrease with time before casting. However, it is possible to maintain the fresh POC in a good state by using a set-retarding admixture. The authors have found that holding good fresh properties is possible by the use of set-retarding admixtures, and is also effective for keeping good workability in casting.

Key words: Porous concrete, Transportation Time, Chemical Agent Addition, Compressive strength, Fresh properties, Hot Weather Condition

1. Introduction

Porous concrete (POC, no-fines concrete or pervious concrete), an environmentally friendly material, has received much attention in recent years due to its applications, such as permeable pavement, water purification, being a vegetation base, or functioning as a habitat for organisms. POC is a special concrete consisting of a cementing matrix (paste or mortar), coarse aggregate, little or no fine aggregate, admixture, water, and continuous voids (see Fig.1).

The applications of POC differ greatly from normal concrete due to a large amount of successive pores. The applications of POC are mainly divided into two categories [1]: reducing environmental loads and co-existing with living organisms. The followings are the main applications:

(1) POC for reducing environmental loads:
  a) Permeable concrete (allows drainage of rain water, underground water, and permeates into the ground)
  b) Noise-absorbing concrete (absorbs the noise from roads, railways and factories)
  c) Hazardous-gas-absorbing concrete (absorbs gases such as NO_x, SO_x, and CO_2 contained in the gas emissions of automobiles, and acts to detoxify them)
  d) Humidity-controlling concrete (absorbs humidity during humid seasons and emits humidity during dry seasons)
  e) Thermal-storage concrete (temporarily stores heat from the sun and heat exhausted from factories by making use of heavy aggregates)

(2) POC that co-exists with living organisms:
  a) Greening concrete (allows grass, plant weeds, and low trees to grow along riverbanks and road slopes)
  b) Concrete to purify water (indirectly purifies water when various bacteria are attached to the pores)
  c) Concrete to create seaweed beds (a certain amount of nutritious salt is mixed into the porous concrete in coastal shores where there is a rocky-shore denudation phenomenon, and the nutrition value is either increased or decreased. The nutritious salt will gradually dissolve into the coastal shores to help early creation of seaweed beds.)
  d) Concrete to provide a habitat for small animals (providing living space for relatively small protozoan, polychaete, crustacean larva, insectan larva, and particularly small providing a bed for a diversity of water organisms.)

In general, the on-site construction POC goes through the process of transportation after manufacture, compaction, and curing (see Fig.2). Each process should be performed appropriately, as it has a great impact on the quality of POC. Compared with ordinary concrete, POC possesses less paste amount and has a large contact surface with air. Thus, it is expected that fresh POC is greatly affected during transportation to the construction site after manufacture [2]. It was reported that the time elapsed between mixing and placement decreases the compressive strength of POC [3,4]. Therefore, production/construction guidelines of POC [2] according to the Japan Concrete Institute (JCI) prescribes time from mixing to placement as 90 minutes. Moreover, it recommends 60 minutes as a more desirable time. However, very few systematic studies have investigated the effects of agitating on various physical properties of fresh POC. Therefore, we quantitatively assessed the influence of transportation time on various qualities of POC aiming at the quality assurance of the on-site construction POC.

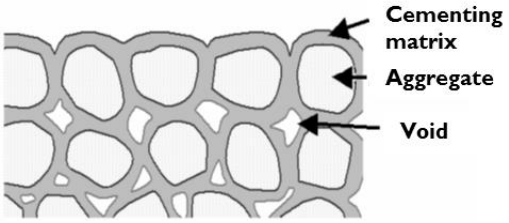


Fig.1 Conceptual figure of composition of POC

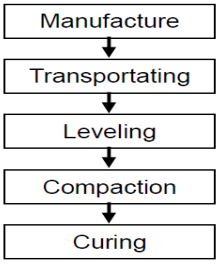


Fig.2 Example of POC construction procedure

2. Outline of experiment

2.1 Experimental factors

Table 1 lists the experimental factors and testing levels considered in this research. For the experiment, crushed stone No.6 (5 mm ~ 13 mm), ordinary Portland cement, and tap water were used for POC. The POC was designed to achieve a void ratio of 25%, and water-cement ratio of 30%. To maintain the fresh properties, we used an admixture (HAE) and examined the effect of the additional amount on the fresh properties and the compressive strength of POC. The agitating time shown in the table indicates the time of agitating fresh POC after mixing at a low speed with a tilting mixer (see Fig.3). Fresh POC was mixed up during 105 minutes with no admixture added.

The unloading time was set by assuming the time required for the fresh POC transported to the construction site. The fresh POC was then removed from the mixer 15 minutes before preparing specimens.

2.2 Specimen preparation method

A 70L tilting mixer was used during mixing and slow agitating of the POC. The mixing was carried out by putting coarse aggregate and cement into a mixer and mixing at 32 rpm for 60 seconds, then adding water and mixing at 32 rpm for 180 seconds. Subsequently, the lid of the tilting mixer was closed and agitated at low speed (1.5 ~ 2 rpm). The mixing, and low-speed agitating were carried out in a thermostatic chamber reproducing the temperature conditions in hot weather with a temperature of 35 to 37 degrees and relative humidity of 50%.

Fresh POC was discharged into a container at the low-speed agitation time of 15, 45, 75 and 105 minutes (if necessary). Then, it was cast in plastic cylindrical mold (φ100×200 mm) within 15 minutes.

The compaction was divided into two layers, and each layer was compacted by pressing and jigging. Three specimens were prepared immediately every 30 minutes after the mixing. Note that, five specimens were prepared at 120 minutes after the mixing because of peeling of aggregate at the end face of the specimen is expected.

The specimens were cured in the molds with their surfaces sealed with vinyl sheets and demolded after 5 days. After demolding, the specimens were placed in water for 27 days. The specimens were capped with sulfur after drying for about 1 hour.

2.3 Experimental method

(1) Measurement of temperature of thermostatic chamber and POC

The temperature inside the thermostatic chamber and of the fresh POC were measured at every 15 minutes after mixing. The air temperature was measured with a resistance thermometer installed in the thermostatic chamber, and for the fresh POC, the sample inside the mixer was measured with a liquid thermometer.

(2) Evaluation of fresh properties

The POC after agitation was subjected to a simple evaluation based on the visual evaluation method [2,5] of fresh properties at 0, 30, 60 and 90 minutes after mixing.

(3) Compressive strength test

Cylindrical specimens for the compressive strength test of POC were subjected by applying sulfur capping to the upper and lower end faces. The test was performed after 28 days based on the laboratory test method of porous concrete according to JIS A 1108 and JCI[2].

3. Results and discussion

3.1 Temperature of thermostatic chamber and POC

Fig.4 shows the change of the temperature inside the thermostatic chamber and of the fresh POC.

From the figure, it can be confirmed that the temperature of the POC becomes almost equal to the temperature inside the thermostatic chamber with an increase of the elapsed time, regardless of the presence or absence of the admixture and the HAE addition rate.

Table 1 Experimental factors and testing levels

Facter	Level
Admixture(HAE)	presence, absence
HAE/C(%)	0.2, 0.3, 0.4
Agitating time(min)	0, 15, 45, 75, (105)

[note]HAE : High-performance AE water reducing agent
HAE/C : HAE addition rate to cement mass

Fig.3 Tilting mixer used during mixing and low speed agitating

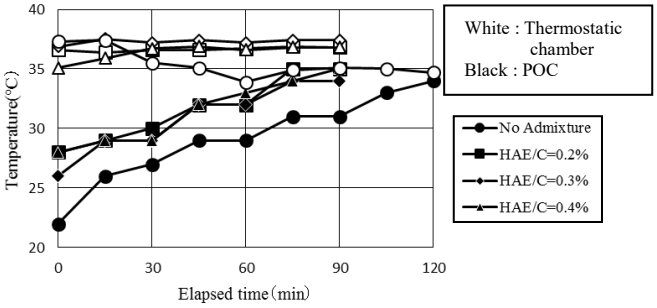


Fig.4 Relationship between temperature and elapsed time

3.2 Visual evaluation of fresh properties

Table 2 shows the changes in fresh properties with elapsed time for each addition rate (HAE/C) of HAE. The range of the photograph in the table is 50 mm×50 mm, and the marks and numbers shown at the bottom of the photograph indicate the visual evaluation of the fresh state of POC and the detailed state of the paste.

From the table, the drying of the paste with no admixture was observed with the increase of the elapsed time. In addition, at 120 minutes of elapsed time, the fluidity and viscosity of the paste decreased, and the workability of the specimens decreased.

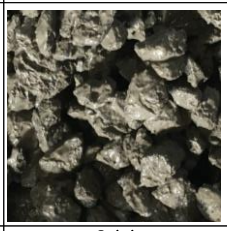
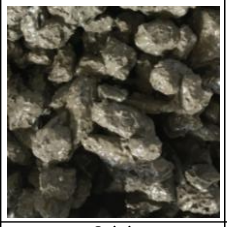
At HAE/C = 0.3% and 0.4%, it was confirmed that good fresh properties were maintained even at 90 minutes after mixing. However, at HAE/C = 0.4%, the fluidity of the paste at 0 minutes was extremely high, and dripping at the bottom of the paste was observed as shown in Fig.5.

3.3 Compressive strength test result

Fig.6 shows the results of compressive strength tests of POC with varying HAE/C.

For the results, the compressive strength of the POC without admixture added decreased with the increase of the elapsed time after mixing. As shown in Fig.7, it is considered that the decrease

Table 2 Changes in fresh properties with elapsed time

HAE/C(%)	Elapsed time(min) 0	30	60	90	120
0 (No Admixture)					
	○(2)	○(2)	○(2)	○(2)	○(2)～×(1)
0.2					
	○(3)	○(3)～○(2)	○(2)	○(2)	
0.3					
	△(4)～○(3)	○(3)	○(3)	○(3)	
0.4					
	△(4)	△(4)～○(3)	○(3)	○(3)	

[note] POC fresh condition (visual evaluation)…△ (4 : The paste draws a thread)
○ (3 : The paste draws a little thread)
○ (2 : The paste does not draw a thread, but it is slightly sticky)
× (1 : The paste is dry)

of the fluidity due to the drying of the paste caused a decrease in the adhesion area between the coarse aggregates.

At HAE/C = 0.2% and 0.3%, the compressive strength decreased with the increase of the elapsed time after mixing, similarly when no admixture was added. Furthermore, at HAE/C = 0.3%, the compressive strength increased by approximately 25% compared to POC without admixture. The reason may be due to the addition of the admixture, where the fluidity of the paste improved, then increased the adhesion area between the coarse aggregates.

At HAE/C = 0.4%, the compressive strength of POC at 0 minutes after mixing is equivalent to that without the admixture, and gradually increases up to 60 minutes. It is considered due to the fact that the variation of the void ratio between the top and bottom of the specimen immediately after mixing is increased because of the paste dripping and which is improved with the increase of the elapsed time. From the results, it is confirmed necessary to consider the influence of transportation time to design the mixture of POC.

Fig.5 Condition of paste on the bottom of the specimen (HAE/C=0.4%, 0 minutes after mixing)

4. Conclusion

The authors investigated the effect of the elapsed time after mixing and the amount of admixture added on the fresh properties and compressive strength of POC under hot conditions (23 ~ 35 degrees). The following are the findings obtained.

(1) The decreasing rate of compressive strength of POC without admixture became 28% at 120 minutes after mixing.

(2) The compressive strength of POC with admixture of HAE/C = 0.3% increased by approximately 25% compared with POC without admixture under constant water-cement ratio and void ratio (W/C = 0.3, V_R = 25%).

In the future, POC is expected to be a social infrastructure material that plays an important role not only in the environmental aspect but also in disaster prevention such as heavy rain countermeasures. The establishment of construction standards based on this research will contribute to the disseminating of POC.

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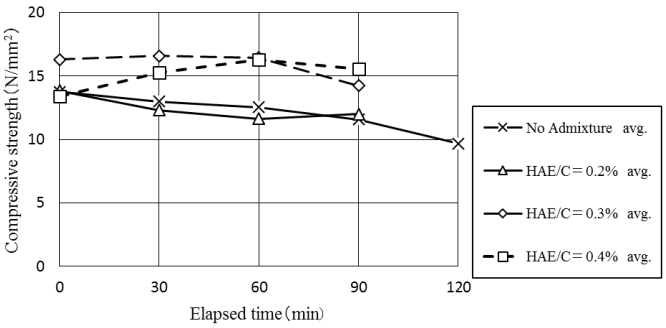
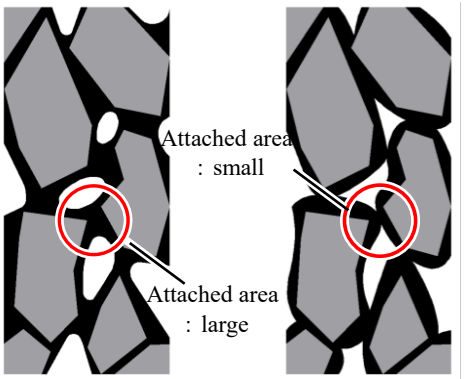


Fig.6 Relationship between compression strength and elapsed time (W/C = 0.3, V_R = 25%)



(a) Paste fluidity：Good (b) Paste fluidity：Bad

Fig.7 Conceptual diagram of adhesion failure between coarse aggregate due to change of fresh properties of POC

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Primary Identification of *Alternaria* Species by the Morphological Characteristics

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Abstract: The fungi *Alternaria* species are widely distributed throughout the world. Most of those species are saprophytes, receiving nutrients from dead organisms, but a few of them are considered important plant pathogens and can produce host specific toxins. The accurate identification and taxonomy of such species are important in order to control the diseases they might cause. However, it is difficult even for professionals to identify whether *Alternaria* species observed from the symptoms are pathogenic or not from morphological characteristics. To solve the problem, this study aimed at making a database for the primary identification of *Alternaria* species by pattern recognition. Isolates of various identified *Alternaria* species were incubated on PCA plates. The images of the characteristics, or each colony, were captured with a camera. Based on these data, a database was built for identification by pattern recognition. These results contribute to the establishment of a primary identification system for the genus *Alternaria* species by pattern recognition.

Key words: *Alternaria*, plant pathogen, primary identification, pattern recognition.

