Technical Report

Changing of Ecosystem in Tidal Flat and Shallow Mesocosms with Steelmaking Slag

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Abstract

Coastal ecosystems were created at Sea Lab. II, which has two experimental tanks and two reservoir tanks. The experimental tanks were composed of different depth zones, one being a tidal flat and the other being a shallow. The experimental tank of the control site was lined with dredged soil as the sediment. On the other hand, the experimental site was lined with a mixture of the dredged soil and steelmaking slag as the sediment. Twenty-seven months later, the ecosystem created in the experimental tanks was different between the control and the experimental site. Zostera marina were reproduced and macrobenthos were submerged in the shallows of the experimental site. It is considered that these results were caused by the improvement of their habitat through the mixture of the dredged soil and steelmaking slag.

1. Introduction

Steelmaking slag is a by-product of the steelmaking process and is produced in amounts of about 40 mt/y in Japan. Steelmaking slag has been widely used in construction works as cement, roadbed, and civil engineering materials.[1)] In recent years, it has become difficult to predict the market supply and demand for steelmaking slag due to changes in social conditions, such as the decline in domestic public works and construction demand, competition with other by-products, and accelerated use of waste concrete and other recycled materials. Given these situations, we have been required to develop new uses for steelmaking slag in order to expand its sales channels.

Against this background, using steelmaking slag and particularly BOF steelmaking slag as “environmental materials”, Nippon Steel Corporation developed a seaweed bed construction material (Vivary™ Unit) by mixing carbonated steelmaking slag with humus, slag-improved soil (dredged soil conditioned with steelmaking slag), and artificial stones (Vivary™ Rock and Vivary™ Block) using steelmaking slag as the aggregate (**Fig. 1**).

The Vivary™ Units are used to counteract macroalgae depletion. The slag-improved soil is used to prevent blue and red tides. Steelmaking slag is not simply used as civil construction material but is applied to remediate the environment where it is used, mainly in coastal areas. These differ significantly from conventional slag utilization technologies. In our development work, we conducted laboratory experiments to clarify the mechanism whereby the effect of steelmaking slag appears. Using large water tank facilities called “Sea Lab. and Sea Lab. II”, we simulated phenomena with the use of steelmaking slag and studied the sustainability of its effectiveness. In this way, we have scaled up our research on steelmaking slag and have deepened our findings about the usefulness of steelmaking slag. Furthermore, we have conducted acute toxicity tests of eluates from steelmaking slag and the long-term effect evaluation

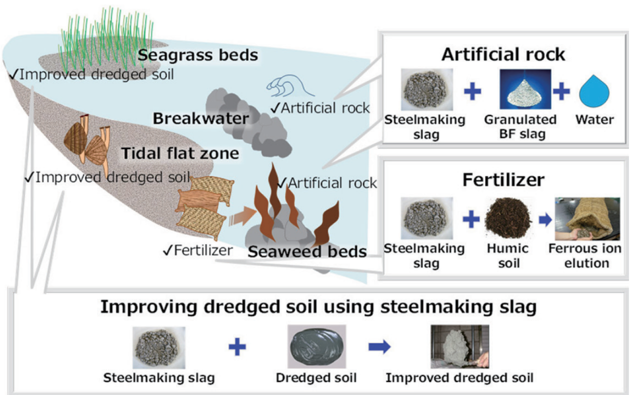

Fig. 1 Slag utilization for marine environmental restoration

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experiment of the slag in Sea Lab. II, and verified the safety of steelmaking slag.[2–5] In this special issue, we reference the studies by Kato et al. As described above, the technologies for utilizing steelmaking slag in coastal areas have been steadily applied to actual coastal areas while performing scientific demonstrations.

In recent years, coastal areas as principal places for the use of steelmaking slag have been expected as places to sequester CO_2 as blue carbon. Blue carbon is the carbon captured by marine organisms into the sea. It was defined for the first time in the report "Blue Carbon" (hereinafter referred to as the UNEP Report) jointly announced in 2009 by the United Nations Environment Program (UNEP), the Food and Agriculture Organization of the United Nations (FAO), and the United Nations Educational, Scientific and Cultural Organization (UNESCO).[6] The UNEP report underscores the magnitude of the impact of blue carbon on the global environment. It also states that of the CO_2 (green carbon) absorbed by living organisms on the earth, half is absorbed on land and the other half (55%) is absorbed in seas and especially in coastal areas. Mangrove forests, salt marshes (in coastal areas subjected to tides but negligibly affected by waves), and seagrass beds are mainly considered as ecosystems that store blue carbon (hereinafter referred to as blue carbon ecosystems).

Whether the use of the blue carbon is added or stated in the Intended Nationally Determined Contribution (INDC) of our own country is a measure of the use of blue carbon or the recognition of its use as a CO_2 emission reduction measure. Of the 151 countries which ratified the Paris Agreement, 58% have shown some blue carbon initiatives. Also, Australia and the United States have started their addition to the Paris Agreement Inventory. Several countries such as China, Indonesia, and the United Arab Emirates (UAE) have announced that they will prepare to start adding to the Inventory. In contrast, Japan has not yet announced its blue carbon initiatives.[7]

In response to the international blue carbon initiatives described above, similar movements have appeared in Japan. In 2017, the Blue Carbon Study Group was established under the sponsorship of the Ministry of Land, Infrastructure, Transport and Tourism (MLIT). In the industry, the Japan Project-Industry Council (JAPIC) established a similar blue carbon study group. The MLIT-sponsored Blue Carbon Study Group has set the main tasks of increasing the number of trial sequestered CO_2 volume calculation cases for many regions and species and improving the accuracy of calculating the sequestered CO_2 volume. The Blue Carbon Study Group has been summarizing the trial sequestered CO_2 volume calculation cases and has been preparing to present national blue carbon initiatives in cooperation with other ministries such as the Ministry of the Environment and the Ministry of Agriculture, Forestry, and Fisheries and to add blue carbon to the CO_2 inventory. Japan has the sixth longest coastline in the world. If we can develop effective coastal area utilization measures by making the best use of this land feature and can create mechanisms to do so, such measures and mechanisms are greatly expected to lead to the reduction of Japan's CO_2 emissions.

