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Effect of Digestate from Methane Fermentation using *Ulva* sp. and Food Waste for Cultivation of Decolored *Pyropia yezoensis* (Edible Laver Seaweed)

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ABSTRACT

Ulva sp. (green seaweed) often proliferates explosively and piles up in shallows. This phenomenon is called “green tide,” caused by increased nutrient flow into an enclosed sea area. Although green tide is one of the environmental problems in coastal areas, *Ulva* sp. can be regarded as carbon-neutral and therefore can serve as an abundant feedstock for renewable energy. Methane fermentation is one of the suitable techniques for converting such seaweed into energy. Digestate from methane fermentation is normally used as fertilizer; however, it is ends up being treated as wastewater due to limited spaces in urban areas. This paper proposes that the digestate from methane fermentation using mixed biomass (*Ulva* sp. and food waste) can be applied to the cultivation of *Pyropia yezoensis* (edible laver seaweed, *nori* in Japanese), which has recently suffered from decolored phenomena because of decreasing nutrients. The absorption of nutrients and the color recovery of *nori* were investigated in laboratory-scale experiments based on comparison with artificial seawater. The results highlight that the significant absorption of nutrients and color recovery occurred because of digestate utilization, indicating its positive effects on decolored *nori*. In addition, the experiments found that not only NH_4-N, but also other substances such as trace metal related to *Ulva* sp. can influence such effects. The findings clearly indicate that digestate can be used in the sea and that the suggested multiple uses of digestate would increase the value of digestate.

Keywords: methane fermentation, digestate, green seaweed, food waste, seaweed cultivation

INTRODUCTION

Ulva sp. (green seaweed) often proliferates explosively and piles up in shallows. This phenomenon is called “green tide,” caused by increased nutrient flow into an enclosed sea area, and has been reported in the enclosed sea area around Japan as well as other eutrophic shallow water areas around the world (Liu et al., 2009; Morand & Briand, 1996). This is because the nutrient levels in enclosed sea areas close to big industrial cities tend to be very high, given large pollution loads that are much higher than their natural purification capacities.

Pyropia yezoensis (edible laver seaweed), known as *nori* in Japanese, also causes environmental problems. *Nori* is commonly used in preparing *sushi*, a traditional Japanese dish. Cultured in the coastal sea area primarily during the winter season, the *nori* industry plays an important role in Japanese fishery. However, decolored *nori*, whose color becomes much lighter than the normal black-colored *nori*, has affected the *nori* industry because of its low quality especially after 1998 (Hori et al., 2008). In 2017, *nori* production generated 116 billion JPY (approximately 1 billion USD; MAFF, 2020), which constituted 23% of the total aquaculture revenue in Japan. Although normal *nori* is rated as 10 JPY/sheet, decolored *nori* is estimated as 3 JPY/sheet (Kawamura et al., 2011). One of the reasons for decolored *nori* is the decreasing level of nutrients, such as dissolved nitrogen and phosphorus, in the *nori* cultured sea area (Hori et al., 2008; Tada et al., 2010; Takagi et al., 2012). The reasons for the occurrence of decolored *nori* are being investigated in various fields such as chemistry, oceanography, meteorology, and biology (Zhang et al., 2004). Hence, unbalanced nutrient distributions can be observed in enclosed sea areas across Japan, such as Osaka Bay.

Ulva sp. is carbon-neutral and can therefore serve as an abundant feedstock for renewable energy. Methane fermentation is one of the suitable techniques for converting such seaweed into energy, because of its high moisture content. However, one of the difficulties in promoting methane fermentation in urban areas such as Osaka, which is one of the most populous cities in Japan, is the generation of digestate (Kuroda et al., 2017). Although digestate can be used as fertilizer because of its nutrient-rich content,