1. Introduction

Fungi have hyphae, which are multicellular and filamentous structures, and disperse the spores, which are somatic and reproductive cells. They are eukaryotic organisms that live mainly by decomposing and absorbing organic substrates. Fungi are generally known as mold. They are often observed on food or in damp rooms, sometimes affect plants, and cause diseases which lead to devastating damage and losses. The genus *Alternaria* are filamentous fungi that are widely distributed throughout the world. Most of them are saprophytes, but several taxa are known as postharvest pathogens for major crops, causative agents of phaeohyphomycosis in immune-compromised patients, or airborne allergens. Some taxa produce host specific toxins that cause heavy pathogenicity or toxic spots on Japanese pears (Woudenberg et al. 2013). On the other hand, the identification of these species and discrimination of pathogens from saprophytes, which is of central importance to control these diseases, is generally difficult because of a lack of knowledge of their morphological characteristics. Although using DNA sequences has recently become a major tool for taxonomy and identification of species, it is still necessary to perform analysis in multiple loci, which is considered difficult.

In conventional methods, the genus *Alternaria* has been classified and identified mainly by its morphology and host specificity. However, as Simmons (2007) indicated, a tool such as a dichotomous key based on those morphological characteristics has not been practically used to identify the species, and the method to estimate the pathogens with that is still difficult and time consuming even for professionals.

Nowadays, in plant pathology, the diagnosis and identification of pathogens with image recognition technology have been attempted based on taken pictures of lesions which show characteristics of disease (Mohanty et al. 2016). However, no attempts have been made in the identification of fungi by image recognition. Therefore, the aim of this study is to consolidate a database of images of morphological characteristics of *Alternaria* isolates, which have been preserved in Mie University, and to develop the preliminary identification method applicable to the laboratory setting.

2. Materials and methods

2.1 Isolates

30 strains of *Alternaria* and its allied genera, *Ulocladium*, *Stemphylium*, and *Pleospora*, were used in this study They have been preserved in the culture collection of the laboratory of plant pathology (MUCC) in Mie University. The species of *Alternaria* were; *A. alternata*, *A. alternata* Japanese pear pathotype, *A. alternata* strawberry pathotype, *A. brassicae*, *A. brassicicola*, *A. cinerariae*, *A. crassa*, *A. cucumerina*, *A. cumini*, *A. dauci*, *A. gaisen*, *A. gomphrenae*, *A. helianthi*, *A. iridicola*, *A. japonica*, *A. nobilis*, *A. panax*, *A. paragomphrenae*, *A. porri*, *A. zinniae*, and *Alternaria* sp. The species of *Ulocladium* were *U. atrum*, *U. botrytis*, *U. chartarum*, and *Ulocladium* sp. Regarding *Stemphylium*, *S. botryosum*, *S. lycopersic*, *S. lycopersici* and *S. vesicarium* were used. *Pleospora* sp. was used as the strain for the genus *Pleospora*.

2.2 Image Acquisition of Characteristics of the Cultured Colonies and Its Data Base Development

In order to take pictures of cultural characters, each mycelial disc hollowed out by sterilized polypropylene-straw was transferred onto the center of each PCA plate so that the surface with mycelia could touch the plate, and then incubated at 25℃ in the dark for 7 days (PCA; Simmons 2007). Both the surface and back of the plates were photographed (Canon EOS 80D) with two kinds of backgrounds, black cloth and white paper. Those images were then trimmed so that only the plates remained and sorted by species to establish the database.

2.3 Image Diagnosis

Based on the information about the pathogenicity connected to the test strains, each was grouped into two categories – one with pathogenicity and the other without. 80% of the images were uploaded as training data to Azure Custom Vision Service. The rest of the images were submitted to the created learning model as a test. The performance of the model was evaluated in terms of precision, recall and average precision (A.P.). Precision was calculated as TP/(TP+FP), which shows the probability that the model predicted it correctly and the answer is correct. On the other hand, recall was calculated as TP/(TP+FN), which is the probability that the precision is correct out of all correct answers; TP, FP, TN, FN are the number of true positives, false positives, true negatives, and false negatives, respectively. Average precision is integral to the area under precision/recall curve, and it reflects performance throughout the

whole model.

3 Results

As a result of uploading and training the collected image data to the Azure Custom Vision Service, the resulting precision of the training model was 94.1%, the recall was 94.1%, and A.P. was 96.7% (Table 1). The precision of the images of non-pathogenic isolates was 59.6%. This precision suggested that it had a high possibility to be a false positive. Its overall performance was 61.4%. It is conceivable that this is because the number of images was small. The group of pathogens had a performance of 98% as a whole.

Table 1. Performance of each category and the training model

	Precision(%)	Recall(%)	A.P.(%)	Image count
Non-pathogen	59.6	73.9	61.4	227
Pathogen	97.7	95.8	98.8	2715
Training Model	94.1	94.1	96.7	2942

After uploading the remainder of the test image data, the training model performed at an accuracy of 90% and above with non-pathogens, while that of almost all pathogens was over 98%. It can be suggested that this was because the amount of data for pathogens was enough for analysis but that for non-pathogens was inadequate. However, only a few of them attained 50 to 80% accuracy and, also, included completely incorrect ones. There was a tendency of low accuracy when the colony was of a dark color and when the background was black as well.

4. Discussion

In this study, a new training model for the primary identification of plant pathogens using morphological characteristics was created using the Azure Custom Vision Service. The accuracy rate of identification was lower for non-pathogens. On the other hand, several pathogens showed low accuracy as well. The reasons are as follows. First, this time, the non-pathogenic species was only *Alternaria alternata* which is indistinguishable from *A. alternata* pathotypes in terms of morphology, which led to much less image data compared with that for pathogens. To solve this problem, it would be effective to use more isolates originated from non-pathogenic species or to keep a balance by using less images of pathogenic species. As the second reason, the color of the colony and the background showed influence. Dark colored colonies with a black cloth or light-colored colonies with white paper background made it difficult for the model to identify because of the similar color. On the other hand, the opposite combination had high performance. Thus, it would solve the problem of backgrounds to make a group of each species.

This time, to make it simple, only pictures of colonies were used and the adjustment of color temperature was not carried out. Simmons (2007) includes conidia and the sporulation patterns observed under the microscope as important factors for the identification of *Alternaria* species. By adding these data, the model performance would be higher and it could be possible to realize not only the identification to whether the sample is a pathogen or not, but also for the identification of the species itself.

When the primary identification with the images of only the morphological characteristics is achieved, it will be possible to make rapid and flexible assessments to deal with diseases in the future. This would enable the prevention and conjecture in advance regarding the spread of disease, which would be a clue for solutions for various kinds of problems, such as food shortages or the reduction of income of farmers associated with yield loss.

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Purification of Capsid F protein of Bacteriophage ϕX174

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Abstract: Food poisoning caused by bacteria is considered a serious problem. We expect that using bacteriophage, a virus which can infect only bacteria, to potentially solve the problem. The bacteriophage ϕX174 recognizes lipopolysaccharides (LPS) on bacteria by capsid proteins and infects the bacteria by changing the protein's conformation. The G and H proteins are known for their ability to recognize LPS. However, recent studies showed that the F protein might have such a capability. To investigate the interactions between the F protein and LPS, this study examines the purification of the F protein. The conditions of culturing and purification of the F protein with the His-tag at the N-terminal (HisF protein) were re-examined. Additionally, a novel plasmid which encodes fusion proteins having the His-tag on C-terminal of F protein was constructed. The HisF was expressed in an insoluble fraction. The addition of urea made HisF denatured and transferred to a soluble fraction. However, the HisF precipitated again and failed to be purified. This study reveals that the HisF can be purified via the denatured condition. The ion-exchange chromatography under such a condition will attempt to solve that issue. The refined F protein will make it possible to study its interactions with LPS.

Key words: Purification of protein, bacteriophage, virus infection

1. Introduction

Every year many people are afflicted with food borne illnesses caused by bacteria. They can grow and increase their numbers in foods and in our bodies. To prevent food poisoning, it is important to control the bacteria. Bacteriophage is a virus which can specifically infect their host bacteria, e.g. *Escherichia coli*, and can kill them selectively. Antibiotics can also control bacteria, but they lack such selectivity. Therefore, using bacteriophage, known as phage therapy, has received attention for solving the food poisoning problem (Wernicki, Nowaczek, & Urban-Chmiel, 2017). We studied bacteriophage ϕX174 which is known to infect *Escherichia coli* in order to investigate the role of viral proteins. The phage recognizes lipopolysaccharides (LPS) on the surface of bacteria by capsid proteins and enters the cell (Fig. 1). When ϕX174 infects host cells, it uses mainly two spike proteins, the G and H proteins, at the vertices of the icosahedral particles. (Neuwaldi, 1975). However, several studies reported that the F protein (Fig. 2), which forms the viral icosahedral capsid, also recognizes a part of LPS (McKenna, 1992). To study the interactions between the F protein and LPS, a sufficient amount of highly purified F protein is needed. A previous study (Sekiguchi, 2006) constructed a bacterium which expresses the F protein carrying Histidine tag on the *N*-terminal, called HisF. However, the prepared F protein was insufficient for its purity and amount.

This experiment performed purification of the HisF protein by changing the conditions of culturing and purification in order to obtain enough to examine the interactions between the F protein and LPS. Also, a novel plasmid expressing the F protein with a Histidine tag on its *C*-terminal (cHisF protein) was constructed because both the *C*-terminal and *N* terminal seemed to exist near the surface of virus according to X-ray results.

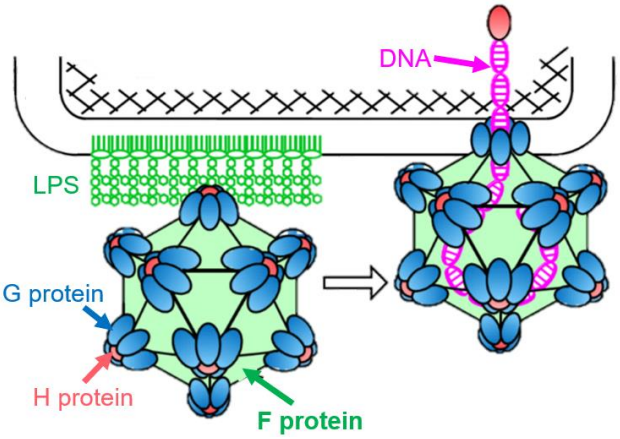

Fig. 1 The extrapolated mechanism of infection of ϕX174.

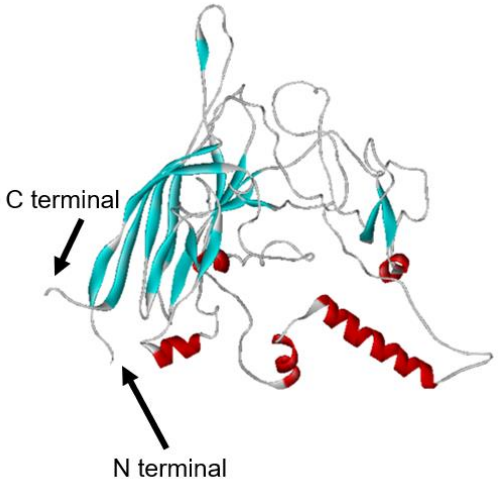

Fig. 2 X-ray structure of capsid F protein of bacteriophage ϕX174. PDB ID 1AL0

2. Materials and Methods

2.1 Purification of HisF protein

Bacteria, which haves the plasmid pQE-30+F constructed in a previous study, were inoculated into LB (lysogeny broth) agar medium containing 100 μg/ml ampicillin. The colonies were cultured in the LB medium with ampicillin and induced the expression of HisF with IPTG (nakalai tesque). After collecting bacteria by centrifugation, the expression of HisF protein was checked using SDS-PAGE (12.5%). Bradford protein assay (Biorad) was then used to know the concentration of proteins. Purification of the HisF protein was performed with the Profinia Protein Purification System (Biorad) which performs automated affinity purification of protein. In order to denature HisF protein, 8 M urea (nakalai tesque) was used. We used dialysis to remove urea from the protein solution with Bio Design Dialysis Tubing (Bio Design, 8000 MWCO). Ion exchange chromatography was conducted using a Hi Trap Q HP column (GE Healthcare).

2.2 Constructing of cHisF protein

The DNA fragment encoding F protein was amplified using the plasmid pQE-30+F, following the PCR (Polymerase Chain Reaction) method. The primers (Table 1) were purchased from Eurofins Genomics. The amplified DNA fragments and plasmid pQE-60 were treated using the restriction enzymes, *Bam* HI and *Bgl* II. After eliminating the enzymes, these two DNA fragments were then fused with T4 ligase (Toyobo). Enzymes were eliminated by phenol-chloroform extraction. The constructed plasmid was transformed into *Escherichia coli* JM109 by electroporation.

Table 1 The sequence of primers

		Tm (℃)	GC-content (%)
Forward	5'-CCCGGATCCATGTCT AATATTCAAACTGG-3'	61	45
Reverse	5'-GGGAGATCTCACGAA GTCATGATTGA-3'	60	46

3 Results

3.1 Purification of HisF protein

In this study, we sought to purify F proteins in order to study the interactions between F proteins and LPS. On the whole, the F protein was easily agglutinated. An expression check of the F protein was performed using SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). Fig. 3 shows that HisF protein was expressed in all colonies having pQE-30+F. The concentration of protein which was used for purification was measured using a Bradford protein assay. It was 1.58 mg/ml (Fig.4).

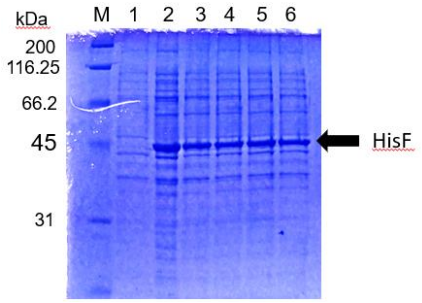

Fig. 3 Check of protein expression. All colonies of bacteria having pQE-30+F express F protein. Lanes: M, molecular markers; 1, whole cell of *Escherichia coli* JM109 with pQE-30 plasmid; 2-6, whole cells of *Escherichia coli* JM109 with pQE-30+F plasmid.

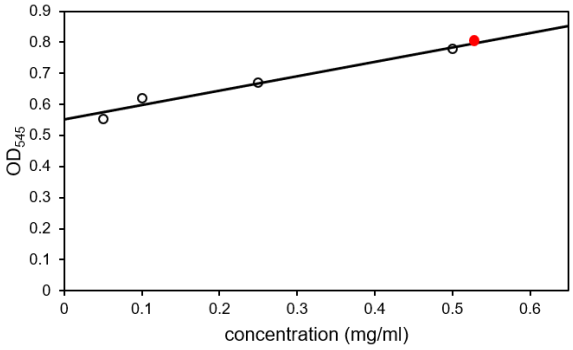

Fig. 4 Concentration determination of HisF protein by Bradford assay. This graph was drawn with the results of BSA. The red point shows the OD_{545} of His F protein. The estimated concentration of HisF was 1.58 mg/ml.