Based on the above trends, Kuwae et al.[8] analyzed data from past literature values to calculate the potentials of blue carbon ecosystems in Japan. As a result, they estimated the annual CO_2 sequestration capacity in Japan's coastal ecosystems (seagrass beds, seaweed beds, mangrove forests, tidal flats) at an average of 1.32 mt-CO_2. They also stated the need for building new blue carbon ecosystems by using recycled materials such as dredged soil and steelmaking slag.

Based on the above, we have developed tidal flat and shallow technologies by utilizing steelmaking slag. The slag-improved soil was developed as an effective measure for utilizing the dredged soil generated in harbors. Since the slag-improved soil solidifies soft bottom soil, it is expected to be a material for building artificial tidal flats and shallows and especially for raising sea beds. Fishermen hoped that the slag-improved soil would serve as material for creating habitats for benthic organisms. To meet their expectations, we developed a method for removing fine particles from steelmaking slag and mixing the remaining steelmaking slag with the dredged soil to alleviate hydration and solidification and to improve the particle size of silty dredged soil.[9] We launched the experimentation at Sea Lab. II to verify the effectiveness of the non-solidifying slag-improved soil as soil for benthic organisms and to evaluate tidal flats and shallows restored as blue carbon ecosystems by using steelmaking slag. Sea Lab. II is located at the Research & Engineering Center of the R&D Laboratories in Futtsu City, Chiba Prefecture. It consists of two indoor experimental tanks and two outdoor reservoir tanks. It is possible to conduct an experiment by setting up an experimental site and a control site under a controlled environment that cannot be realized in an actual sea area (influent seawater, seawater temperature, lighting, flow direction, and flow velocity) (**Fig. 2**). Unlike a mesocosm tank generally composed of zones of the same water depth, each experimental tank is composed of two zones of different water depths (a tidal flat zone and a shallow zone). The experimental tank was designed to reproduce the environment of a shallow coastal area in a single water tank and to evaluate complex changes in an overall coastal ecosystem of a tidal flat and a shallow (**Fig. 3**). One of the two experimental tanks was used as the experimental site and filled with the slag-improved soil on the bottom. The other was used as the control site and filled with the dredged soil on the bottom. A tidal flat and a shallow were created in each tank. The water quality, benthic organisms, and other conditions were regularly monitored. We determined the CO_2 sequestration capacity under different bottom material conditions while tracking the changes in the ecosystems created in the tanks.

In the following sections, we summarize the changes in the ecosystems created in Sea Lab. II during a period of about 2 years and 3 months from the start of the experiment in November 2017 and discuss the sufficiency of our data to calculate the CO_2 sequestration capacity. Lastly, we present an outlook of future blue carbon research.

Fig. 2 Experimental tanks in Sea Lab. II

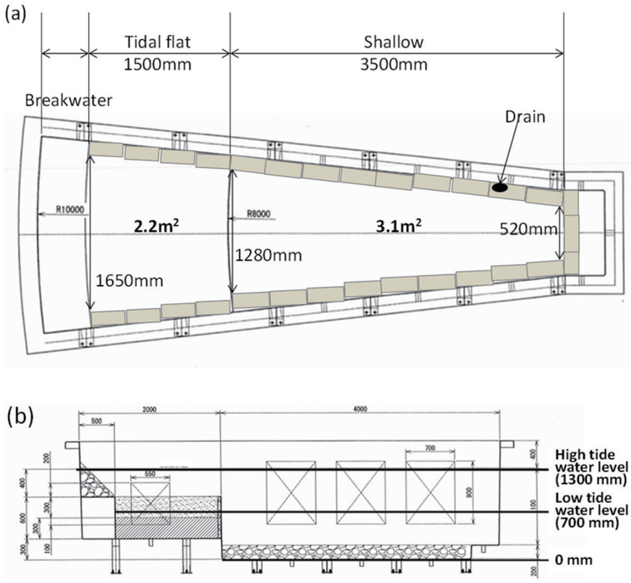

Fig. 3 Plane view (a) and cross-sectional view (b) of experimental tank

2. Main Subjects

2.1 Experimental conditions and research methods

2.1.1 Tides and seawater introduction and exchange

The two experimental tanks of Sea Lab. II have the same shape as that shown in Fig. 3. Each tank was divided into a tidal flat zone and a shallow zone. The two zones were different in depth. The tidal flat zone was designed so that the bottom material dried up at a low tide.

Tides were created in the experimental tanks by exchanging the seawater between the experimental tanks and the reservoir tanks and by flushing part of the seawater. This experiment set the high tide at 1 300 mm and the low tide at 700 mm. The water level was changed at intervals of 12 h per tide. When the experimental tank changed from high tide to low tide, it was set to discharge the seawater to outside of the system (50% per tide) and to completely replace the seawater in one day. The seawater used in the experiments is fed from an intake pump installed near the revetment on the north side of the Research & Engineering Center, is temporarily stored in the reservoir tanks without filtration and any other treatment on its way, and is introduced into the experimental tanks according to the operating conditions.

2.1.2 Waves

Among the water flows in the experimental tanks were reciprocating water flows (maximum wave height of about 2 cm) perpendicular to the longitudinal direction of the transverse section of the tank and generated by the translational motion (2 s/time) of a wave-maker and water flows vertical to the longitudinal direction of the transverse section of the tank and generated by the exchange of water with a water temperature controller.

2.1.3 Light

Artificial lighting metal halide lamps were installed above each experimental tank. The height of the lighting was maintained to ensure a light quantum density of 100 to 150 μmol/m^2/s (approximate brightness under cloudy weather) on the sea surface at high tide. For the actual quantity of light, we measured the light quantum density (μmol/m^2/s) at a water depth of 50 cm in three places in the tank using a JFE Advantech AAQRINKO multi-item water quality meter in June and November 2018, February and June 2019, and January 2020.

Light and dark periods were reproduced by turning the lights on and off. The duration of the light period was set at 10 to 14 h depending on the season (12 h in February to May, 14 h in June to August, 12 h in September to November, and 10 h in December to January).

2.1.4 Water temperature

The water temperature in the experimental tanks was controllable within ±1°C of the set temperature by the water temperature controller and was preset with reference to the surface layer water temperature in Tokyo Bay.[10] The air conditioner set temperature was changed to stop the room temperature from deviating too much from the water temperature. The water temperature in the experimental tanks during the experimental period mostly followed the set water temperature (**Fig. 4**).