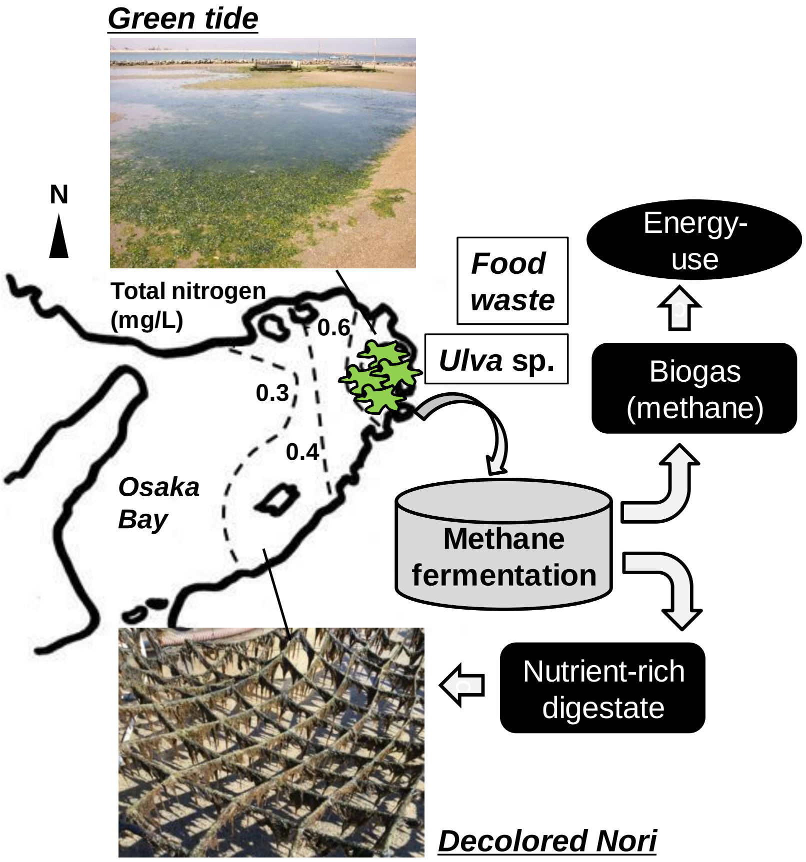

Figure 1. Concept for methane fermentation system

it is normally treated as wastewater because the available land area in urban spaces is limited, and even when digestate can be used as fertilizer, the value of digestate is estimated to be low because of its high moisture content, which is affected by the additional cost for hydration (Czekała et al., 2020). Therefore, discovering valuable uses of digestate is necessary.

This paper proposes that the digestate from methane fermentation can be applied to decolored *nori* to improve its color. In general, this objective has been achieved through the application of artificial fertilizers. However, the use of digestate as a fertilizer to improve decolored *nori* can both contribute to the recycling of local material and reduce the use of artificial fertilizers. This paper is a new attempt at proposing a circular economy with a marine viewpoint. Although researchers have attempted to use digestate for cultivation of marine creatures related to microalgae (Nguyen et al., 2013, 2015; Nguyen, Lin, & Lay, 2019; Veronesiv et al., 2017; Yang et al., 2017; Zhou et al., 2019; Zhu et al., 2019), few studies have applied digestate to macro algae such as *nori*.

Because high ammonium concentration is a disadvantage in using digestate for the cultivation of microalgae, Praveen et al. (2018) proposed the pretreatment of digestate through nitrification. Svehla et al. (2017) also attempted nitrification of digestate for soil use to minimize the ammonium losses during the application process. Nitrification, however, requires operational costs and energy. By contrast, *nori* does not require nitrification because it can use ammonium for growth (Sakaguchi et al., 2003), which is one of the reasons why *nori* is assessed in this study. According to our review of the literature, few studies have investigated digestate use for decolored *nori*, especially the relation between color recovery and nutrient absorption.

The purpose of this study is to find valuable means of digestate use for decolored *nori* by proposing an anaerobic digestion system that considers the importance of marine aspects in a circular economy that leads to solving unbalanced nutrient distribution. For this purpose, laboratory-scale experiments of *nori* cultivation are conducted to evaluate the availability of digestate as a nutrient supplier to *nori*. This study quantitatively investigates the effect of digestate from methane fermentation by using *Ulva* sp. and food waste in *nori* cultivation, focusing on the absorption of nitrogen and phosphorus and color recovery of decolored *nori*.