We attempted to purify F proteins from soluble fraction using a Ni-NTA affinity chromatography with profinia^{TM}. In Fig. 5(a) two bands were revealed. However, the F protein band was a minor one. The obtained amount of HisF protein was less than expected. Consequently, we checked the protein solution and found that most of HisF proteins were in the insoluble fraction. (Fig. 5(b)). Ni affinity chromatography cannot be used for insoluble fraction. We changed the culturing conditions of bacteria expressing HisF protein to solubilize the HisF protein. First, the change of final concentration of IPTG from 1 to 5 mM was conducted. However, the HisF protein was still included in the insoluble fraction in all conditions (Fig. 6(a)). Next, the concentration of LB medium and culture temperature were changed. However, the HisF protein was found in the insoluble fraction (Fig. 6(b), (c)).

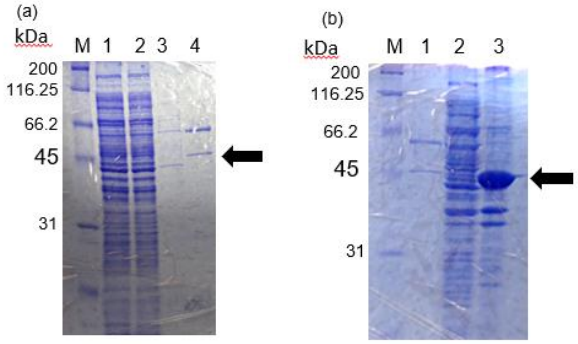

Fig. 5(a) SDS-PAGE of purified HisF protein. Lane: M, molecular marker; 1, eliminated protein by first wash; 2, eliminated protein by second wash; 3, eliminated protein by third wash; 4, purified protein. (b) SDS-PAGE of protein solution. Lane: M, marker; 1, purified fraction; 2, soluble fraction which was used for Ni affinity chromatography; 3, insoluble fraction.

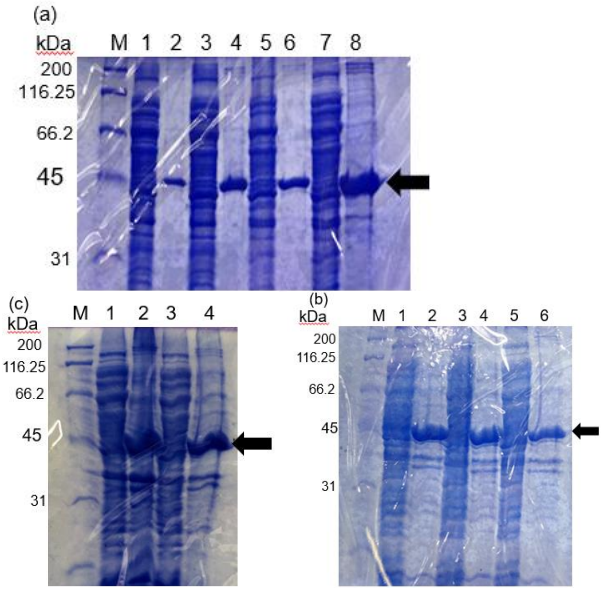

Fig. 6(a) Optimization of the concentration of IPTG. Lane; M, marker: 1 and 2, 0.1 mM IPTG soluble and insoluble fraction: 3 and 4, 0.2 mM IPTG soluble and insoluble fraction: 5 and 6, 0.3 mM IPTG soluble and insoluble fraction: 7 and 8, 0.5 mM IPTG soluble and insoluble fraction. (b) Optimization of the concentration of LB medium. Lane; M, marker: 1 and 2, soluble and insoluble fraction of bacteria cultured in LB medium diluted in the rate of 2: 3 and 4, soluble and insoluble fraction of bacteria cultured in normal LB medium. (c) Optimization of culture temperature. Lane; M, marker: 1 and 2, soluble and insoluble fraction of bacteria cultures at 20℃: 3 and 4, soluble and insoluble fraction of bacteria cultures at 25 ℃: 5 and 6, soluble and insoluble fraction of bacteria cultures at 37 ℃.

We attempted to purify under denatured conditions. At first, the HisF protein was denatured and solubilized by urea (Fig. 7(a)). The solubilized HisF protein was then purified using Profinia™. However, most of HisF protein passed through the Ni column and eluted with contaminants (Fig. 7(b)).

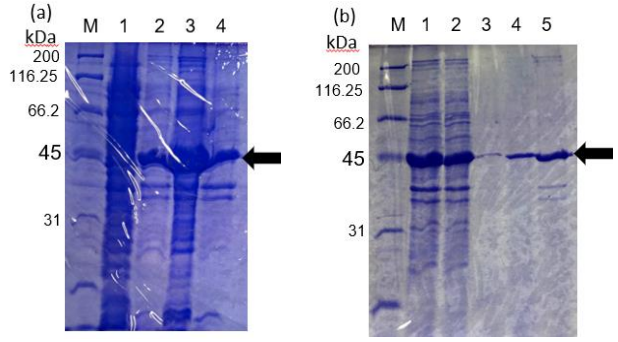

Fig. 7(a) Solubilization of HisF protein. Lane; M, marker: 1, soluble protein: 2, insoluble protein: 3, solubilized protein: 4, protein which could not be solubilized. (b) Purification of solubilized HisF protein. Lane; M, marker: 1, eliminated protein by first wash: 2, eliminated protein by second wash: 3, eliminated protein by third wash: 4, purified protein: 5, the insoluble protein which was solubilized.

3.2 Constructing of cHisF protein

As a result of the HisF protein experiment, we changed the way to construct a new plasmid which has the F protein with an His tag on its C terminal. DNA fragment encoding F protein was amplified using PCR. pQE-60 was extracted from *Escherichia coli* JM109 having pQE-60 plasmid. The amplified DNA fragments (1,295 bp) and pQE-60 (3,431 bp) were checked by agarose gel electrophoresis (Fig. 8(a)). The amplified DNA fragments and the plasmid were digested by restriction enzymes, *Bam* HI and *Bgl* II. Before the treatment, the plasmid showed three bands, 2,400 bp, 3,400 bp, and 5,000 bp. But after the treatment of enzymes, the DNA fragment of 3,400 was increased (Fig. 8(a)). This means the treatment of restriction enzyme was successful. After purification of the plasmid, a ligation reaction was conducted to join the two kinds of DNA, and the constructed plasmid was transformed into *Escherichia coli* JM109 through electroporation. If the transformation succeeded, the bacteria would resist ampicillin. However, no colonies were obtained in the LB plate with ampicillin.

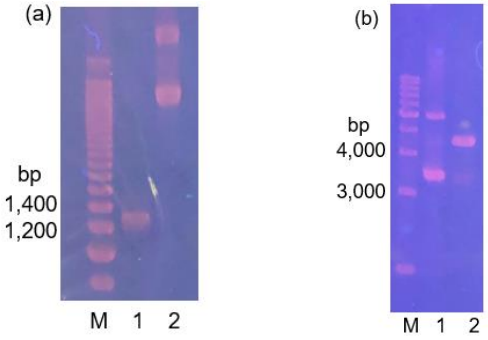

Fig. 8(a) Electrophoresis of DNA fragment encoding F protein and plasmid pQE-60. Lane; M, marker: 1, F protein with the cleavage site of the restriction enzymes: 2, plasmid pQE-30+F. (b) Restriction enzymes treatment of pQE-60. Lane; M, marker: 1, F protein with the cleavage site of the restriction enzymes: 2, digested F protein with the cleavage site of the restriction enzymes.

4. Discussion

We attempted to prepare an F protein by changing the culturing and purification conditions and constructing a new bacterium which would express the F protein in order to study the interactions between it and lipopolysaccharides (LPS). However, we did not attain any highly purified F proteins because of their high hydrophobicity. To solve this problem, we will attempt to purify under denatured conditions. With sufficient amounts of prepared high-level purified F protein, we will be able to study the interactions between the F protein of bacteriophage ϕX174 and LPS on the *Escherichia coli*. This investigation could eventually reveal the important role of F proteins in infection. Progress in the study regarding the phage therapy which can be used for treatments of food poisonings caused by bacteria is expected in the future.

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Soils are living! Microbial activities in soils containing various organic matters

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Abstract: Farmers sometimes add organic matter. Organic nitrogen decomposes to Ammonia nitrogen (NH_4-N), and transforms to Nitrate nitrogen (NO_3-N). To predict organic matter decomposition, monitoring the NH_4-N in the soil is necessary. However, this measurement is time consuming and costly. On the other hand, these reactions in soil have been found to be caused by microorganisms and the microbial activity (ATP amount) has become easier to measure due to advances in technology. Therefore this study investigated the amount of ATP in soils containing various organic matters and the relationship to the change in NH_4-N.

The samples were Iwate Andisol mixed with several organic matters. They were packed into 50 mL columns, and settled in a 25 °C chamber. Some samples were taken out more than six times, and NH_4-N and NO_3-N concentrations were measured using a absorptiometer while the ATP amount was measured by firefly luciferase. The change of NH_4-N was analyzed through a first-order reaction equation. Results showed that the ATP amount varied with changes in NH_4-N concentrations. The change in ATP amount was divided to three stages. These results imply that the amount of ATP can be used to predict the NH_4-N concentration in soil.

Key words: organic matter decomposition, ATP, first order reaction, microorganisms

1. Introduction

In agriculture, chemical fertilizer is applied to increase crop production and save labor. However, continuous use of chemical fertilizers in soils leads to the degradation of soil properties, such as hardness, water retention, permittivity, and ion exchange capacities, making sustainable farming unfeasible. To improve soil properties, organic matters are often applied to soil. Carbon and nitrogen components in organic matter are utilized by the soil microorganisms, thereby increasing soil microbial activity and diversity.

Nitrogen composed in the organic matter decomposes to Ammonia nitrogen NH_4-N (mineralization), and then transforms into Nitrate nitrogen NO_3-N (nitrification). As useful as NO_3-N is for plants, it also pollutes ground water. Therefore, it is necessary to predict NH_4-N and NO_3-N concentrations in order to apply suitable amounts to the soil. Moreover, understanding the suitable amount of organic fertilizer to apply reduces the cost for cropping.

The mineralization rate of soil organic matter depends on soil moisture content, temperature, and C/N which is the ratio of carbon to nitrogen in the organic matter. NH_4-N in soil can be taken up by microorganisms (immobilization), especially where there is high C/N. Hirose (1973) showed that organic matter having a C/N higher than 10 tends to be immobilized. The mineralization rate of different soil organic matters can be predicted by applying a first order reaction equation to the change in the NH_4-N and NO_3-N concentrations in the soil. To solve the reaction equation, we need to determine the rate constant, which requires sequential measurements of NH_4-N concentration in soil. However this measurement is time consuming and costly, which has made it difficult to analyze many kinds and conditions of soil.

Mineralization is caused by soil microorganisms. Recently, an easy and quick method to measure the microbial activity (ATP amount) has been developed by Kikkoman Biochemifa Co. Ltd. This method has been applied in various environments, such as in the food sanitation industry. Recently, Muto et al. (2018) tried to use this method for investigating nitrification in soil and reported that the amount of soil ATP was proportional to the nitrification rate. However, the relationship between the amount of ATP and soil organic decomposition rate is still unclear.

Therefore, the goals of this study are to monitor ATP amount in soils using this method during decomposition of various types of organic matter and to investigate the relationships of the ATP to the soil organic matter decomposition ratio, and to its rate constant.

2. Materials and methods

Iwate Andisol was used in this study. The Andisol was collected from the A horizon at an experimental field at Iwate University, Japan. The experimental field has been managed as a weeded fallow field for several years. The soil was sieved through 2-mm mesh. Oil meal, clover, rice bran and rice straw were added to the Andisol with 400 mg organic-C/100$g_{drysoil}$. The clover and rice straw were crushed through 2-mm mesh after air-drying. Details of the organic matters are shown in Table 1.

Tab.1 C/N and N content of organic matters

Symbol	Organic matter	C/N	The amount of org-N (mg/100$g_{-drysoil}$)
A	Oil meal	7	54
B1 B2	Clover*	16	21 – 26
C	Rice bran	18	22
D	Clover with roots	23	16
E	Rice straw*	35	11

*Crushed into 2 mm fragment after air drying

The samples were packed into 50 mL-stainless columns with water content of 0.4 and dry bulk density of 0.9 g/cm^3, and settled in a desiccator at a temperature of 25 °C (Batch experiment). A sample was taken out at more than six days, and the soil pH and EC were measured. The soil water was extracted from samples and the concentrations of NH_4-N and nitrate nitrogen NO_3-N were measured using a adsorptiometer (DR6000, Hach) while ATP was measured by firefly luciferase (Rumitester C-110, Kikkoman Biochemifa). The change in NH_4-N by mineralization and nitrification was then analyzed through the first-order equations (1) and (2)