2.1.5 Bottom material

Table 1 shows the materials used in the experimental and control sites. The dredged soil used in the experiment was the dredged soil collected off the coast of Hirohata, Hyogo Prefecture in July 2017. The steelmaking slag used in the experimental site was BOF steelmaking slag from the Kimitsu Area of the East Nippon Works.

In the tidal flat zone, the dredged soil and slag-improved soil (mixed with 30 v/v% of 0 to 30 mm steelmaking slag particles) were spread to a thickness of 25 cm in the control and experimental sites, respectively. These soils were then covered with river sand (fine aggregate for concrete) to a thickness of 20 cm. In the shallow zone, dredged soil and slag-improved soil (mixed with 20 v/v% of 10 to 30 mm steelmaking slag particles) were spread to a thickness of 25 cm in the control site and experimental sites, respectively. The slag-improved soil in the tidal flat zone at the experimental site was applied to fully solidify so that it would function as a filling material for the tidal flat. In the shallow zone, on the other hand, the slag-improved soil was applied not to solidify so that *Z. marina* and benthic organisms could be recruited.

2.1.6 Water quality and bottom material monitoring

The water was sampled from the experimental tanks at almost

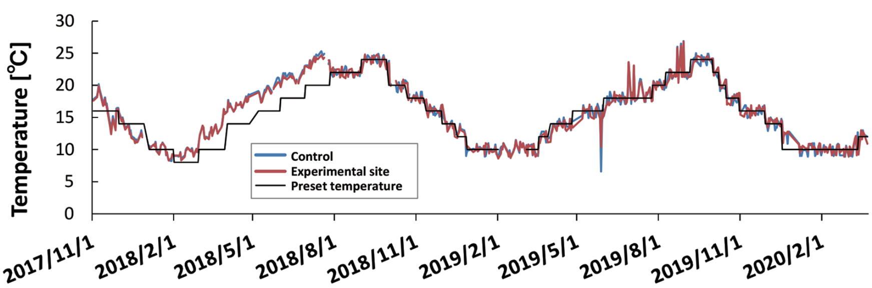

Fig. 4 Water temperature

Table 1 Materials using mesocosm experiments

	Control site		Experimental site	
	Tidal flat	Shallow	Tidal flat	Shallow
Sand	$0.45\,m^3$ (20 cm thick)		$0.45\,m^3$ (20 cm thick)	
Dredged soil	$0.55\,m^3$ (25 cm thick)	$0.77\,m^3$ (25 cm thick)		
Slag improved soil			Dredged soil: $0.38\,m^3$ Steelmaking slag (0–30 mm): $0.16\,m^3$ (25 cm thick)	Dredged soil: $0.61\,m^3$ Steelmaking slag (10–30 mm): $0.16\,m^3$ (25 cm thick)

the same time and at a predetermined place. The influent seawater was sampled once a week from an intake installed ahead of the reservoir tank. The tank seawater and the influent seawater were each suction-filtered with a 0.45 μm membrane filter (ADVANTECH, cellulose mixed ester type) and then subjected to analysis. The analysis items were pH, turbidity, tristate nitrogen (sum of NH_4-N, NO_2-N, and NO_3-N, or collectively referred to as DIN), phosphate phosphorus (PO_4-P), and silicon (D-Si). The turbidity was determined from the 660 nm absorbance and the calibration curve prepared as specified in JIS K 0101 “Testing methods for industrial water”. The DIN was analyzed by a SEAL Analytical QuAAtro39 AutoAnalyzer, PO_4-P was analyzed in a 5 cm quartz cell by molybdenum blue colorimetry, and D-Si was analyzed by a Shimadzu ICPE-9000 ICP emission spectrometer.

In January 2019, we started determining the amount of chlorophyll *a* (Chl. *a*) in seawater, an indicator of the abundance of phytoplankton, by extracting with N, N-dimethylformamide (DMF). The influent seawater, the seawater in the control site, and the seawater in the experimental site were sampled in amounts of 100 mL each. Each sample was suction filtered with a Whatman φ47 mm glass fiber filter paper GF/F. The sample was sandwiched between filter papers to remove the water. The Whatman filter paper was immersed in DMF pipetted in a 15 mL centrifuge tube and left overnight in a −30°C freezer to extract the Chl. *a*. On the next day, the fluorescence intensity of the Chl. *a* was measured at an excitation wavelength of 436 nm and a fluorescence wavelength of 680 nm. The Chl. *a* (μg/L) was determined from a pre-prepared calibration curve.

In June 2019, we measured the suspended solids (SS) and volatile suspended solids (VSS) in the experimental tanks to determine the turbidity components. We sampled 2 L and 1 L of the seawater at the experimental and control sites, respectively. Each seawater sample was suction-filtered with a Whatman 1.0 μm glass fiber filter paper GF/B. The filter paper was dried in a dryer at 105°C for 2 h and allowed to cool in a desiccator. The sample was re-weighed and the pre-measured filter paper weight was subtracted from the sample weight to determine the SS. Additionally, the filter paper was heated (ashed) at 600°C for 30 min, allowed to cool in the desiccator, and weighed to determine the VSS. The VSS was subtracted from the SS to determine the ignition loss (JIS K 0101).

The hardness of the bottom material was measured in June and November 2018 and February and June 2019. At three predetermined points each in the shallow zone and the tidal flat zone, the hardness of the bottom material was measured three times each with a Yamanaka hardness tester.

2.1.7 Transplantation and growth evaluation of *Z. marina*

In this experiment, we transplanted *Z. marina* and evaluated its subsequent growth to form its communities and beds in the experimental tanks. *Z. marina* is a typical organism that stores blue carbon. *Z. marina* seeds were collected in Shima City, Mie Prefecture. From August 2017, the seeds were sown, germinated, and grown at the Hayama Marine Science Laboratory of Kajima Corporation. The seedlings were grown larger in the tanks at the Research & Engineering Center for 5 months from November 2017 and were transplanted in the experimental tanks at Sea Lab. II in April 2018. After transplantation, the number of shoots was counted to evaluate the growth of *Z. marina* with different bottom materials (June, September, November, and December 2018, February and July 2019, and January 2020).