CONCEPT OF ANAEROBIC DIGESTION SYSTEM USING DIGESTATE

Figure 1 presents the concept of anaerobic digestion system proposed in this study based on the previous study (Kuroda et al., 2016). Osaka Bay has unbalanced nutrient distribution and therefore green tide can be observed in the north of Osaka Bay; by contrast, low nutrient concentration causes decolored *nori* in the southern Osaka Bay. In this system, *Ulva* sp. is harvested before accumulation and decomposition and is used as the input of anaerobic digestion along with food waste. The methane gas produced can be used as an energy resource. In this study we proposed the use of digestate for decolored *nori* as fertilizer even

Table 1. Chemical composition of simulated biomass

	Mixed biomass	*Ulva* sp.	Vegetable waste	Fishery waste
Mixed ratio [%]	-	30	50	20
Total solids [%]	8.0	11.5	5.4	30.0
Volatile solids [%]	7.1	10.0	3.5	27.0
Total organic carbon [mg/g-TS]	410.9	323.1	383.9	567.6
Total nitrogen [mg/g-TS]	29.8	13.9	24.6	86.7
Total phosphorus [mg/g-TS]	3.0	1.0	3.1	25.1
Fe [mg/100g]	-	4.3	0.6	0.5

Figure 2. Picture of *nori* cultivation in Nishitottori port, Hannan city

though digestate is generally used as liquid fertilizer for agriculture on land. By using digestate on land and at sea, the application of digestate expands, and the proposed system contributes to the nutrient cycle in the coastal area.

MATERIAL AND METHODS

Digestate

Anaerobic sludge was procured from Higashi-Nada sewage plant in Hyogo, Japan, and had been inoculated with mixed biomass in a 5 L anaerobic digester at Osaka Prefecture University. The mixed biomass contained *Ulva* sp., vegetable waste, and fishery waste, which was simulated based on the production around Osaka Bay (Kuroda et al., 2014, 2017). *Ulva* sp. was harvested at Rinku Park in Osaka in the spring of 2015 and was kept frozen until the experiments started. Vegetable wastes were a mixture of cabbage and lettuce in a 1:1 ratio, which was based on total solid weight. Fishery wastes were a part of red sea bream, known as *tai* in Japanese. The entire biomass was mixed and was treated as slurry state and the chemical composition of the mixed biomass (see summary in **Table 1**). Continuous anaerobic digestion was conducted at 35°C, and the amount of biomass, determined as the organic concentration in the digestion tank, was 3.0 g-VS/L-sludge. The digestate was centrifuged at 4,000 rpm for 30 min, and the supernatant was ultimately used as the additive for *nori* cultivation.

Nori Seed

Nori seeds in this study were collected and provided by a fisherman in Hannan city, located in southern Osaka Bay, in December 2017. As is the common practice, *nori* seeds attached to cultivation nets are grown on the sea surface with the absorption of dissolved nitrogen, phosphorus, and other trace substances (**Figure 2**). After the seeds grow to a sufficient length, the *nori* is harvested, washed, and minced. Minced *nori* is then molded, pressed, and dried, and becomes the *nori* sheets used in *sushi*. A part of the cultivation net (a small mesh [square] 14 cm long), where *nori* seeds are grown to a length of 2 to 3 mm, was used in this study (**Figure 3**). The seeds were kept frozen until the experiments started. Prior to conducting the experiments, the frozen *nori* seeds were washed with tap water and further cultivated until the *nori* reached lengths of approximately 5 cm.