increased to 0.56 mg/100g $_{drysoil}$, dropped to 0.1 mg/100g

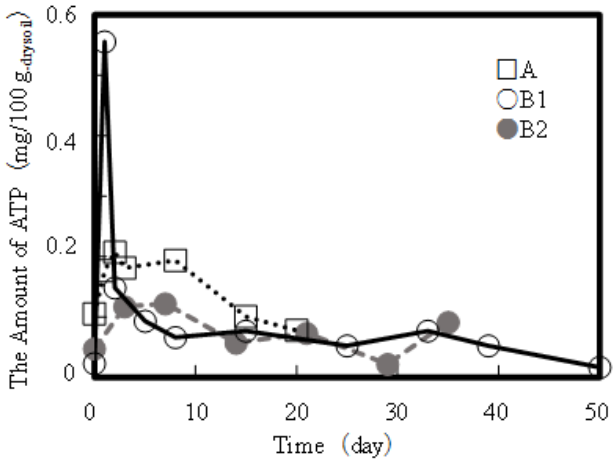

Fig.1 Amount of ATP in A,B1,B2

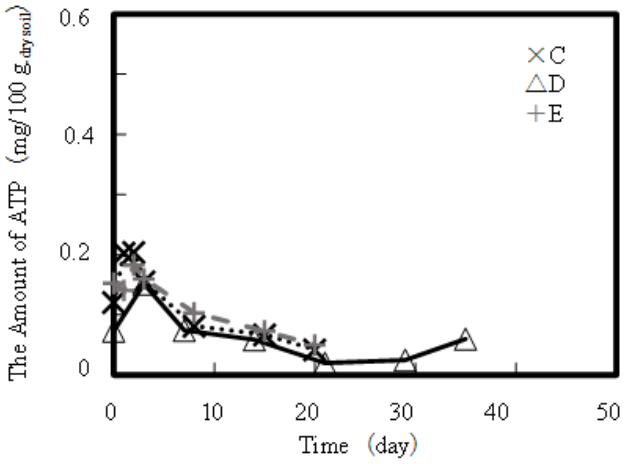

Fig.2 Amount of ATP in C,D,E

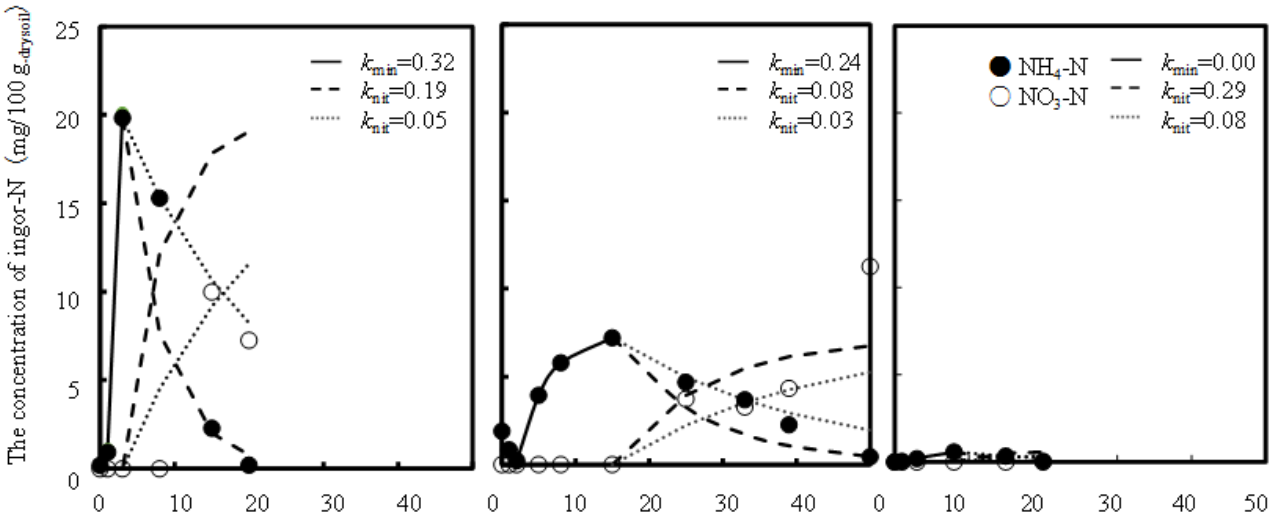

Fig.3 Inorg-N concentration in A
Fig.4 Inorg-N concentration in B2
Fig.5 Inorg-N concentration in C

$$\frac{dC_{org\text{-}N}}{dt} = -k_{min} C_{NH4\text{-}N}$$
$$\frac{dC_{NH4\text{-}N}}{dt} = k_{min} C_{NH4\text{-}N} \quad \cdots(1)$$

$$\frac{dC_{NH4\text{-}N}}{dt} = -k_{nit} C_{NH4\text{-}N}$$
$$\frac{dC_{NO3\text{-}N}}{dt} = k_{nit} C_{NH4\text{-}N} \quad \cdots(2)$$

where $C_{org\text{-}N}$, $C_{NH4\text{-}N}$, and $C_{NO3\text{-}N}$ are concentrations of organic matter, NH_4-N, NO_3-N, t is the time, and k_{min} and k_{nit} are decomposition and nitrification rate, respectively.

3. Result and discussion

During batch experiments, water content and temperature of all samples were kept constant (approximately 0.4 cm^3/cm^3 and 25 °C). The changes in ATP amount in samples were shown in Fig.1 and 2. In the sample with clover added (B1), the amount of NH_4-N was 0.02 mg/100g $_{drysoil}$ at the beginning, quickly

$_{drysoil}$ and then remained constant.

Fig.3, 4 and 5 show the change in NH_4-N and NO_3-N concentrations in the sample added oil meal (A), clover (B1), and rice bran (C). Symbols indicate the measured data categories. In sample with oil meal added (A), the concentration of NH_4-N increased from the beginning, and reached 20.1 mg/100$g_{drysoil}$ on the third day. It is considered that mineralization was superior to nitrification and other biochemical relations in this duration. After the NH_4-N reached a peak concentration (the third day), the concentration of NO_3-N increased with the decreasing NH_4-N concentration. This may come from nitrification. The concentration of NH_4-N remained high until the eighth day. This corresponded to the period when the amount of ATP was high (Fig. 1), which implies active growth of soil microorganisms and production of NH_4-N by soil microorganisms. The constant amount of ATP after the eighth day would relate to the activity of nitrification bacterium. In sample with clover added (B2), similar changes in ATP, NH_4-N and NO_3-N were observed as (A), except during first decline period in NH_4-N: the concentration of NH_4-N was 2.0 mg/100$g_{drysoil}$ at the beginning, and then decreased to 0.3 mg/100$g_{drysoil}$ after two days. In the first NH_4-N decline period, it can be considered that microorganisms intensely grew in the soil with newly added organic matter (immobilization), drastically increasing the ATP amount, shown in Fig. 1. In the sample with rice bran added, the concentration

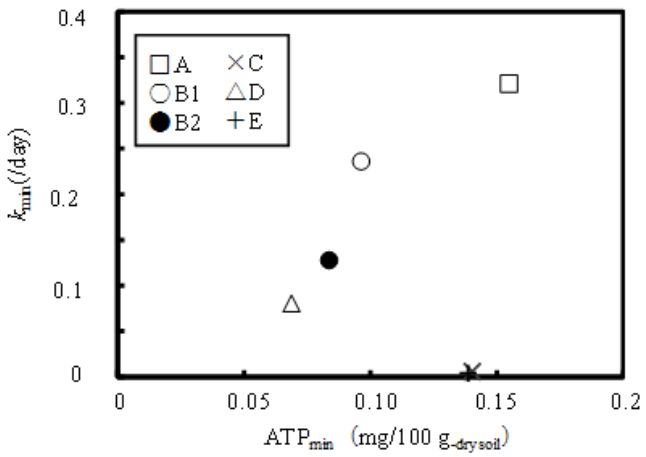

Fig.6 Relationship between ATP_{min} and k_{min}

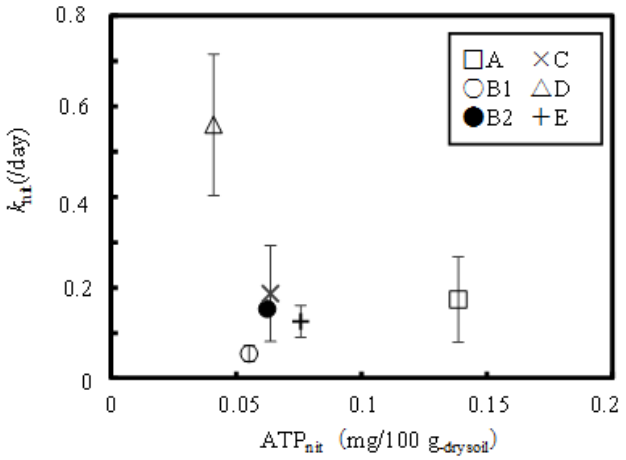

Fig.7 Relationship between ATP_{nit} and k_{nit}

of NH_4-N did not increase. According to the ATP amount (Fig. 2), soil microorganisms were active, so immobilization was supposed to be superior to mineralization. Additionally, NO_3-N was not produced for this sample, which means nitrification did not occur. This was also seen in the samples with rice straw where C/N was high.

The observed data was fitted by Equation (1) (solid line in Fig.2) and Equation 2 (dashed and dotted lines in Fig. 2). The Equation (1) nicely reproduced observed data, and we can determine k_{min} for each type of organic matter. However, the Equation (2) vaguely reproduced the observation with a wide range of k_{nit} (0.03 to 0.08 in Fig. 4). This implies that nitrification following organic matter decomposition is more complex than the nitrification observed in Muto et al. (2018), where soil contained NH_4-N and no organic matter.

Whether nitrogen was more used for immobilization or mineralization depended on the C/N of each type of organic matter. The peak concentration of NH_4-N was related to the C/N for each sample, which means k_{min} is different for each kind of organic matter.

Fig.6 and 7 showed the relationship between k_{min} and ATP_{min}, and k_{nit} and ATP_{nit}. The ATP_{min} and ATP_{nit} represent the average of ATP amount during mineralization and nitrification. ATP_{min} was proportional to k_{min}. This is because more NH_4-N was produced by more microorganisms. There is some possibility for predicting NH_4-N concentration in soils by monitoring soil ATP, which is much easier than measuring the NH_4-N. On the other hand, no relationship was found between ATP_{nit} and k_{nit}.

4. Conclusion

In this study, we aimed to monitor ATP (microbial activity) in organic matter decomposition process and to understand the relationships between ATP and mineralization, and the nitrification rate. The amount of ATP was monitored during immobilization, mineralization, and nitrification during batch experiments of soils containing various kinds of organic matter.

When organic matter was added to the soil, the amount of soil ATP increased rapidly, decreased soon after, and then kept constant. This result might indicate that the microorganism activity increased with organic matter addition, and then reached a stable value during the mineralization and nitrification periods. The k_{min} (constant rate of mineralization) was determined by fitting the increase of NH_4-N data to Equation (1) for each organic matter. The peak concentration of NH_4-N and k_{min} depended on the kind of organic matter. On the other hand, the average of ATP in mineralization was proportional to the k_{min}. This implies that we can predict k_{min} from monitoring ATP, which is easier and cheaper to measure than NH_4-N itself. If the first-order constant can be predicted easily, it makes the application of organic fertilizer easier as well. Applying suitable amounts of organic matter to soils leads to the prevention of ground pollution and the reduction of fertilizer cost. More farmers may be trying to improve soil properties by using organic fertilize which is difficult to treat. If soil characteristics are improved, farming will be sustainable and vegetables will continuously be produced. It remains necessary to clarify the decomposition of organic matter under various conditions such as rainfall to apply it to the actual field of farming. In the future, experiments using a rainfall device etc. will be conducted, and the mechanism of decomposition of organic matter with regards to microorganism and chemical changes will be applied.

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Soil physics

Short comment
I would like to communicate with many people.

Kunio Watanabe

Professor
Graduated school of Bio resources
Soil physics

Short comment
We also study about frozen soil.

Development of Vision-based Localization and Mapping System for Autonoumous Agricultural Robot

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Abstract: The Japanese agricultural sector faces several problems such as depopulation, aging workforce, and physical workloads. In order to contend with these problems, we have developed a satellite-based agricultural robot to assist workers by using GNSS (Global Navigation Satellite System). However, the system cannot be used in some areas due to geographical problems. In this study, a vision-based localization and mapping system were developed to overcome such problem, by adding the functions that it can not only localize its position but also map objects along the driving route without using GNSS. The functions of localization and mapping were developed using a visual- SLAM (Simultaneous Localization and Mapping) and an object detection deep learning model. The system and model were tested in a single targeted rural area. As the result, the robot successfully achieved the function of localizing the movement of workers. Moreover, it is capable of mapping the trajectories of workers as well as objects along the driving route. We conclude that our developed system can be used without using GNSS, in particular in the case of agricultural fields. Further research will be needed for different types of agricultural fields to monitor the performance of localization and the mapping system.

Key words: Autonomous agricultural robot, ORB-SLAM, deep learning, YOLO.

1. Introduction

The Japanese agricultural sector faces several serious problems, such as depopulation, a rapidly aging workforce, and extreme physical workloads. In order to solve these problems, agricultural robots have been developed for over ten years. In 2018, Japanese agricultural machine manufacturers released robot tractors, robot combine harvesters, and robot planting machines with automated driving systems of SAE (Society of Automotive Engineers) Level 2. These robots are able to drive autonomously by using GNSS (Global Navigation Satellite System) which can measure positions with high accuracy up to several centimeters. Nevertheless, the position accuracy decreases in some places where it is difficult to receive GNSS signal, such as near buildings and trees, inside garages, and in hilly areas.

In our previous studies, we developed several types of systems for worker tracking and worker behavioral recognition using image processing (Morio et al., 2012, 2016, 2017). The system autonomously could control cameras on vehicles in order to track workers and identify workflows based on worker behavior in the fields. Moreover, we developed an agricultural scene recognition system (Morio et al, 2018). The system could identify its position on generated 3D map with GNSS, to match current and recorded field scenes. However, the system could only be applied in agricultural fields which could receive satellite information as GNSS.

Therefore, this study developed an agricultural autonomous robot using a camera in order to solve the problem. Vision-based localization and mapping systems were combined to obtain two functions at once. The first function was to localize the robot itself by using a stereo camera. The second function was to map objects along the driving route by detecting and estimating their positions. A visual-based SLAM (Simultaneous Localization and Mapping) system and deep learning model for object detection were applied for developing these functions. In the visual SLAM system, the ORB-SLAM2 method was applied by using a stereo camera (Mur-Artal and Tardós, 2017). The method was customized and tuned so that the system could robustly work for some specific scenes. Meanwhile, in deep learning, the YOLOv3 model family was used for recognizing and detecting objects around the driving route. (Redmon et al., 2016, 2017, 2018).

2. System Overview

Our vision-based localization and mapping system was used to estimate the positon of a robot, and to generate a 3D map for navigating such robots. The system has three processes: the image capturing process with a stereo camera, the localization and mapping process with ORB-SLAM2, and the object recognition and mapping process with YOLOv3. In the image capturing process, an experimenter carried a stereo camera to capture a targeted field. In the localization and mapping process, the camera trajectory was drawn on a 3D navigation map by using our customized ORB-SLAM2. The generated navigation map was also applied to estimate the robot's position by using the localization mode of the ORB-SLAM2 algorithm. In the object recognition and mapping process with YOLOv3, road edge, observed along a working route in the targeted area, was identified using our trained YOLOv3 models. Besides, recognized road edge was estimated the 3D positon, and drawn on generated 3D map with ORB-SLAM2.

2.1 Image capturing process using stereo camera

The stereo camera was built using two web cameras (Microsoft LifeCamStudio). The cameras were fastened with a baseline length of 100 mm using steel frames and rubber cushions. The stereo camera was mounted on the gimbal (DJI RONIN-MX). The image processing system was developed to capture an image and calculate the disparity of the stereo camera by using the open-source computer vision library of OpenCV 3.4.6.

In this study, a worker walked along a targeted working route with the stereo camera in his hand. The worker only captured a field scene around the route once. After that, the localization and mapping processing mentioned below was performed offline to tune all of the parameters of our system.