2.1.8 Benthic organism survey

We investigated benthic organisms (benthos) that were naturally recruited into the experimental tanks via seawater, especially meiobenthos (32–500 μm) and macrobenthos (0.5–1 mm<). Benthos is a general term for organisms that live in the water bottom. Some live burrowed in the bottom (endobenthic), some live on the surface of the bottom (epibenthic), and some live in tubes in the bottom.

For meiobenthos, cores were taken down to 1 cm from the surface with a core sampler with a diameter of 3 cm (inner diameter 2.6 cm) at three randomly selected points each in the shallow and tidal flat zones. Each core was transferred into a 60 mL ointment bottle and fixed with neutral formalin to a final formalin concentration of 10%. The unnecessary parts of the collected cores were returned to the original sampling points. The fixed sample was passed through a sieve with a mesh size of 1 mm. The undersize was further passed through a sieve with a mesh size of 500 μm and then through a sieve with a mesh size of 32 μm. Organisms remaining on the last sieve were dyed with rose bengal. The species and population size of the organisms were identified and counted with a stereomicroscope and a biological microscope, respectively. The number of species that appeared at the control site and the experimental site was evaluated over a total area of 15.9 cm^2 at three points each in the shallow and the tidal flat zones.

For the macrobenthos, a 20 cm × 20 cm square frame was placed at one point in the shallow zone and at one point in the tidal flat zone. The bottom soil in each square frame was sampled with a scoop. Each bottom material sample was screened with a sieve with a mesh size of 1 mm. The organisms that remained on the sieve were transferred into a 1 L polypropylene bottle and fixed with formalin to a final formalin concentration of 10%. In the laboratory, the species and population size of the organisms were identified and counted under the stereomicroscope, respectively.

2.2 Results

2.2.1 Tank environment (pH, water quality, bottom material hardness)

The pH changed in a standard seawater pH range of 7.9 to 8.3 at both the control and experimental sites. No alkaline effect of the steelmaking slag used at the experimental site was observed (**Fig. 5** (a)). The dissolved components DIN, PO_4-P, and D-Si showed no significant differences between the influent seawater and the experimental tank seawater. They behaved similarly throughout the measurement period (Figs. 5(b) to 5(d)). The seasonal variation of the DIN was higher in fall and winter (September to February) than in spring and summer (March to August). The trends were reversed for the PO_4-P and D-Si. They tended to be higher in spring and summer (March to August) than in fall and winter (September to February).

Regarding the suspended components, the Chl. *a* and turbidity were higher in the influent seawater than in the experimental tank seawater on many days. The Chl. *a* in the experimental tank seawater was less than 1.7 μg/L on average throughout the measurement period. It was lower than the standard of less than 3 μg/L for short-neck clam fishing grounds specified in the Fisheries Water Standards.[11] On the other hand, the Chl. *a* was a total average of 6.7 μg/L in the influent seawater (**Fig. 6**(a)). The turbidity in the experimental tank seawater continued to be higher at the control site than at the experimental site for a long period of time (Figs. 6(b) and (c)). Especially, it exceeded that of the influent seawater in March to July 2019. The seawater turbidity difference between the experimental tanks during this period was visually apparent (**Fig. 7**). When the seawater turbidity components in the experimental tanks were investigated in June 2019, the SS and the VSS were more than 5 times higher and more than 2 times higher at the control site than at experimental site, respectively (**Table 2**). Furthermore, the VSS and SS at the control site showed that 60% of the turbid components at the control site were inorganic components.

For the quantity of light in the experimental tanks, the daily light integrals at each measurement time were calculated from the measured photon quantum density and the set light period (**Table 3**). As a result, almost no differences were observed between the experimental tanks in June 2018. In November 2018 when the turbidity at the control site started to exceed that at the experimental site, the daily light integrals at the control site were lower than at the experimental site. The same trend continued until June 2019 when a significant turbidity difference was observed. In January 2020, the difference in the daily light integrals decreased daily and reflected the turbidity result.

The hardness of the bottom material in the tidal flat zone measured during the experimental period was stable at a hardness of about 8 mm between the control and experimental sites. The shallow zone at the experimental site maintained a hardness index of about 6 mm and showed no extreme hardness. The bottom material in the shallow zone at the control site was so soft that its hardness index could not be measured (**Fig. 8**).

2.2.2 *Zostera marina*

The *Z. marina* transplanted onto the entire surface of the shallow zone in April 2018 was once depleted but remained in a relatively large amount in the central part. It continued to be depleted until about November 2018 as the seawater temperature rose. Regenerat-