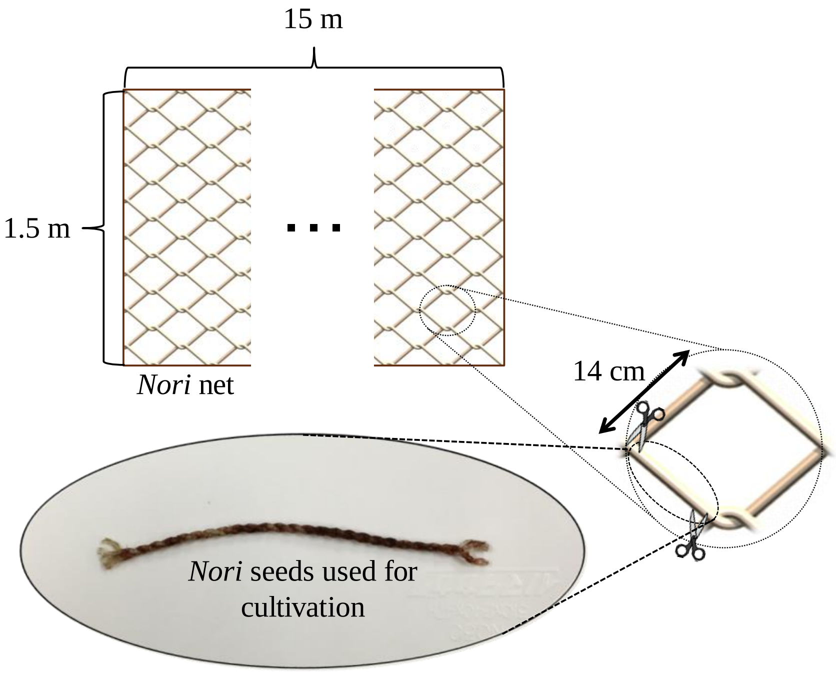

Figure 3. Schematic representation of the *nori* net and seeds

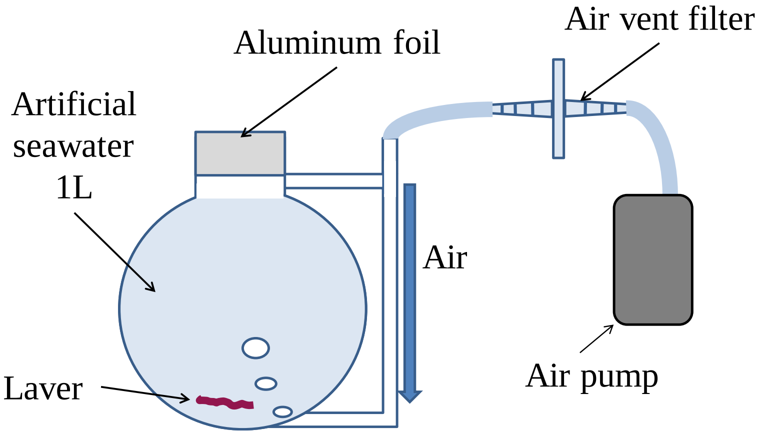

Figure 4. Experimental apparatus set-up for *nori* cultivation

Experimental Set-up and Procedure

Nori cultivation

Pre-cultivated *nori* was divided into two portions (each 7 cm in length): one for cultivation with digestate, and the other for cultivation with artificial seawater. The laboratory-scaled cultivation set-up comprised a 1 L round-bottomed glass flask with an air vent filter of 0.2 μm (Aervent, Merck Millipore) that prevents microorganism contamination by air (**Figure 4**). The artificial seawater was based on M-ESWA culture, developed for *nori* cultivation (Fujiyoshi et al., 2014). The composition of M-ESWA is summarized in **Table 2**. The dissolved inorganic nitrogen in M-ESWA is sodium nitrate ($NaNO_3$). In this experiment, digestate was determined as an alternate source of nitrogen in M-ESWA, and the required amount of digestate was estimated as the same amount of nitrogen present in M-ESWA. We assumed that any organic compounds were not included in the digestate used for the cultivation because the digestate was provided from the reactor, in which methane fermentation was finished.