2.2 Localization and mapping system using ORB-SLAM2

The robot's positon while carrying the stereo camera along the working route was estimated and drawn on a 3D map using ORB-SLAM2 (Mur-Artal and Tardós, 2017). In our system, a blank area was applied as a non-processing area to control ROI

(region of interest) of the ORB-SLAM2, as shown in Fig. 1. The positioning of the blank area was set so that the upper area of an image could be covered by the blank area. The width of the blank area was fixed to be the same width as the image, and the height was individually tuned for each field type so that the ORB-SLAM2 could robustly and precisely estimate its position in an agricultural field.

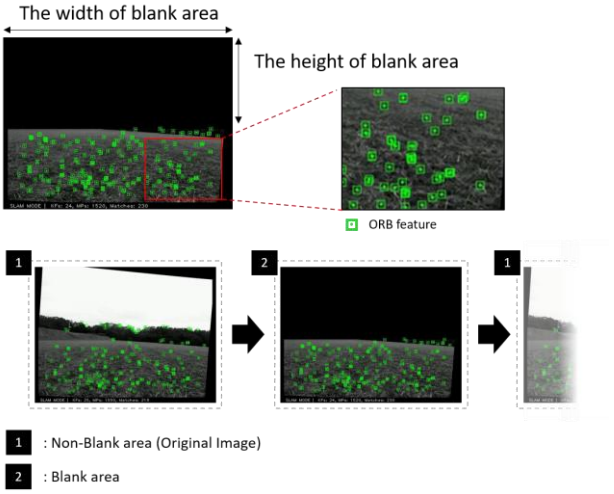


Fig. 1. Our customized ORB-SLAM2 system using blank area algorithm.

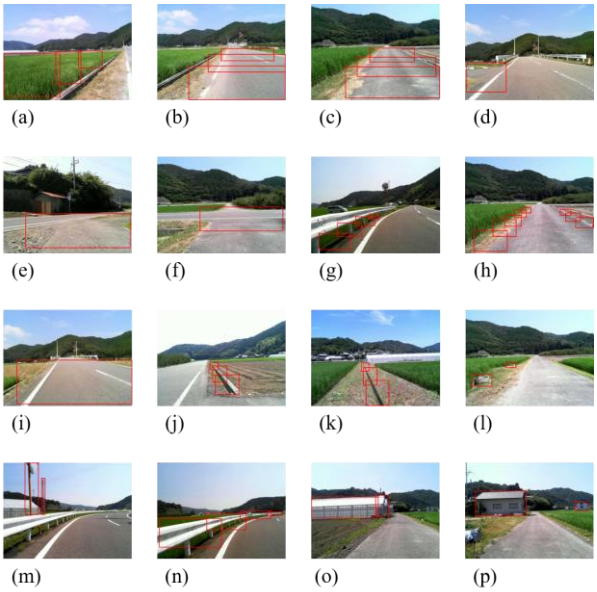


Fig. 2. The YOLOv3 model for detecting targeted 15 types of objects.: (a) paddy field, (b) major road, (c) minor road, (d) entrance from a major road to minor road, (e) exit from a minor road to major road, (f) intersection of a road, (g) road edge on major road, (h) road edge on minor road, (i) gradual uphill, (j) gutter, (k) channel, (l) agriculture faucet of paddy field, (m) utility pole, (n) guardrail, (o) greenhouse, (p) buildings

2.3 Road edge recognition and mapping using the YOLOv3 model

In this system, the YOLOv3 model was designed to recognize 15 types of objects: paddy field, major road, minor road, entrance from a major road to minor road, exit from a minor road to major road, intersection of a road, road edge, gradual uphill, gutter, channel, agriculture faucet of paddy field, utility pole, guardrail, greenhouse, and buildings, as shown in Fig. 2. The 15 types of objects were always observed by workers driving a vehicle in the rural area to understand the field scenes, recognize a working route, and safely drive a vehicle along the route. For making the dataset of YOLOv3 model, we used a yolo_mark, which is GUI for marking bounded boxes of objects in images for training YOLOv3. The bounded boxes of 15 types of objects are also shown in Fig. 2.

In order to identify and map the road edge, our developed localization and mapping system used a 5 steps applied customized ORB-SLAM2 method and the designed YOLOv3 model, as shown in Fig. 3. Firstly, the localization and mapping algorithm calculated the centerline of road. By applying the RANSAC method, the centerline was calculated using center coordinates of bounded boxes of a major or minor road. Secondly, the road edge was separated into left and right based on the calculated centerline. Thirdly, in order to estimate each position of the road edge accurately, this system extracted the limited region of road edge individually. Fourthly, the system calculated each average of X axis extracted from each set of road edge coordinates, and these averages were smoothed with the moving average method. Finally, the distance of each road edge from coordinates with the origin of camera was transformed into the generated 3D map by ORB-SLAM2.

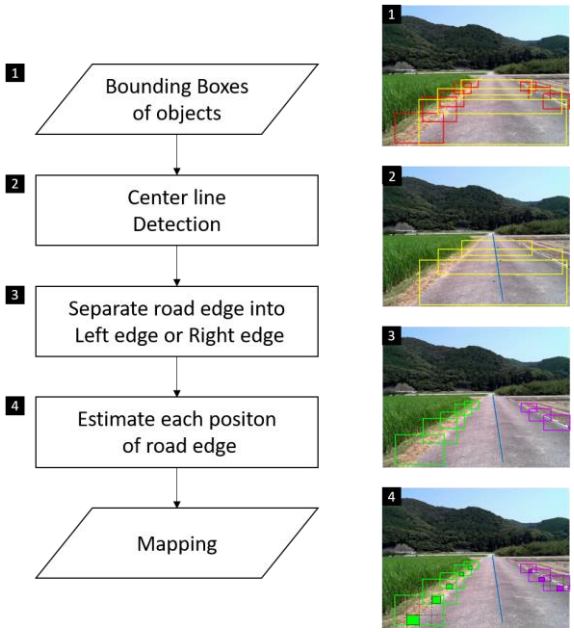


Fig. 3. Road edge recognition and mapping algorithm.

3. Experimentation

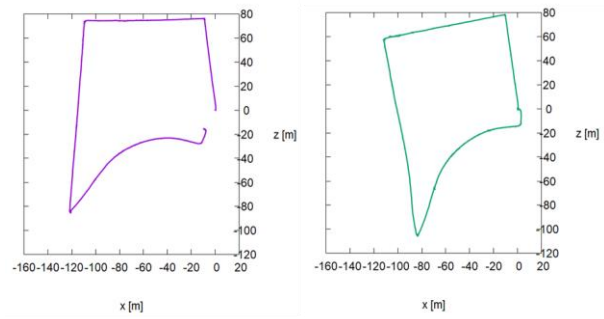
The potential, performance, and applicability of our developed localization and mapping system was tested through three experiments in a targeted agricultural field. This field was 480 meters long. The first experiment tested our customized ORB-SLAM2 system, as described in subsection 2.2. The second experiment focused on the potential of the object detection method in summer, fall, and winter seasons by using our designed YOLOv3 model. In the third experiment, the localization and mapping system was demonstrated by using the results of robot's position estimating with the ORB-SLAM2 and detecting objects with the YOLOv3 model.

4. Results and discussion

4.1 Performance of our customized ORB-SLAM2 system

Figure 4 shows the camera trajectories measured by the ORB-SLAM2 method. In this targeted field, the camera trajectory could not be successfully estimated by the original

ORB-SLAM2 system. Extracted ORB features were generated from the boundary between the sky and the mountain in each camera image. Moreover, the ORB features were distant from the origin coordinates of the camera. As a result, the matching algorithm which estimated the 3D coordinates of the robot, could not work well. However, by setting the blank height of 190 pixels, the result was obtained with high accuracy even when compared with the satellite image. In this field, the blank area covered upper area included the area between the sky and the mountain. Besides, the ORB features were generated on pixels which have accurate depth value.



(a) (b)

Fig. 4. Compare camera trajectories with ORB-SLAM2: (a) original system, (b) customized system with blank area

4.2 Performance of object detecting method of the YOLOv3 model

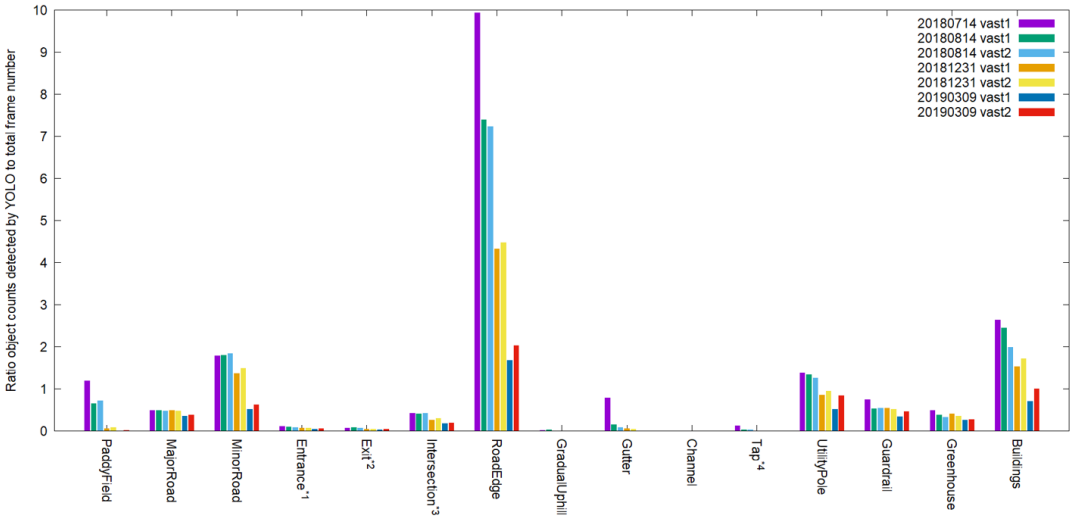
Figure 5 shows that the percentage of converting the total number of each category detected in all frames in each season into one frame was shown as a histogram. Many categories of the YOLOv3 model succeed to be identified regardless of different seasons. In particular, the recognition results of minor roads, buildings, guardrails, greenhouses and utility poles were exactly identified, and stable recognition results were obtained. Moreover, the result of detecting road categories such as major road, minor road and gradual uphill, was identified despite we configured the YOLOv3 model which was classified in detail. On the other hand, these objects: paddy field, agriculture faucet of paddy field, and gutter, were not recognized stably. The field view of a targeted agriculture field affected recognition rate of these objects count. Although the result of road edge was also affected by above reason, the localization and mapping system could draw 3D map using the result of road edge in each season.

This system containing the YOLOv3 model was developed to identify objects around a driving route for an autonomous robot. Therefore, in order to detect objects with YOLOv3 correctly, the model must be trained by using field view imagery for every season. Through this experiment, the YOLOv3 model managed to identify objects in the case where the field view image of experimental data was similar to the training data.

4.3 Performance of localization and mapping system

Figure 6 shows the results obtained from mapping the left and right edges of the roads. With our designed YOLOv3 model, the road edge, major roads and minor roads were detected stably, and the centerline of roads was calculated correctly. These results showed that the road edge was estimated well along most driving routes, and drawn correctly on the map, although the distance from the camera position to the calculated each road edge was smaller than the actual scale. On the other hand, this result indicated that the localization and mapping system has great potential to navigate robots safely. The resolution of the stereo image affected the calculated distance of road edge. The resolution of the estimated road edge became higher when the camera was close to the road edge on the road. Therefore, the accuracy of the calculated distance could be improved if the traveling route was close to the edge, a dangerous place where robots could fall down.

The positions of centerline and edge of the roads failed to be calculated correctly when turning at each road intersection. In these places, our localization and mapping system could not divide the road edge into left and right halves because there was the road edge the front if traveling route went along toward intersection of a road straightly. In addition, when turning at road intersections, there were not important objects such as road edge, major road and minor road, in the camera view. This result showed that the localization and mapping algorithm based on the camera could not map all of the road edge correctly.



*1 : Entrance from a major road to minor road
*2 : Exit from a minor road to major road
*3 : Intersection of a road
*4 : Tap of paddy field

Fig. 5. Recognition rate of object counts detected by the YOLOv3 model to total frame number

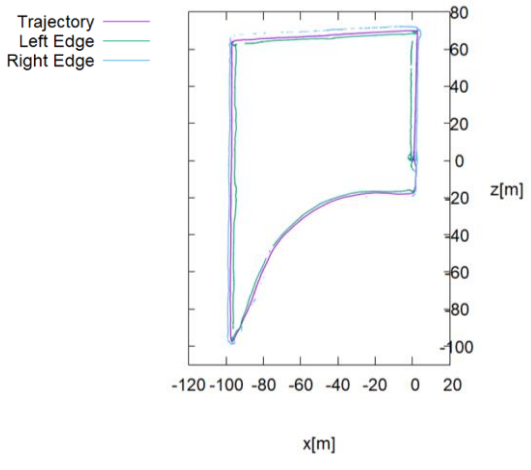


Fig. 6. Detected road left-hand edge and right-hand edge using localization and mapping system

5. Conclusion

This study tried to generate a map for an autonomous robot using a stereo camera in order to solve the geographical problem of not receiving GNSS satellite information.

Through the experiments, the localization and mapping system could successfully generate a 3D map and draw road edges on the map. The camera trajectory was robustly estimated in the targeted agricultural field by using the customized ORB-SLAM2 system. In addition, objects along a driving route could be identified by using our designed YOLOv3 model. Finally, the localization and mapping system which combined the ORB-SLAM2 system with the YOLOv3 showed good potential to be applied to autonomous driving. However, this system was only tested in a single targeted agricultural field. Therefore, in order to test the performance of this system, this localization and mapping system will be tested on different types of agricultural fields.

In future studies, we will develop an autonomous agricultural robot having the localization and mapping system for assisting in a specific agricultural field such as a pea field or paddy field. If the robot can detect workers in agricultural fields, approach them workers autonomously, and decide by oneself what assistance is best for the workers, the robot becomes essentially one of the workers themselves. Therefore, our next goal is to develop a worker assistance system in which the robot can monitor the conditions of workers, make a decision of how to assist the workers, and help the workers by performing the most suitable type of assistance. A robot using our system may be able to overcome the serious problems being faced by the Japanese agricultural sector.

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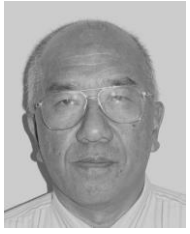
Omura Hiroma

Brief bio data
1st grade of Master's student
Graduate School of Bioresources
Image processing

Short comment
I hope people all over the world always keep smiling.



Katsusuke Murakami

Brief bio data
Professor
Graduated School of Bioresources
Environmental Control in Agriculture

Short comment
Artificial control of plant growth is important for sustainable development.