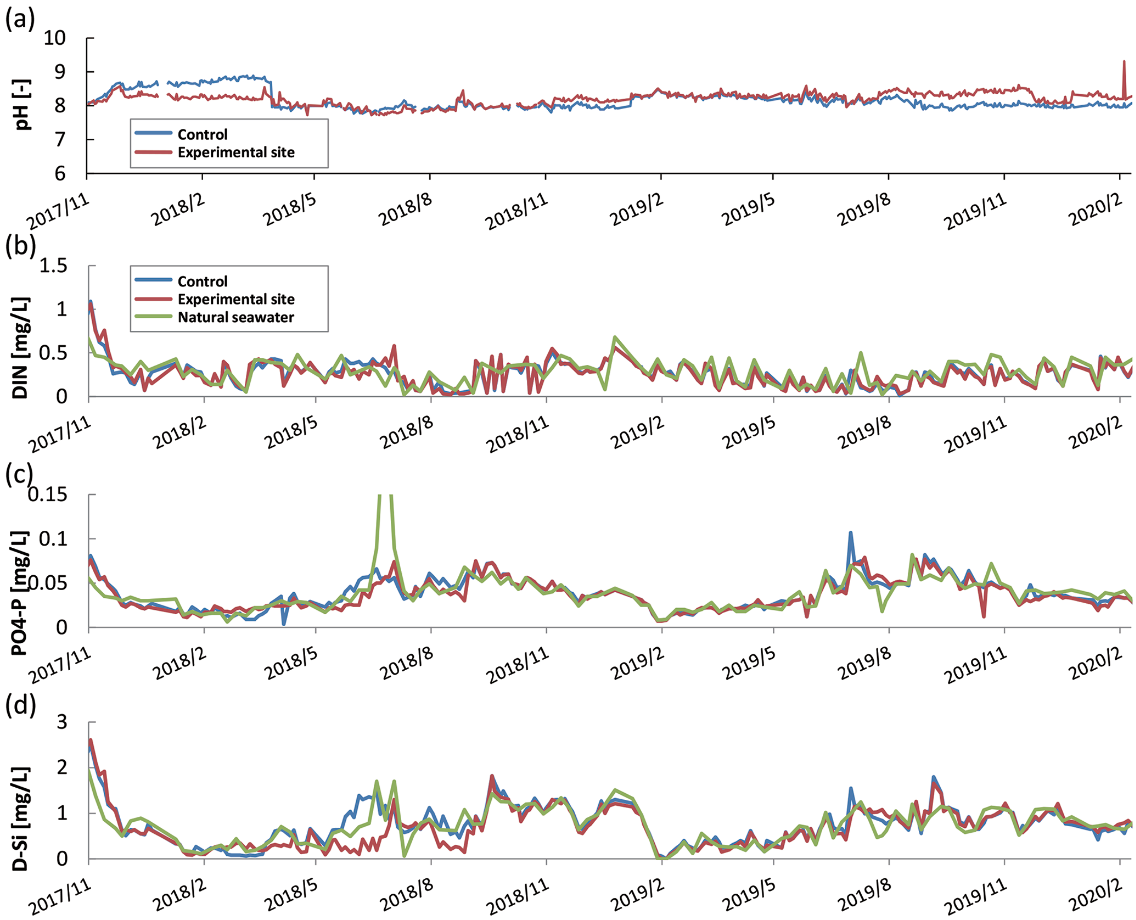

Fig. 5 Changes in dissolved components (pH (a), DIN (b), PO_4-P (c), D-Si (d))

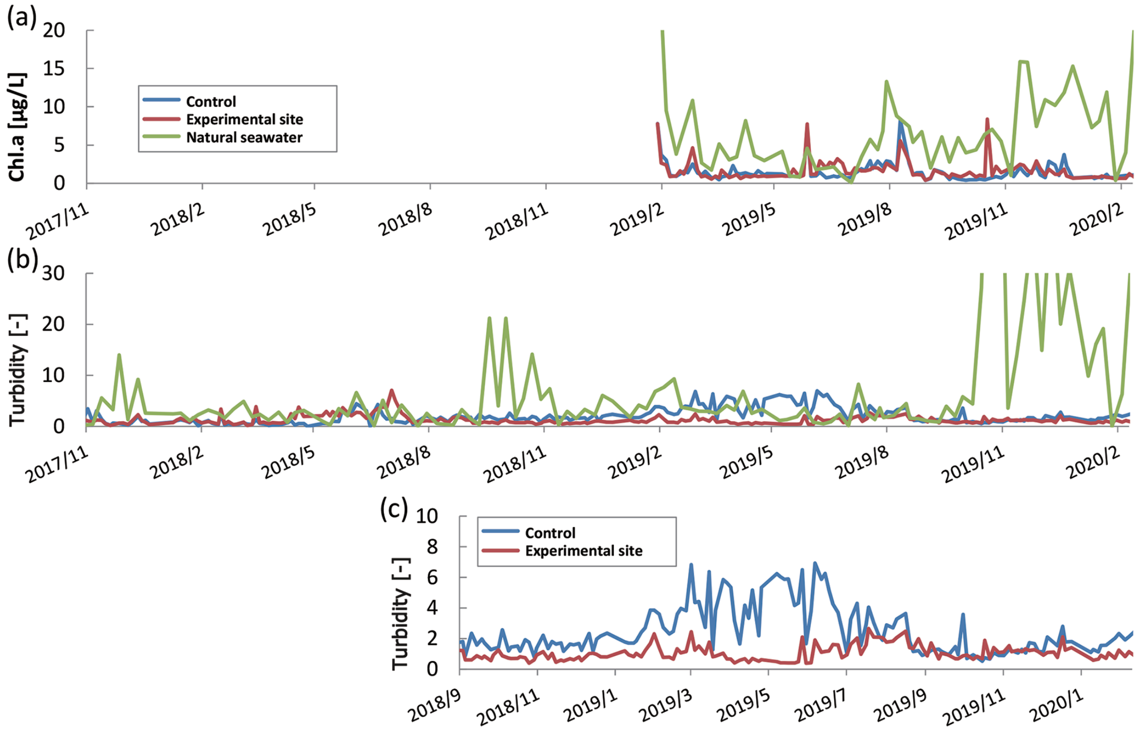

Fig. 6 Changes in suspended components (Chl. *a* (a), turbidity (b), turbidity (c) enlargement)

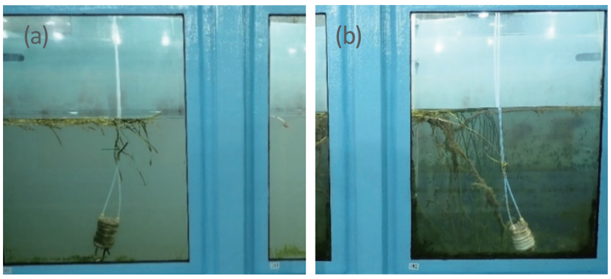

Fig. 7 Difference of turbidity between control site (a) and experimental site (b) (June 2019)

Table 2 Difference of turbidity elements between control site and experimental site (June 2019)

	Control site	Experimental site
SS [mg/L]	5	<1
VSS [mg/L]	2	<1

Table 3 Accumulated light amounts in control site and experimental site

	Light period [h]	Accumulated light amount [mol/m²/d]	
		Control site	Experimental site
2018/6	14	2.3	2.6
2018/11	12	2.4	3.0
2019/2	10	1.5	2.0
2019/6	14	2.0	2.8
2020/1	10	2.0	2.2

ed individuals started to appear in October 2018 at both the control and experimental sites. In December 2018, many regenerated individuals were confirmed near the large individuals that survived the year. As of December 2018, there were few regenerated individuals at the control site. The number of shoots was clearly different from that at the experimental site. In February 2019, the number of shoots reached a maximum of 25 at the experimental site. At that time, the number of shoots already began to decrease to 7 at the control site. In March 2019, flowering shoots were confirmed in both tanks. In July 2019, the number of shoots again became 0 at the control site due to seasonal fluctuations. At the experimental site, 13 shoots remained (**Fig. 9**). In around November 2019, regenerated individuals began to be seen again. The regeneration of the *Z. marina* was repeated in the experimental tanks. Particularly at the experimental site, adult individuals remained to stably retain the *Z. marina* biomass.