Nori was cultured at 18°C in a temperature controlled chamber (NK-SYSTEM, LH-241S) for 3 weeks. Light intensity, with florescent lights as the source, was 60 μmol/m^2/s and day and night time hours were 11 and 13 hours, respectively. The seawater used for cultivation was changed once per week and was analyzed for nutrient absorption

For the color recovery experiments, grown *nori* was purposely decolored by eliminating nitrogen from the artificial seawater for a few days. Because the lack of nitrogen is reported as the main reason for decolored *nori* in western Japan including in Osaka Bay (Ishii et al., 2008), this study emphasized the importance of nitrogen supply. The grown and decolored *nori* was then divided into three parts and cultivated using three types of nitrogen sources: $NaNO_3$, NH_4Cl, and digestate. Duration of cultivation was 1 week, and the color recovery was evaluated before and after the additives of each nitrogen source.

Table 2. Composition of M-ESWA (1L)

Distilled water	1000	mL
$NaNO_3$	0.047	g
$NaH_2PO_4 \cdot H_2O$	0.0031	g
NaCl	21.2	g
$MgCl_2 \cdot 6H_2O$	9.59	g
Na_2SO_4	3.55	g
KCl	0.6	g
$CaCl_2 \cdot 2H_2O$	1.34	g
$Na_2SiO_3 \cdot 9H_2O$	0.03	g
$NaHCO_3$	0.174	g
HBO_3	0.023	g
KBr	0.086	g
$SrCl_2 \cdot 6H_2O$	0.022	g
NaF	0.0028	g
$MnSO_4 \cdot 4H_2O$	132	µg
$ZnSO_4 \cdot 7H_2O$	17	µg
$FeCl_3 \cdot 6H_2O$	55.9	µg
$CoSO_4 \cdot 7H_2O$	336	ng
Na_2SeO_3	79	ng
$Na_2MoO_4 \cdot 7H_2O$	584	ng
$NiCl_2 \cdot 6H_2O$	370	ng
Na_2EDTA	6.1	mg
Nicotinic acid	2	µg
Calcium pantothenate	2	µg
Folic acid	100	ng
Biotin	10	ng
Vitamin B_{12}	18	ng

Analytical method

Dissolved inorganic nitrogen (DIN: NO_3-N, NO_2-N, and NH_4-N) and dissolved inorganic phosphorus (DIP: PO_4-P) in cultivation seawater were measured by following Japanese standard methods: JIS K 0102 (JIS, 2013), JIS K 0104 (JIS, 2011), and JIS K 0400-42-60 (JIS, 2000). Color recovery of *nori* was determined quantitatively by using a spectrophotometer (CM-700d, KONICA MINOLTA), which is commonly used for the evaluation of the color of *nori* (Kotani, 2002; Sakaguchi et al., 2003; Takagi et al., 2012). The values of L*a*b* are used as per the definition of the Commission Internationale de I'Eclairage (CIE). L* indicates lightness, with values ranging from 0 (dark) to 100 (light), and a* and b* indicate brightness. The negative and positive values of a* denote green and red, respectively. Likewise, the negative and positive values of b* denote blue and yellow, respectively. According to Kotani (2000), L* below 60 indicates the first stage of decolored *nori* and a value above 73 indicates heavily decolored condition.

RESULTS AND DISCUSSION

Nutrient Absorption

The change in the DIN and DIP values per week are shown in **Figures 5** and **6**, respectively. Clearly, *nori* with digestate absorbed both DIN and DIP twice more than the *nori* with artificial seawater. This finding suggests that digestate promotes nutrient absorption. The reason DIN absorption rates between *nori* with digestate and with artificial seawater differ can be explained in terms of the contrasting composition of DIN (see summary in **Table 3**). The nitrogen in digestate is in the form of NH_4-N, which is more efficiently absorbed than NO_3-N by *nori* (Yamamoto, 1992), indicating the advantage of digestate as a fast-acting fertilizer. By contrast, DIP absorption rates differ but the initial DIP contained in both digestate and artificial seawater is similar. The reason for this phenomenon may be the variation in the absorption ratio of DIN to DIP according to the chemical composition of *nori*. The ratio of DIN to DIP remained between 7 and 8, which implies that nitrogen and phosphorus are absorbed by maintaining the ratio of nitrogen to phosphorus.