Yosinari Morio

Brief bio data
Associate Professor
Graduate School of Bioresources
Image processing

Short comment
We have developed intelligent agricultural robots using image processing since 1997.

Glucose production using thermophilic anaerobic bacteria

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Abstract: Biofuel is a type of renewable energy that will be used in the future instead of fossil fuels. Production of biofuel requires three steps: pretreatment of biomass, saccharification of plant polysaccharides and fermentation of sugar into biofuel. However, enzymes used for saccharification are expensive. The purpose of this study is the efficient production of glucose as the fermentation feedstock for producing biofuels and chemical compounds from cellulose, one of the major plant cell wall polysaccharides.

We used the BSES (Biological Simultaneous Enzyme production and Saccharification) strategy. *Clostridium thermocellum* and *Escherichia coli* which express a thermostable β-glucosidase for relieving feedback inhibition were used. *C.thermocellum* produces cellulolytic enzyme complexes, cellulosomes which can degrade cellulose into cellobiose efficiently. The β-glucosidase expressed in *E.coli* hydrolyzes cellobiose into glucose. *C.tthermocellum* uses cellobiose as a major carbon source, but does not use glucose. Thus, glucose is accumulated in culture broth. This combination was able to produce glucose efficiently.

When the *E.coli* cells collected from 30mL of culture broth were added to a medium containing cellulose with *C.thermocellum*, 38.0g/L of glucose was accumulated from 60g/L of cellulose at 60℃ for 9 days.

Further study is needed to increase glucose yield, including a larger β−glucosidase addition and optimization of culture condition.

Key words: Biofuel, Fermentation feedstock, *Clostridium thermocellum*

1. Introduction

This study aims to prevent global warming and improve the self-sufficiency rate of energy. Fossil fuels will become scarce in the near future, and biofuels have received much as a renewable energy alternative to replace suchfossil fuels [1].

According to carbon neutrality, a biofuel do not discharge carbon dioxide [2]. The production of biofuel requires three steps: pretreatment of biomass, saccharification of plant polysaccharides, and fermentation of sugars. The feedstocks of biofuel are ubiquitous in the world. However, sugar cane and corn are edible feedstocks, and so their use competes with food production. Therefore, we want to produce biofuel from inedible feedstocks such as rice straw, corn stover and forest thinning residues. These are promising sources of biomass because they do not compete with food production [2].

The plant cell wall is a chemically complex structure composed of cellulose, hemicelluloses, and lignin as the main polymers. Those polymers, together with other components, provide the plant cell with the robustness required for its diverse functions [3]. Therefore, the plant cell wall is difficult to be degraded. As a result, pretreatment of cellulosic biomass is needed.

There are two type of pretreatments: chemical or physical. Physical pretreatment includes fine grinding, steaming, or radiation. Pretreatment is designed to loosen the rigid structure of the plant cell wall and to increase surface area. Consequently, saccharification efficiency increases because enzymes react better [4].

After pretreatment of polysaccharides, saccharification of plant polysaccharides is needed to produce soluble fermentable sugars such as glucose. Enzymes including cellulase, hemicellulase, pectinases, lignin-decomposing enzyme and additional accessory enzymes are required. Three types of cellulase exist: cellobiohydrase, endoglucanase and β−glucosidase. Cellulases are classified by their sequence homology and biochemical characteristics. Using these enzymes has three advantages. (1) Sugar yield is higher than that from other methods. (2) Waste liquid treatment is easier than that from other methods. (3) Enzyme reaction proceeds at normal conditions.

However, there are three disadvantages. (1) Enzymes are costly. (2) A large amount of enzymes is required. (3) Enzyme reaction takes a long time.

After saccharificating, the soluble sugar mixture is used as a carbon source for alcoholic fermentation. Yeasts such as *Sccharomyces cerevisiae* are used to ferment. Microorganisms produce ethanol, n-butanol and isopropanol as metabolic products from the soluble sugars [5]. Ethanol can be used as a fuel for automobiles.

In this study, *Clostridium thermocellum* and *Escherichia coli* which produces β−glucosidase from *Thermoanaerobacter brockii* were used. This method is called biological simultaneous enzyme production and saccharification (BSES) [6]. *C. thermocellum* is a thermophilic, anaerobic, spore-forming and cellulolytic bacterium [7], first isolated in 1926 and producing cellulose decomposing enzyme complexes called cellulosomes [8]. Cellulosomes are composed of approximately twenty enzymes such as cellulase, hemicellulase and chitinase. Therefore *C. thermocellum* is one of the bestexplored and well characterized cellulose-degrading bacterium in nature [9]. The β−glucosidase from *T. brockii* has high glucose tolerance and thermostablity. Optimal pH and temperature are 5.5 and 75℃ respectively [10].

The BSES can directly produce glucose from cellulosic material. *C. thermocellum* was cultivated in the BM7CO medium containing ball-milled cellulose (BMC) and *C.* produced cellulosome and degraded cellulose materials into cellobiose. Cellobiose is nutrition for this bacterium but it inhibits cellulosome enzyme activity. The β−glucosidase hydrolyzes cellobiose into glucose and relieved feedback inhibition of cellubiose. *C. thermocellum* cannot consume glucose, thus glucose is accumulated in the medium. This combination is able to produce glucose efficiently with no addition of enzymes.

2. Methods

2.1 Organism strain, plasmid DNA

C. thermocellum DSM1313 was used and obtained from Deutsche Sammlungvon Mikroorganismen und Zellkulturen GmbH (Germany). The β−glucosidase (CglT) gene was inserted in pQE30 (an expression vector). pQE30-CglT was used and obtained from Ichikawa (Mie university).

E. coli JM109 was used as an enzyme expression cell.

2.2 Transformation

E. coli JM109 was transformed using the calcium method. The JM109 strain was taken out from -80 °C freezer and melted slowly on ice. After melting, w2μL of plasmid was added and mixed, and left for 30min. After 30 min, *E. coli* was incubated at 42 °C for 30 sec and cooled on ice. 1mL of SOC medium was added to *E. coli* and then shacking cultured (220rpm) for 1h. 50mL of this liquid was scattered on the LB medium containing 50μL/mL of ampicillin using a turntable and cell spreader. It was then incubated overnight at 37 °C.

2.3 *E.coli* culture

Transformed *E.coli* was obtained from colony or glycerol stock which is a long-tern storage method of bacteria. E.coli cells were grown overnight at 37 °C and 220rpm in Luria-Bertani medium containing ampicillin (50μg/ml). The pH was controlled at 7.2-7.5 by 1N NaOH addition. After one night, centrifugation(12,000, 5minutes) was carried out. Only *E. coli* was added to LB medium for mass culture. When Optical density(OD_{660})was near 0.5, isopropyl thiogalactoside (IPTG final concentration 1mM) was added to the medium and continued to culture for 4 h.

2.4 Preparation of culture medium

Ball-milled cellulose (BMC) was used as carbon source of *C.thermocellum*. 3 % BMC was prepared by ball mill processing of the KC flock (Nippon Paper Chemicals, Tokyo, Japan) with distilled water for 72 h at 4 °C. 10mL of 3% BMC in a screw test tube was centrifugated (2,500 rpm 30 minutes) and 6mL of supernatant was removed. 5-fold concentration BM7CO medium was added and extensively flushed with nitrogen. The pH was controlled at 7.0 by addition of 6N HCl. The tubes were then autoclaved (120 °c, 20min).

2-5 Culture method

C. thermocellum was statically cultured in the BM7CO medium containing 10g/L microcrystalline cellulose powder for 2 days at 60 °C. 100μL of this pre-culture fluid was added to the BM7CO medium containing 6 % BMC and *C. thermocellum* was statically cultured at 60 °C. After two days, the culture broth of *E. coli* expressed β–glucosidase was added.

2-7 Glucose concentration measurement

The concentration of the released sugar was measured by Glucose C2 (Mutarotase-GOD method).

3 **Results**

In order to investigate the concentration of glucose, we changed the amount of added β–glucosidase.

When the *E. coli* cells collected from 10mL of culture broth were added to a BM7COmedium containing 6% BMC with *C. thermocellum*, 35.2g/L of glucose was accumulated after 7 days.

When the *E. coli* cells collected from 20mL culture broth were added to a BM7COmedium containing 6% BMC with *C. thermocellum*, 36.9g/L of glucose was accumulated after 7 days. 6%BMC was not completely degraded.

When the *E. coli* cells collected from 30mL culture broth were added to a BM7COmedium containing 11.6g/L of 3-(N-morpholino)propanesulfonic acid and 6% BMC with *C. thermocellum*, 38.0g/L of glucose was accumulated after 9 days.

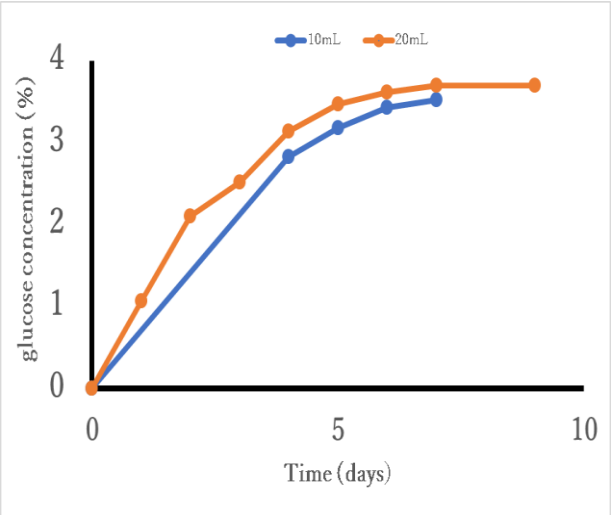

Fig.1.Glucose concentration of adding 10mL or 20mL of *E. coli* cells

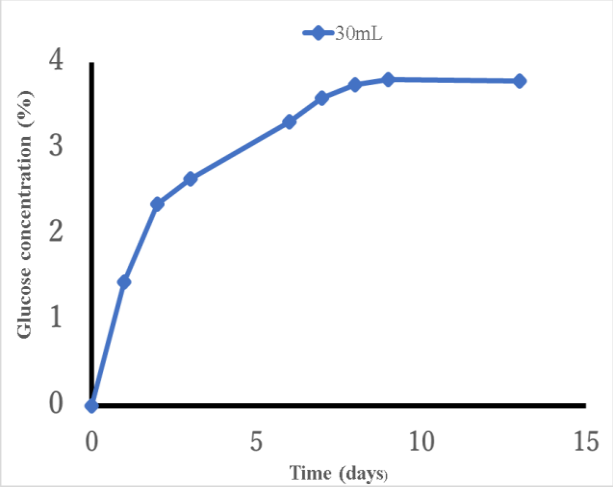

Fig2 Glucose concentration of adding 30mL of *E .coli* cells

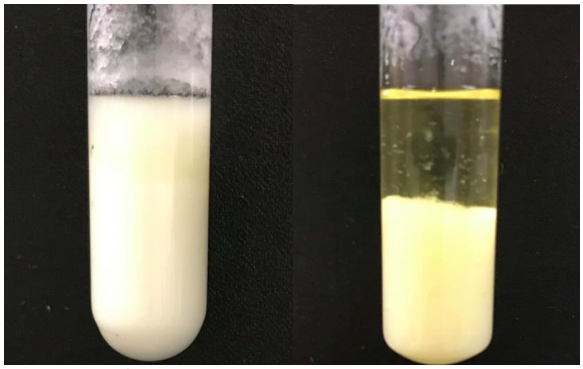

Fig 3 left 6% BMC BM7CO medium
right after 13days adding 30mL of *E. coli* cells

4 Conclusion

This study aimed to increase glucose production from cellulose, using *Clostridium. thermocellum* and *Escherichia coli*, which produces β–glucosidase. This combination is called biological simultaneous enzyme production and saccharification (BSES).

In a previous study, 5% of ball milled cellulose (BMC) was completely degraded and about 40g/L of glucose was produced over 6 days. Therefore, we used a medium containing 6% of BMC. As a result, 6% of BMC was not completely degraded, and 38.0g/L of glucose was produced after 9 days. This is the highest concentration of this time. We changed the amount of added *E. coli*, but it changed little the glucose production.

Future studies will continue to increase glucose production. If we are able to extract a large amount of glucose from biomass, a sustainable society in the future can be better imagined.

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Applied Microbiology and Fermentation
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Sound communication in narrow-ridged finless porpoises (*Neophocaena asiaeorientalis*)

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Abstract: The narrow-ridged finless porpoise is an endangered species which is easily influenced by human activities because its habitat usually overlaps with human activity areas. To conserve the species, it is important to understand their ecological niche. The sounds they emit are limited to pulsed sounds, used mainly for echolocation. At the time of the current study, no other study has examined the communication function of these pulsed sounds. We investigated whether the finless porpoise uses pulsed sounds for contact call by recording the pulsed sounds of six captive porpoises in the two contexts isolation and free-swimming. In total, their sounds were recorded for 4.5 hours over two days in the isolation context and for 11 hours over four days in the free-swimming context. Several burst-pulse sounds were heard in the isolation context, making burst-pulse sounds a candidate for contact call. Our further study will attempt to shed light on the purpose of the contact call for the narrow-ridged finless porpoise after careful comparison of sounds with behaviors and social contexts.

Key words: narrow-ridged finless porpoise, *Neophocaena asiaeorientalis*, acoustic communication, burst-pulse sound

1. Introduction

The loss of biodiversity is one of the most serious problems facing the world today. If biodiversity continues to be lost, humans are placed more and more at risk as we depend heavily on ecosystem services derived from such biodiversity. The narrow-ridged finless porpoise is a member of the dolphin family (Fig.1). The IUCN Red List of Threatened Species lists narrow-ridged finless porpoise (*Neophocaena asiaeorientalis*) as "Endangered". Narrow-ridged finless porpoises are easily and negatively impacted by human activities, because they live in coastal areas which humans also use intensively. In order to conserve this species, we must better understand their ecological niche.

Odontocetes, including narrow-ridged finless porpoises, strongly rely on sounds for their living, thus anthropogenic noise would have negative impacts on their behavior (Madsen et al., 2006). Sakai et al. (2011) suggested that this animal has more complex sociality than our current scientific knowledge conventional would predict. If they communicate acoustically, the impact of such noise can be considered to be greater than previously thought. However, very few studies have been conducted on acoustic communication for narrow-ridged finless porpoises.