2.2.3 Benthic organisms

The meiobenthos is classified by morphological characteristics at the phylum and class levels. The abundance of the meiobenthos by phylum in our 2-year survey is summarized in **Figs. 10** and **11**. The abundance of the meiobenthos did not increase in both the tidal flat zone and the shallow zone. No difference was observed in the abundance between the control and experimental sites (Fig. 10). When the composition of the meiobenthos was investigated, arthropods were found more in the shallow zone than in the tidal flat zone. They also tended to appear more at the experimental site than at the control site (Fig. 11).

When the total number of macrobenthos species measured in the 2-year survey was examined, no significant difference was found in the tidal flat zone between the control and the experimental sites. There was no increasing or decreasing tendency. On the other hand, in the shallow zone, especially at the experimental site, the abundance of the macrobenthos tended to increase in winter and decrease in summer (**Fig. 12**). When the appeared species were closely examined, many endobenthic species were observed at the experimental

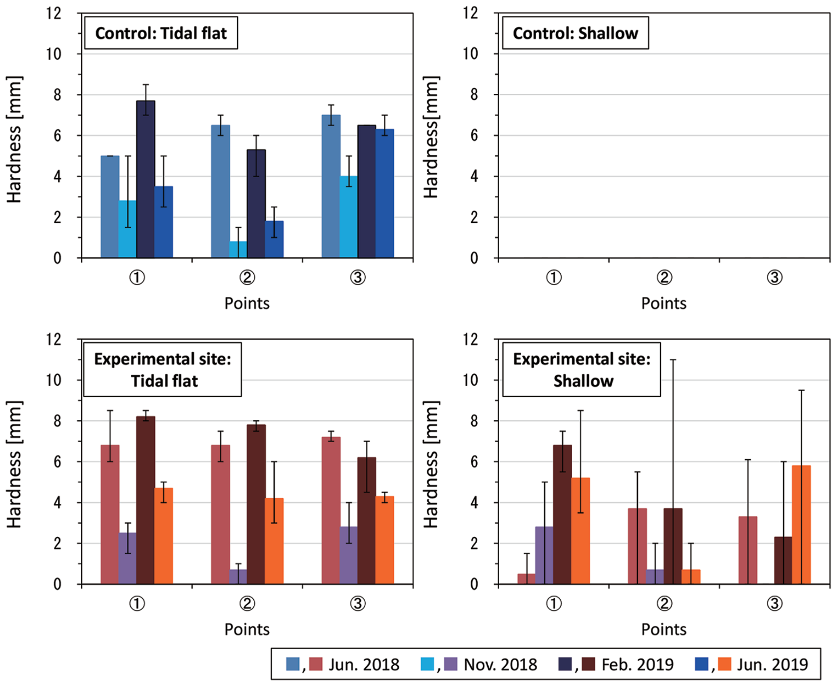

Fig. 8 Changes in hardness of sediments in tank (The values in the graph show the average of the three measurements. Error bars indicate the range from minimum to maximum.)

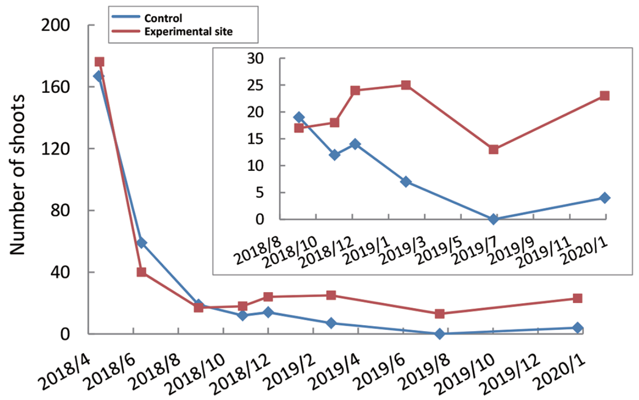

Fig. 9 Changes in number of *Zostera marina* (Upper right graph shows enlargement graph after August 2018.)

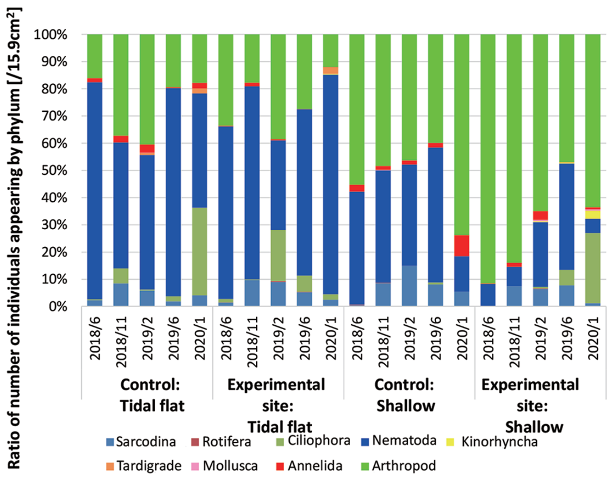

Fig. 11 Composition of number of individuals appearing by the phylum of meiobenthos