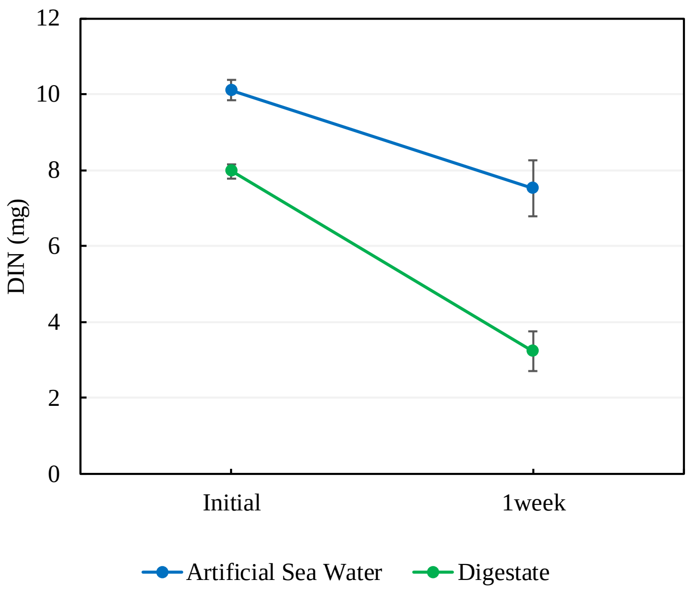

Figure 5. Dissolved nitrogen before and after the *nori* cultivation

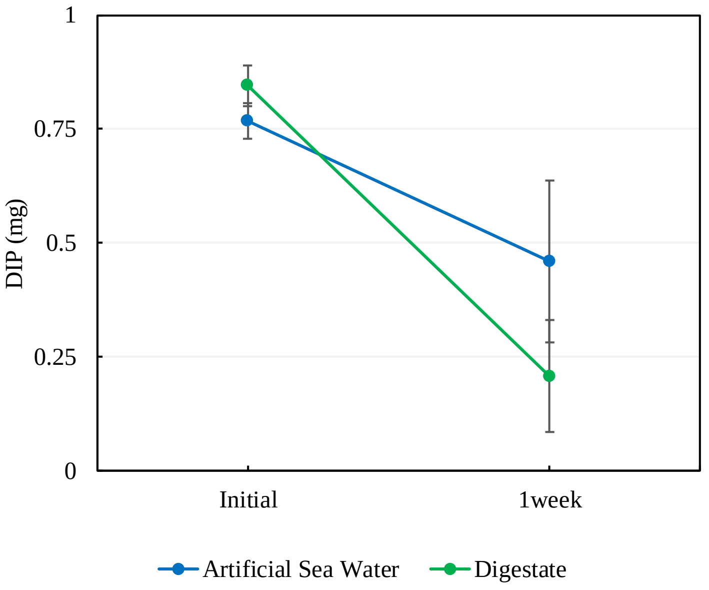

Figure 6. Dissolved phosphorus before and after the *nori* cultivation

Table 3. Absorption of dissolved nitrogen and phosphorus in mg, and the ratio of DIN to DIP (N/P)

	DIN			DIP	N/P
	NO_2-N	NO_3-N	NH_4-N	PO_4-P	
Artificial SeaWater	0.0	2.5	0.094	0.31	8.4
Digestate	0.0	0.0	4.7	0.64	7.4

Color Recovery

Table 4 describes color change expressed as L*a*b* before and after the additives of the three types of nitrogen sources. No change in L* and a* was observed in artificial seawater containing $NaNO_3$, and b* slightly changed. The L* of digestate changed sharply from 80.0 to 52.6, and the L* of artificial seawater containing NH_4Cl demonstrated little change, from 78.1 to 70.6. These results suggest that color improvement can be more easily ensured by using ammonium related sources than by nitrate related sources. Additionally, only digestate contributed to the change in the color of *nori* from the decolored condition to the normal state. This finding indicates that not only NH_4-N, but also other substances can be influenced by the use of digestate. As suggested by Zhang et al. (2004), reduction in the color of *nori* can also be induced by Fe deficiency. Isagi et al. (2006) reported that seawater did not include Fe, Mn, and Zn when decolored *nori* occurred and concluded that deficiency of these elements could be a major reason for decolored *nori* in Ariake Sea. Therefore, candidates of other substances could be trace metals such as Fe. In **Table 1**, *Ulva* sp. has a higher Fe content than the other biomass. These findings implies that digestate with *Ulva* sp. as its origin influences