Several groups of toothed whales, including narrow-ridged finless porpoises, have been found to not produce a "whistle" (commonly using for communication) but only to produce a "pulsed sound" (mainly using for echolocation). So far, there is little understanding regarding acoustic communication by pulsed sounds for these species. Several species, such as the harbor porpoise (*Phocoena phocoena*), Heaviside's dolphin (*Cephalorhynchus heavisidii*) and Hector's dolphin (*C. hectori*) do not produce a whistle. It has been suggested that these species may communicate acoustically using very short sounds with Inter Pulse Interval (IPI) such as "burst pulse sounds" (Clausen et al., 2010, Dawson 1991, Martin et al., 2019, Sørensen et al., 2018). From the results, it is thought that such pulsed sounds have the function of a "contact call". These sounds were produced when they conducted social behavior. Our study was conducted with captive narrow-ridged finless porpoises to investigate whether or not this animal communicates acoustically. It was expected that this species would produce more contact calls in an isolation context, similar to the white whale (*Delphinapterus leucas*, Mishima et al., 2015). This study was carried out in two contexts. One is the isolation context, and the other is a free-swimming context, with several porpoises in order to compare the two. The current study hypothesizes that narrow-ridged finless porpoises communicate acoustically such as in burst pulses.

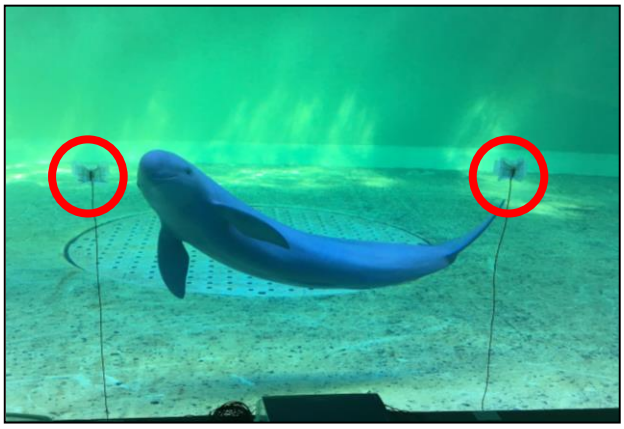

Fig. 1. Narrow-ridged finless porpoise in Toba aquarium. It has no dorsal fin and has gray smooth body. Red circles indicate hydrophones for sound source localization.

2. Materials and methods

2.1 Study site and subject animals

Video and acoustic recordings of seven narrow-ridged finless porpoises were conducted at the Toba Aquarium, Mie Prefecture, Japan, for eight days from May 22 to June 28 2019. In the present study, the narrow-ridged finless porpoise pool was composed of three pools: the holding pool (22 m^2 surface area and 1 m deep), the main pool (67 m^2 surface area and 4.5 m deep) and the J pool (58 m^2 surface area and 3.4 m deep) (Fig. 2). We studied three adult females (#Y: 34 years old, #C: estimated 17 years old, #R: six years old), two juvenile females (#K: two years old, neonate (it has not been named yet): 1.5 months old), one adult male (#G: estimated 20 years old) and one juvenile male (#H: three years old). #Y, #R, #H, #K and neonate were born at Toba Aquarium. #H and neonate are the calves of #C.

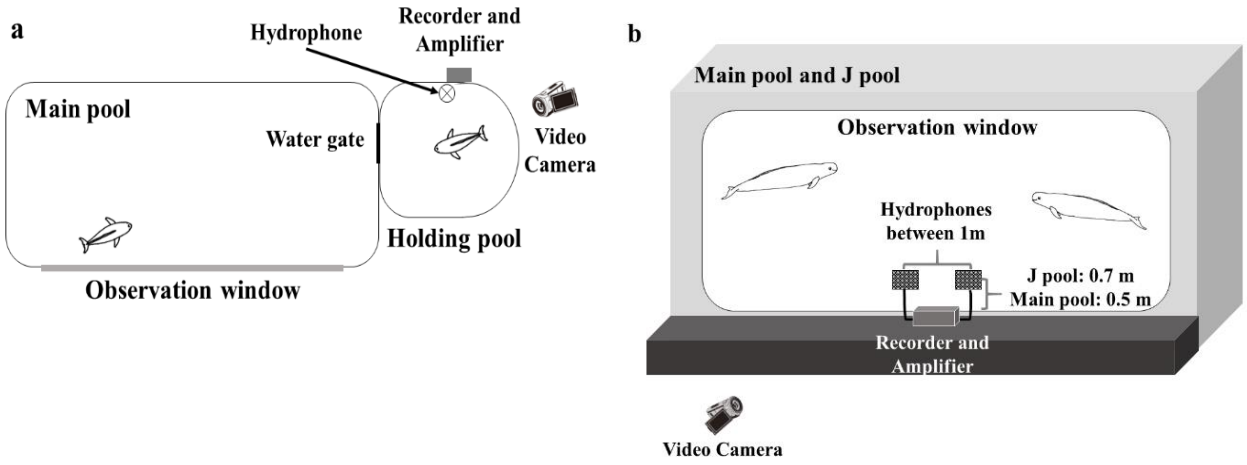

Fig. 2. Schematic view of the narrow-ridged finless porpoise pools. a: The main pool and the holding pool are viewed from above. The water gate was made of wood and fiber reinforced plastics, and these individuals cannot see each other. b: The main pool and the J pool are viewed from the side. The main and J pools have similar structures. The J pool is distant from the main and holding pools.

2.2 Study contexts and data collection

We conducted experiments with several porpoises in two contexts: in isolation and in free swimming. In the isolation context, #R and neonate were recorded for 2.5 hours of video and for 4.5 hours of acoustic on two observation days in the holding pool. Acoustic recordings were taken using one AQH 200K hydrophone (Aquasound Inc., Japan). The hydrophone was placed at a depth of 0.5 m. In the free-swimming context, #Y, #C, #R, #K, #G, and #H were recorded with video and acoustic for 11 hours over a four days period in the main and J pools. Acoustic recordings were taken using two AQH 100K-DTP hydrophones (Aquasound). These hydrophones were attached to the window with a distance between them of 1 m. Their height from the bottom of the window were 0.5 m in the main pool and were 0.7 m in the J pool. In the two contexts, the signal was analog bandpass filtered from 1 kHz to 200 kHz, and it was amplified to 70dB using an Aquafeeler IV preamplifier (Aquasound) and recorded into separate channels of an EZ7510 data recorder (NF Corp., Japan).

2.3 Sound analysis

We analyzed only sounds recorded on May 22 2019 and used Avisoft SASLab Pro version 5.2.13 (Avisoft Bioacoustics, Germany). Sound spectrograms were generated with FFT size of 1024 and a Hamming window function. Li et al., (2005) indicated that most sounds have IPIs smaller than 0.1 seconds and all sounds have peak frequencies over 70 kHz. In the present study, we eliminated any IPIs over 0.1 seconds from IPIs graphs and all sounds below 70 kHz were removed to eliminate non-specific noises (Fig. 3. II, V).

3. Results and discussion

Pulsed sounds were divided into two types as follows: Burst pulse sounds type ("packet type") and Echolocation type. Most packet type sounds had IPIs smaller than 0.01 seconds and many series of short burst pulses (Fig. 3 III). On the other hand, the echolocation type had steady IPIs of about 0.03 seconds (Fig. 3. VI). Packet type sounds was produced for several seconds when the porpoises stopped and headed to the main pool near the water gate, whereas the echolocation type was produced when they were purely swimming. In the case of harbor porpoises, when a calf was physically separated from its mother, the calf produced contact calls more frequently towards its mother in a different pool (Clausen et al., 2010). In addition, burst pulse sounds of Heaviside's dolphins are similar to those in our study, particularly regarding the sound spectrogram and the IPI (Martin et al., 2019). Here, we supposed that packet type sounds have the same function of contact call as found with harbor porpoises and Heaviside's dolphins. In this study, packet type sounds are louder than echolocation type sounds (Fig. 3. I IV). Packet type sounds were produced near the water gate in the opposite direction whereas the echolocation type were produced near and towards the hydrophone. As such, it is thought that the difference of volume cannot be explained by the distance between #R and the hydrophone. This difference may indicate that #R produced louder sounds for conspecific individuals to hear. For the above reasons, it is possible to understand that packet type sounds of narrow-ridged finless porpoise have the function of contact call, like those of harbor porpoises, Heaviside's dolphins and Hector's dolphins.

4. Conclusion

This study sought to reveal whether or not narrow-ridged finless porpoises communicate acoustically. We found the possibility that narrow-ridged finless porpoises have contact call sounds. In the future, we plan to record and analyze enough data to understand such acoustic communication. The reactions of individual porpoises in the main pool must be subsequently observed during burst pulse production. The hydrophone will be set in the main pool to verify whether sounds in the holding pool can reach the main pool as well. In free-swimming contexts, we will examine their sounds with a focus on the burst pulse sounds. Our future study hopes to examine the contact calls of the narrow-ridged finless porpoises by comparing sounds, behaviors, and social contexts.

5. Acknowledgments

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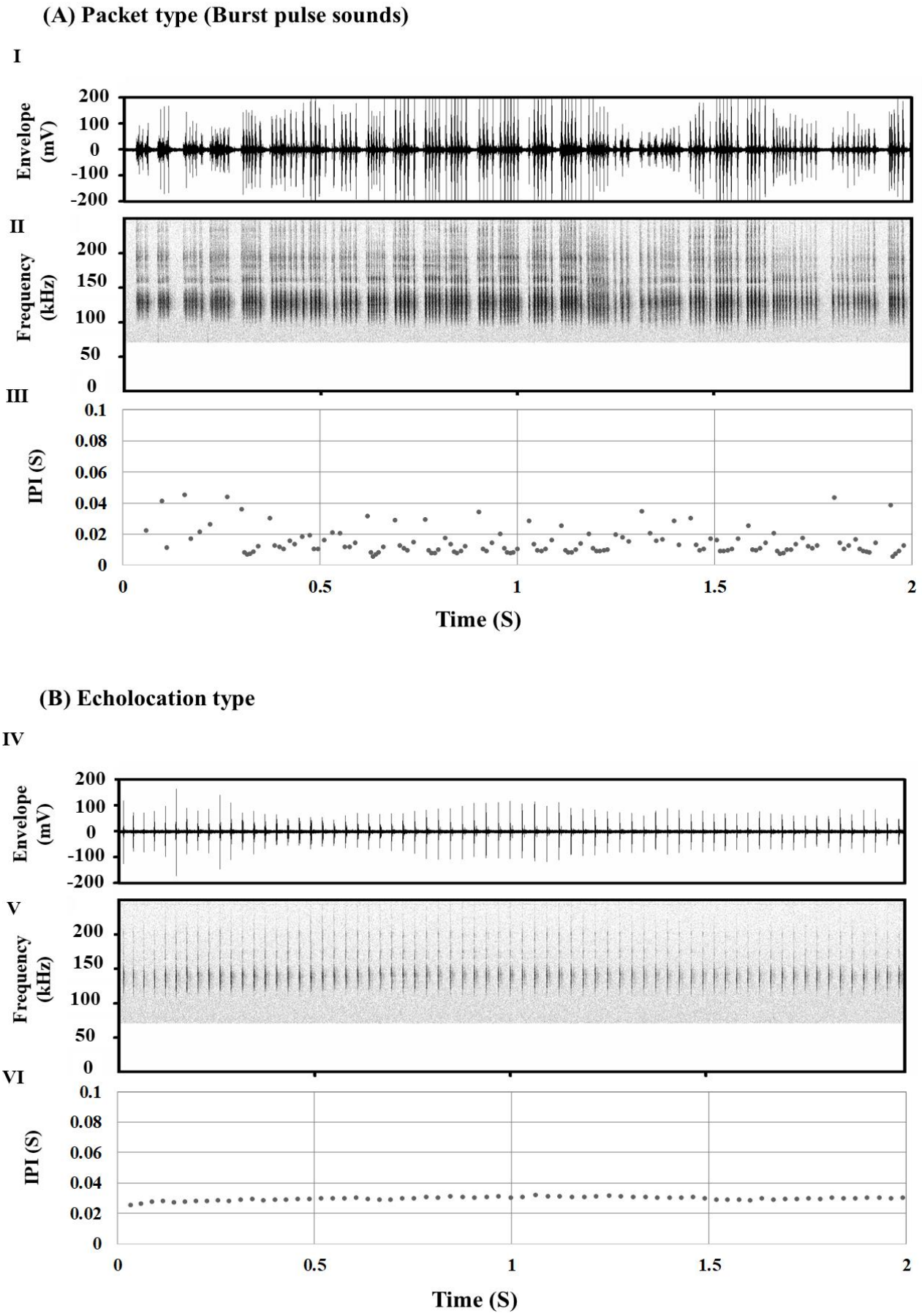

Fig. 3. Pulsed-train of each sound type of #R. X-axis: time, Y-axis: Envelope; pressure level of signal (mV), IPI; Inter pulse interval (S).

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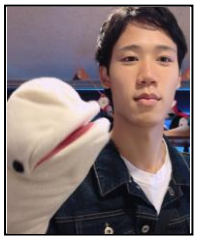

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Repeated prolonged fasting-induced immunological changes and growth rate in Japanese abalone, *Haliotis discus discus*

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Abstract: The present study aims to investigate the effect of a three-day periodical short term starvation on innate immune response changes in Japanese abalone, *Haliotis discus discus* over a thirty-six-day period. The animals were put in three distinctive experimental groups: Continuous Food (CF), Prolonged Fasting (PF), No Food (NF). This study analysed Specific Growth Rate (SGR) and several innate immune parameters such as total haemocyte count, lysozyme activity, phenoloxidase activity, respiratory burst activity, and protein concentration. Results showed that the PF group has increased values in SGR and protein concentration compared with other groups, and slightly higher in lysozyme activity, respiratory burst activity and phenoloxidase activity than the CF group. However, the PF group was somewhat lower than the NF group considering these parameters. Conversely, SGR, total haemocyte count, and protein concentration values in the NF group were the lowest. These data demonstrated that repeated prolonged fasting may have a direct impact on the innate immune response of abalone related to a survival mechanism and could be used as an alternative way to enhance innate immunity in disease prevention of farmed abalone.

Key words: Prolonged fasting, immune response, Japanese abalone, *Haliotis discus discus*.

1. Introduction

Since the late 20th century, the abalone industry has been severely affected by continuous disease outbreaks associated with viruses and bacteria (Oakes et al., 1996; Hooper et al., 2007). Innate immunity is the first line of defence in abalone to combat infectious diseases. Several previous studies have shown that nutrient deprivation or starvation can change non-specific immune parameters in fish and molluscs (Eslamloo et al., 2016; Butt et al., 2007). Japanese abalone, *Haliotis discus discus*, is a commercially important and highly favoured marine shellfish species in the Japanese market and around the world for its nutrient content. Therefore, the global demand for abalone continues to increase despite its high price. Because of the severe decrease of natural stocks due to overexploitation combined with its slow growth rate under natural conditions, the intensive aquaculture of abalone has developed worldwide (Oakes et al., 1996). However, it is known that aquatic animals cultured in intensive culture ponds are exposed to stressful conditions, rendering them susceptible to infectious diseases.