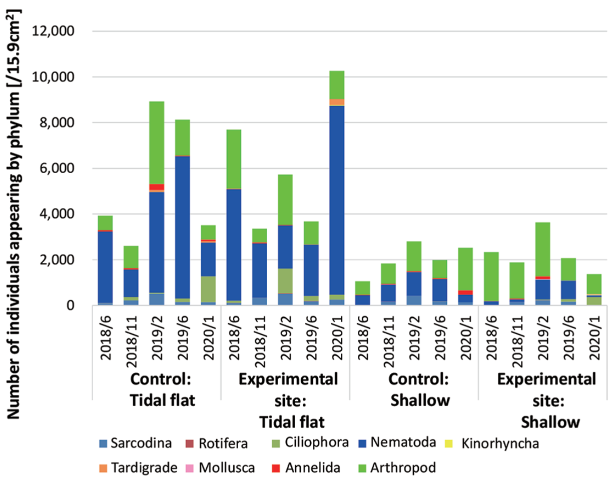

Fig. 10 Changes in number of meiobenthos

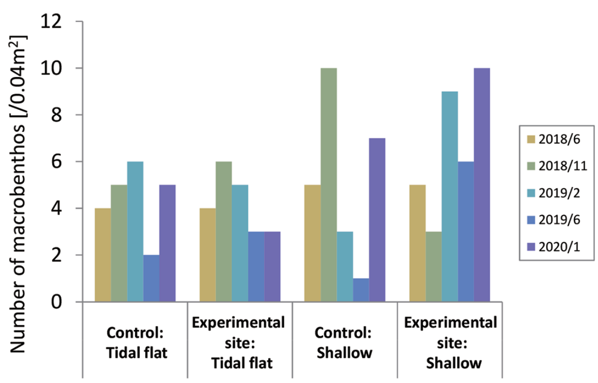

Fig. 12 Changes in number of macrobenthos

site (for example, *Nectoneanthes latipoda*, *Prionospio pulchra*, *Clymenella collaris*, and *Amphitrite* sp.).

2.3 Discussion

Throughout this experiment, we periodically analyzed the influent seawater once a week for 2 years and clarified the seasonal fluctuations and other characteristics of the influent seawater. No differences were observed in the dissolved components between the experimental and control sites. The concentrations of the dissolved components approximated those in the influent seawater. According to the tidal conditions of this experiment (2 tides/day, 50% of the seawater in the experimental tanks was replaced by one tide), it was confirmed that the quality of the seawater in the experimental tanks was mostly the same as that of the influent seawater.

The sources of the DIN are considered to be the inflow loads from rivers (domestic wastewater, agricultural wastewater, rain) and the mineralization of organic matter deposited at the bottom. The former shows no clear seasonal variations and has too many varying factors to make its prediction difficult. In the latter case, the activity of bacteria in the bottom material and the elution of the dissolved components from the bottom material both increase at high water temperatures in summer.[12)] In the surface layer of Tokyo Bay in summer, the productivity of organisms increases to decrease the DIN.[13)] The results of our analysis showed no significant increase in the Chl. *a*. The decrease in the DIN in summer is considered to reflect the above tendency.

The main source of PO_4-P in Tokyo Bay is the inflow load from land areas.[14)] In summer, the products of decomposition of organic matter by bacteria in the bottom layer are added as another source of PO_4-P.[12–15)] Silica from rivers and silica from the redissolution of the carcasses of diatoms collected at the bottom are sources of the DIN.[12)] Particularly, the redissolution of silica from diatoms is shown to progress as the water temperature rises.[16, 17)] The PO_4-P and D-Si observed at Sea Lab. II were both high from spring to summer. This result reflected the tendency of the PO_4-P and D-Si in Tokyo Bay. As described above, the seawater in Tokyo Bay was drawn into Sea Lab. II by the pump installed on the north revetment of the Research & Engineering Center. It was then supplied to the reservoir tanks via about 250 m of vinyl chloride pipe. It is suggested that the PO_4-P and D-Si in the supplied seawater changed similar to the seasonal fluctuations of the PO_4-P and D-Si in the seawater in Tokyo Bay.

Our analysis failed to identify any correlation between the water quality components and the behavior of the Chl. *a*. The nutrient salts DIN, PO_4-P, and D-Si are supplied to sea areas through complicated routes. Nutrient salt loads from rivers and their supply from the sea bottom are unknown. These conditions made it difficult to estimate the Chl. *a* concentration from seawater quality analysis alone.

As shown in Fig. 6, the turbidity at the control site exceeded that at the experimental site and that of the influent seawater, especially from March to July 2019. This strongly suggests the formation of suspended solids in the tanks. The turbidity at the control site continued for a long period of time. The suspended Chl. *a* concentration did not differ between the control and experimental sites. On the other hand, the SS and VSS concentrations were both high at the control site. The inorganic components accounted for 60% of the turbid components. From these data, it can be inferred that the main cause of the turbidity at the control site was the swirling up of the bottom material and that the instability of the bottom material exerted an effect on the turbidity.

On the other hand, high clarity was maintained at the experimental site throughout the experimental period. The non-solidifying slag-improved soil used as the bottom material at the experimental site maintained a hardness index of about 6 mm. The hardness index is 15 mm under the conditions for the solidification of the conventional slag-improved soil (0 to 30 mm and 30 v/v% mixture). It was thus confirmed that the slag-improved soil used as the bottom soil was not solidified under the conditions of the experimental site. Since many endobenthic macrobenthos that live burrowed in the bottom material were observed, the bottom material was confirmed to have no problems as a habitat of benthic organisms. These results are probably due to the reduction of the specific surface area of the steelmaking slag from which fine particles (0 to 10 mm) were removed and due to the alleviation of the hydration and solidification that otherwise occurs with the conventional slag-improved soil. In addition, mixing the dredged soil with steelmaking slag with a particle size distribution of 10 to 30 mm helped to diversify the particle size composition of the bottom material and hence to stabilize the bottom material. As a result, it can be inferred that the swirling up of the bottom material was suppressed to allow a sufficient amount of light to reach into the tank. In fact, at the experimental site, the *Z. marina* grew in abundance and regenerated.