Table 4. Color change analysis with a spectrophotometer

Source	Initial condition						After 1 week					
	L*		a*		b*		L*		a*		b*	
	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD
$NaNO_3$	75.2	0.11	9.8	0.033	15.6	0.01	74.7	0.24	10.3	0.13	12.6	0.67
NH_4Cl	78.1	0.49	8.7	0.11	15.4	0.017	70.6	0.76	14.2	0.43	15.0	0.16
Digestate	80.0	0.022	8.0	0.00	15.3	0.014	52.6	0.24	14.5	0.25	13.9	0.44

Ave. means averaged value.
SD means standard deviation.

color recovery; however further experimental and theoretical research in this respect including heavy metal (Watanabe et al., 2014) is necesssary.

Discussion

This study provided the first quantitative report on digestate use for decolored *nori* and makes novel contributions to the literature. First, digestate can become a fast-acting fertilizer that contributes to a quick recovery from decolored conditions because its rich NH_4-N content is as same as commercial fertilizer, which has been indicated in the literature (Sakaguchi et al, 2003; Yamamoto, 1992). The mechanism is related to nitrogen assimilation processes that have been under investigation that require a molecular perspective that focuses on an ammonium transporter gene (Kakinuma et al., 2017).

Second, the value of a* had a strong relationship with pigments related to photosynthesis such as chlorophyll a, phycoerythrin, and phycocyanin (Sakaguchi et al., 2003). Thus, the results demonstrated that a nitrogen source derived from ammonium is effective in producing pigments that promote photosynthesis. Third, the significant color recovery provided by digestate was presented by the decrease in the value L*. This study proposed a new hypothesis: trace metal elements, especially Fe, in *Ulva* sp. can contribute to color improvement. Most studies have investigated the risk of heavy metals in the digestate for agricultural use (Chen et al., 2019; Vaneeckhaute et al., 2019); however, few studies have focused on the reuse of trace metals such as Fe, Mn, and Zn. Digestate use from the aspect of the reuse of trace metals can increase the value of digestate. Notably, further research is necessary to assess which elements in the digestate are effective in color improvement.

One of the reasons for the decoloration of *nori* in Harima-Nada (a part of Seto Inland Sea), is the competitive utilization of DIN by large-size phytoplankton (Hori et al., 2008). Therefore, the idea of digestate as a supplier of DIN for *nori* can be a valuable approach that contributes to decreasing the external substrate (artificial fertilizer) emitted into the sea. In addition, utilization of *Ulva* sp., which is carbon neutral, for anaerobic digestion can produce renewable energy and closing nutrient cycle. The same advantage in digestate use on land was demonstrated by Czekała et al. (2020). By contrast, this study proposed a new potential use of digestate in the sea. To design a blueprint of the proposed concept (**Figure 1**), economic and environmental aspects that contribute to circular economy should be investigated in detail.

CONCLUSIONS

This study investigated the effect of digestate from methane fermentation on decolored *nori* through experimental analyses. The laboratory-scale cultivation experiments highlight that the significant absorption of nutrients and color recovery occurred because of digestate utilization, indicating its positive effects on decolored *nori*. In addition, the experiments found that not only NH_4-N, but also other substances such as trace metal related to *Ulva* sp. can influence such effects. In addition to its use as an alternative fertilizer, further in-depth research on digestate utilization that focuses on the effect of *Ulva* sp., including the mechanisms of color recovery, is necessary. The findings clearly indicate that digestate can be used in the sea and that the suggested multiple uses of digestate would increase the value of digestate. This attempt contributes to the literature on the closing nutrient cycle and is an effective tool for the circular economy and sustainable society.

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