Infectious disease is one of the serious problems to be overcome in the abalone farming industry. The use of antibiotic for diseases prevention and reducing mortalities rate many years has led to the negative sides in resistant bacteria pathogen and antibiotic residual in farmed animal and human. Consequently, there is a trend in aquatic animal health management to apply the eco-friendly and sustainable strategy in terms of health and water quality management in aquaculture industries (Rasul and Majumdar, 2017). Prolonged fasting or short-term starvation is a condition without eating for more than 2 – 4 days. It is successful to prevent many diseases, as well as stimuli innate and specific immune system in human and mammalian (Kuballa, 2012). However, most studies on nutrition deprivation in abalone have merely focused on growth, survival, and physiological stress (Takami et al., 1995). Little attention has been focused on immunological changes as a result of repeated prolonged fasting of abalone.

The present study aims to investigate the effect of a three-day periodical short term starvation on innate immune response changes in Japanese abalone, *Haliotis discus discus*, over a thirty-six-day period. In order to achieve this goal, an experiment was carried out at a wet laboratory in Mie University, Japan. Eighteen abalone were placed in each of the three distinctive experimental tanks: Continue Food (CF), Prolonged Fasting (PF), and No Food (NF) in triplicates. Initial weight and shell length were measured to calculate specific growth rate (SGR) for a thirty-six-day period time of observation. This study analysed several innate immune parameters, including total haemocyte count, lysozyme activity, phenoloxidase activity, respiratory burst activity, and protein concentration. Inducing a slight stressor such as starvation is a novel finding of this study.

2. Materials and methods

2.1 *Experimental design*

The experiment was performed at the wet laboratory of the graduate school of Bioresources, Mie University, Japan. The abalone (average initial weight 264.97±35.57 g) were supplied by the abalone farming industry in the town of Minami-Ise. Prior to the experiment, the abalone were acclimatized for 7 days and fed a natural diet of "Kombu" (*Laminaria* sp.) *ad libitum*. After acclimation, the initial weight and shell length were determined and randomly distributed into three distinctive groups with three replicates per group: continuous food (CF), feeding continuously with 20 gr a natural diet (*Laminaria* sp.); (PF) prolonged fasting group, 3 days or 72 hours of fasting and 4 days of refeeding to apparent satiation with a natural diet (*Laminaria* sp.) *ad libitum* total 7 days in a cycle for 5 cycles; and (NF) un-fed group, 36 days of fasting. All treatments were observed for 36 days. The animals were placed in 100 L volume, PVC cultivation tanks with water recirculation systems. Water quality was measured and faeces removed three times a week.

2.2 *Sample collection and analysis*

At the end of the experiment after a thirty-six-day period of

observation, all of the abalone were weighed and measured. A random sample of 4 abalone was then taken from each treatment group and tested for total haemocyte count, phenoloxidase, respiratory burst, lysozyme, and phagocytic activities. Haemolymph for all immunological assays was withdrawn from the pedial sinus using 1 mL sterile syringe with 23 G x $1^1/_4$ in. needles. At each time point, the haemolymph from four animals was pooled and immediately placed on ice to retard cell clumping and agglutination. One millilitre of haemolymph from each replicate was immediately used for determining cellular immune parameters. The rest of haemolymph was centrifuged at 3000 x g for 10 min at 4 °C to separate the cellular fraction (haemocytes) from the plasma (haemolymph). The resulting supernatant i.e., plasma (serum) was then stored at –20 °C until its use in the assays (Le Bris et al., 2014).

2.2.1. Specific Growth Rate (SGR)

Initial body weight and shell length of abalone in each group were measured to calculate the specific growth rate (SGR), where final body weight (W_t) – initial body weight (W_0)/period time of observation (t) x 100%. W_t and W_0 are final and initial weights of abalone, and t is the duration of the experimental, a 36-day period.

2.2.2. Total haemocyte count (THC)

The THC for each replicate was determined according to the method of (Xue et al., 2008). Briefly, undiluted haemolymph (100 µl) was mixed with an equal volume of Tris-EDTA pre-cooled (4 °C) (18 mM Tris, 0.45 M Sodium chloride, 13 mM Potassium chloride, 16 mM D-glucose, 20 mM EDTA, at pH 7.5) to avoid haemocyte agglutination and then added to a Neubauer haemocytometer and counted under a microscope.

2.2.3. Respiratory burst activity

Respiratory burst activity of haemocytes was quantified by measuring the reduction of nitroblue trazolium (NBT) to blue formazan as a measure of superoxide anion according to (Pipe, 1992). Briefly, 100 µl of whole haemolymph was added to flat bottom 96 well microplates. The microplates were left at room temperature (22 °C) for 30 min to allow haemocyte settling and adhering on the wells. Twenty microliters of nitroblue tetrazolium (Sigma Aldrich 5 mg mL-1) were then added into each well and incubated at room temperature for 2 h. Supernatants were then drawn off, leaving the stained haemocytes to dry. One hundred microliters of dimethyl sulphoxide (Sigma Aldrich) were then added into each well to dissolve the cytoplasmic formazan formed during the reaction. Final measures of formazan production were expressed as OD_{620}.

2.2.4. Lysozyme activity

Lysozyme activity measurements were based on the turbidimetric method described by (Helal and Melzig, 2008) with some modification. Briefly, the substrate of lysozyme was a 0.2 mg ml-1 freeze-dried Micrococcus lysodeikticus (Sigma Aldrich M3770) suspension in 0.5 M Phosphate Buffer Saline < pH 6.2 (PBS, Sigma Aldrich). Serum (20 µL well-1) was placed in triplicates in a 96 well plate and 130 µL of bacterial suspension was added. As a negative control, PBS replaced serum. The decrease in OD was recorded at 450 nm after 1 and 5 min at 22 °C with a microplate reader. A unit of lysozyme activity was defined as the amount of lysozyme that caused a decrease in absorbance of 0.001 min-1.

2.2.5. Phenoloxidase activity

Phenoloxidase activity was measured by recording the diphenolase activity with the substrate, by L-3,4-dihydroxyphenylalanine (L-DOPA) as described by (Peter and Raftos, 2003). The chromogen, 3-methyl-2-benzothiazolinone hydrazine (MBTH, Sigma Aldrich) was added to the substrate. Briefly, one hundred microliters of whole haemolymph were added in 96-well microplates followed by the addition of 100 µL of L-DOPA (4 mg ml-1 in PBS), containing 1 mM MBTH. The absorbance of the reaction mixture was measured at 490 nm immediately after the addition of substrates using a microplate spectrophotometer. A second reading was made after the plates has been incubated for one hour at room temperature 22 °C. Enzyme activities were expressed as Unit mg $protein^{-1}$.

2.2.6. Protein determination

The total protein concentration (PC) of the abalone serum was determined based on the Hooper et al., (2014) method. Briefly, haemolymph was centrifuged (10.000 x g, 5 min, 4 °C) and 10 µl of haemolymph plasma was transferred in duplicate into 96-well micro-plates to which 200 µl of dye reagent (BCA protein assay reaction, Bichinconinic Assay Kit, Sigma) was added. Samples were then incubated at 37°C for one hour and absorbance was measured spectrophotometrically using a microplate reader at 562 nm. The OD of the haemolymph plasma samples were compared to a standard curve of Bovine Serum Albumin (Sigma Aldrich) and results expressed as mg of protein•ml^{-1}.

2.2.7. Statistical analysis

The Shapiro-Wilk test was used in the first step to estimate the normality of data. According to the obtained data, statistical differences among the three groups were performed by one-way analysis of variance (ANOVA) and expressed as the arithmetic mean ± standard deviation (SD). Post-hoc comparisons between means were assayed by Tukey's test with *P*-value < 0.05 being accepted as significant level using IBM SPSS software for windows.

3. Results

3.1 *Growth performance*

There was a significant difference of SGR value in the PF treatment compared with the CF and NF treatments (Table 1). The obvious absence of food in the NF treatment led to a negative value of SGR which indicates no growth during the period time of observation.

Table 1. Effects of repeated prolonged fasting on all parameters for Japanese abalone, *Haliotis discus discus*.

Parameters	Treatments		
	CF	PF	NF
Specific Growth Rate (%/day)	0.38 ± 1.52^a	0.48 ± 2.08^b	-0.58 ± 1.23^c
Total Haemocyte Count (x10^6 cells/ml)	20.15 ± 11.50^a	11.35 ± 2.51^b	4.27 ± 0.86^c
Respiratory burst activity (OD 620nm)	0.20 ± 0.08^a	0.22 ± 0.05^a	0.24 ± 0.11^a
Lysozyme activity (mU/mg protein)	0.12 ± 0.14^a	0.54 ± 0.81^b	5.22 ± 9.56^c
Phenoloxidase activity (U/mg protein)	0.14 ± 0.02^a	0.19 ± 0.04^a	0.27 ± 0.05^b
Protein concentration (mg/ml)	4.04 ± 0.75^a	4.14 ± 1.28^a	2.35 ± 0.43^b

Different superscripts in the same row indicates significant differences (P<0.05)

between feeding strategies. CF, fed continuously with 20 gr of *Laminaria* sp.; PF, 72 hours fasting and re-feeding with *Laminaria* sp. to apparent satiation; NF, un-fed over a 36-days period.

3.2 *Cellular immune response*

Results indicated that the total haemocyte count (THC) in the three distinctive groups showed significant differences ($P < 0.05$, Table 1) between the treatments during the experiment in which the total number of haemocyte in the CF treatment was the highest. There were no significant differences in respiratory burst activity between all groups, even though the value of respiratory burst activity observed in the PF and NF treatments tended to increase.

3.3 *Humoral immune response*

The changes in some humoral immune responses of a short-term periodical fasted and re-fed group are shown in Table 1. Feeding strategies led to no significant differences in phenoloxidase activity and respiratory burst activity. Data showed the highest concentration of protein appeared in the PF treatment followed by the CF treatment, then the NF treatment with the lowest concentration. However, there was no significant difference between the CF and PF groups. Conversely, the distinct results were demonstrated in lysozyme activity, and phenoloxidase activity. The NF treatment showed a significant highest level of enzyme activity in comparison with the other treatments. Lysozyme activity between groups was significantly different (Table 1), with the highest level of lysozyme activity appearing in the NF group in which a 36-day starvation period applied.

4. **Discussion**

The cycle of fasting and re-feeding in the prolonged fasting (PF) treatment led to an increased SGR level. This pattern can be used as a management feeding strategy to induce compensatory growth and decrease feed costs (Silva et al., 2019; Morshedi et al., 2017; Jafari et al., 2018). Compensatory growth (CG) is an accelerated growth phase under normal conditions occurring after a period of growth depression caused by food restriction and critical environmental conditions (Montserrat et al., 2007). Growth enhancement as a response to the absence of feed is a consequence of increased feed intake during the period of hyperphagia (post-restriction period) (Ali et al., 2003). The CG after fasting or nutrient deprivation varies according to the feed restriction protocol (duration and intensity of regimen) and species as well (Oh et al., 2007).

The obvious absence of feed in the NF treatment has led to a negative SGR level, and could be caused by metabolism changes to maintain energy in a cellular homeostasis as a survival mechanism. In this study, immunological changes occurred during a period of distinct feed regime treatment. Circulating haemocytes in haemolymph possess a vital importance in immunological homeostasis (Wang et al., 2018; Lemaitre and Hoffman 2007). They are involved in both humoral and cellular immune responses, such as synthesis of antimicrobial peptides, encapsulation, and phagocytosis (Ottaviani, 2011; Song et al., 2010) including nutrient transport, digestion, and shell repair (Hooper et al.2007)

Haemocytes consist of hyalinocytes and granulocytes, which are characterized functionally as a phagocytic and encapsulating population (Sahaphong et al., 2001). Additionally, ROS production and lysozyme activity are mainly concentrated in granulocytes under normal and immune-activated situations (Wang et al., 2018). ROS at low levels maintains control normal cellular function. Conversely, it leads to oxidative stress at high levels, which is linked to a variety of diseases, aging, and cell death. ROS production is necessary for functional aerobic metabolism (Delmanstro-Greenwood and Piganelli, 2013). Moreover, an accumulation of ROS eventually brings about cell dysfunction and death. However, nutrient limitation protects from the over accumulation of naïve T cells, allowing for the turnover of older and development of new cells (Rathmell et al., 2000).

The lowest level of total number of haemocytes,counted in the NF treatment, is a haematopoiesis condition occurred as an immune response to fulfil their defence role involved in wound healing, pathogen killing or stressors elimination through phagocytosis and encapsulation (Bouallegui, 2019; Pilla et al., 2016). Consequently, this condition can promote the highest level of respiratory burst, lysozyme, and phenoloxidase in NF treatment compared with other treatments. The lowest level of protein content in the serum of NF treatment can be led by the change of metabolic energy reserves as a response to starvation (Monstserrat et al., 2007; Ali et al., 2003; Oh et al., 2007) and the extended periods of starvation in abalone mobilizes to use muscle protein as their primary fuel source (Morshedi et al., 2017).

5. **Conclusion**

In conclusion, the investigation into the effect of a three-day periodical short term starvation on innate immune response changes in Japanese abalone, *Haliotis discus discus*, over a thirty-six-day period, has provided novel information concerning the feasibility of a sustainable strategy of disease prevention. The significant difference of SGR value and some innate immune responses in the PF group compared with the other groups of feeding methods indicate that short-term starvation cycles and re-feeding can drive growth rate and induce immune responses as well. This approach is consistent with a previous study which found that prolonged fasting-refeeding cycles had an implication on compensatory growth in fish (Morshedi et al., 2017). However, the result of the immune response factor tends to contradict another study that found that immunological defence in Sydney rock oysters (*Saccostrea glomerata*) decreased significantly after starvation for two to four weeks (Butt et al., 2007).

This is the first experiment that provides evidence for periodical short-term starvation and re-feeding cycles, giving different responses in innate immune changes and growth rate in abalone. In this study, inducing a slight stressor such as short-term starvation and re-feeding was shown to be a useful tool for boosting compensatory growth and immune responses in abalone related to disease prevention, a novel finding. This finding is promising and should be explored more deeply to develop a correlation between repeated prolonged fasting with pathogens infection and autophagy activity in the future. This feeding method has potential to be applied in the abalone aquaculture industry.

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