In December 2018, the number of *Z. marina* shoots was considerably larger at the experimental site than at the control site. Thereafter, adult individuals appeared at the experimental site. At the control site, on the other hand, the *Z. marina* shoots gradually decreased and completely disappeared in July 2019. As described above, especially from March to July 2019, the concentration of turbidity in the seawater at the control site markedly exceeded that in the influent seawater. The turbidity in the tank seawater was thus noticeable. As shown in Fig. 9, the number of *Z. marina* shoots decreased significantly during the same period. This period is normally an overgrowth period. It is strongly suggested that the turbidity increase at the control site decreased the amount of light and adversely affected the growth of the *Z. marina*. There are several factors that affect the depletion of *Z. marina* beds, such as high water temperature,[18)] low salt content,[18)] and dissolved oxygen concentration.[19)] The decline of *Z. marina* beds due to the increased turbidity has also been reported for natural *Z. marina* beds.[20)]

According to his survey and literature search on the amount of light required for the growth of the *Z. marina*, Kawabata[21)] reported minimum daily light integrals of 3.0 and 1.5 μmol/m^2/d as measures of the amount of light required for spring to summer and from fall to winter, respectively.[21)] Nakamura et al.[22)] defined and verified the amount of light required to maintain the *Z. marina* biomass by considering the characteristic of the *Z. marina* of frequent leaf generation and the amount of its leaf shedding. Their results supported the values reported by Kawabata[21)]. The amount of light required for *Z. marina* beds as reported by Kawabata[21)] was only exceeded by the amounts of light at the control site in November 2018 and at the experimental site in November 2018 and February 2019 in our experiment. It is speculated that the *Z. marina* at the control site was exposed to a light-deficient environment for a longer period of time than at the experimental site, especially from February to June 2019, normally a sprouting period. This situation is considered to have greatly affected the complete disappearance of the *Z. marina* in July 2019.

The difference in the *Z. marina* growth between the tanks was probably affected by the amount of light due to turbidity as well as by the stability of the bottom material as a *Z. marina* habitat. The rhizomes of the *Z. marina* were rarely exposed at the experimental site. In addition, the regenerated individuals germinated from the inside of the bottom material rather than from the surface of the bottom material. This means that the bottom material at the experimental site is good for the growth of the *Z. marina*. At the control site, in contrast, regenerated individuals were poorly grown, and individuals exceeding 1 m were dislodged from the bottom material. The growing environment for the *Z. marina* in nature is usually sandy (0.075 to 2 mm). The bottom material at the control site in our experiment was dredged soil and was silty with a particle size of 0.005 to 0.075 mm. This bottom material condition affected the growth of the *Z. marina*. Another probable cause was that there were no anchors around which the *Z. marina* could wrap their rhizomes.

In our experiment, the *Z. marina* was greatly depleted immediately after transplantation at Sea Lab. II. But the *Z. marina* that remained at both the control and experimental sites regenerated and successfully formed *Z. marina* beds. Concerning the growing of the *Z. marina* in a mesocosm tank, Nakamura et al.[23] reported their study with a mesocosm tank owned by the Port and Airport Research Institute, a national research and development agency, in Yokosuka City, Kanagawa Prefecture. The repeated regeneration of the *Z. marina* under a completely artificial weather and under artificial lighting with metal hydride lamps as conducted at Sea Lab. II is an extremely rare example and is expected to provide academically valuable data. A bottom material mainly composed of fine particles is not essentially suitable for the growth of the *Z. marina*. Instead, a bottom material with appropriate hardness, such as slag-improved soil, can be used to provide and maintain a bottom environment suitable for the growth of the *Z. marina*. We think that our study has demonstrated this capability of slag-improved soil.

More macrobenthos were observed in the shallow zone at the experimental site than those in the shallow zone at the control site in and after February 2019, more than a year after November 2017. It can be evaluated that a rich ecosystem was formed on the whole with the growth of the *Z. marina*. However, the abundance of benthos is smaller than in a natural environment. The recruitment of meiobenthos and macrobenthos from the influent seawater was extremely gradual throughout the experimental period of about 2 years and 3 months. Kuwae et al.[24] reported that the diversity of organisms in a mesocosm tank was lower than in a neighboring ecosystem even after 6 years of monitoring. At Sea Lab. II, the distance from the intake pump to the reservoir tanks is long. The seawater from the intake pump is stored in the reservoir tanks. After about 6 h, the seawater is transported from the reservoir tanks to the experimental tanks. Given these equipment problems, it is thus considered more difficult to improve the diversity of organisms than reported by Kuwae et al.[24] Mesocosm experiments such as those conducted at Sea Lab. II are designed to simulate a natural environment. It is important to establish the constraints and understand the characteristics when comparing with the actual environment and demonstrating the phenomena involved. In the next step of our study, we will calculate and analyze the carbon sequestration capacity of the blue carbon ecosystem at Sea Lab. II according to the results obtained in the present step.

As described above, during the experimental period of 2 years and 3 months, we were able to grasp in detail the changes in water quality, the accompanying changes in the experimental tanks, and the changes in the ecosystem. We laid non-solidifying slag-improved soil in the shallow zone at the experimental site and proved the usefulness of the non-solidifying slag-improved soil as soil for constructing shallow beds. The formation of ecosystems in the experimental tanks by the regeneration of *Z. marina* and the recruitment of benthos are conducive to the evaluation of the blue carbon ecosystem that is the purpose of this study. The biomass and other conditions in the mesocosm are different from those in the natural environment. We will evaluate the diversity and carbon sequestration capacity of ecosystems by quantitatively comparing the experimental and control sites under the same conditions while taking advantage of the original advantages of the mesocosms.

Nippon Steel's blue carbon research originated from the development of technology for utilizing steelmaking slag in sea areas. Our blue carbon research is not only limited to supporting the commercialization of our steelmaking slag, but is also critical in sustaining the steel industry rooted in harmony with the local regions and the environment. This is especially so for Nippon Steel that has coastal steelworks. Our efforts are consistent with Goal 12-Responsible consumption and production, Goal 13-Climate action, and Goal 14-Life below water, among the Sustainable Development Goals (SDGs) advocated by the United Nations.[25] Green carbon and other CO_2 emission reduction measures are not yet properly established in Japan. Nippon Steel has several barriers to overcome before it can offset its CO_2 emissions. We will continue to proceed with our research to lead Japan's blue carbon ecosystem construction technology by using our performance-proven technology for utilizing steelmaking slag in sea areas.

3. Conclusions

At Sea Lab. II, we conducted our experiment for 2 years and 3 months to create a blue carbon ecosystem by using steelmaking slag and to calculate the CO_2 sequestration capacity of the blue carbon ecosystem. We found that a rich ecosystem was formed as a whole at the experimental site by using steelmaking slag. We proved the usefulness of the non-solidifying slag-improved soil as soil for forming shallow beds. Based on the experimental equipment characteristics of Sea Lab. II, we will evaluate the carbon sequestration capacity of the ecosystems created in the experimental tanks and will push ahead with our blue carbon research.

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