Journal of Environmental Science and Technology

ISSN 1994-7887

science alert

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OPEN ACCESS Journal of Environmental Science and Technology

ISSN 1994-7887
DOI: 10.3923/jest.2019.17.25

Research Article
Optimum Condition for Valuable Seaweed Growth to Utilize Treated Sewage as a Nutrient Source

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Abstract

Background and Objective: Treated sewage (TS) has great potential as a nutrient resource for seaweed cultivation. However, there is little information on using the nutrients in the treated sewage for seaweed cultivation because seaweed cannot live in freshwater, such as TS. In this study, the optimum salinity with mixing seawater and TS for the growth of the valuable seaweed *Pyropia yezoensis* (seaweed laver "nori") was determined using a micro-well plate bioassay. **Materials and Methods:** *Pyropia yezoensis* conchospores were released from the mature conchocelis in the filtered and sterilized natural sea water. To carry out a bioassay using juvenile sporophytes, the spores were cultivated in a micro-well plate. The maximum fluorescence intensity of chlorophyll was determined to reflect response in the bioassay using a micro-well plate. The effects of salinity on spore and sporophytes were tested over a range of salinity concentrations (0-60 per-mil, %). The effect of TS addition on spore growth was examined using the spore bioassay, varying the mix ratio of TS and artificial seawater. The nutrient requirement for the early development stage of *P. yezoensis* was investigated using the spore bioassay. The concentration of the nutrients in the enriched seawater was prepared by adjusting the concentration of PES medium. **Results:** An appropriate ratio of treated sewage: Seawater kept the salinity at 20%, which was optimum for spore germination and sporophyte growth. In addition, the nutritional requirement at the early stage of *P. yezoensis* growth and development was investigated. The nutrients contained in 100% TS were insufficient for maximum growth of *P. yezoensis*. **Conclusion:** Since the TS is useful to promote growth of the variable seaweed as a fertilizer, lowering of salinity becomes the limit factor of the growth. Mixing TS with seawater to keep the salinity at 20% and to maintain as high a nutrient concentration in the culture environment as possible, could promote *P. yezoensis* production.

Key words: *Pyropia yezoensis* (seaweed laver "nori"), nutritional requirement, seaweed, treated sewage, salinity influence, spore germination and sporophyte growth

Citation: Yoshihiro Suzuki, Koki Nakada, Kei Nukazawa and Hiroyuki Yamanishi, 2019. Optimum condition for valuable seaweed growth to utilize treated sewage as a nutrient source. J. Environ. Sci. Technol., 12: 17-25.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A public sewage-treatment system is the hub of social infrastructures controlling public health. Therefore, the construction and improvement of sewage-treatment systems has been promoted continuously all over the world, in both developed and developing countries. Since the amounts of treated sewage (TS) increases as sewerage systems expand, the importance of TS as a source of water[1,2] or nutrients[3] and for hydrologic circulation[4] rises.

Studies of natural ecosystems consider TS containing nitrogen and phosphorus to be one of the most important causes of eutrophication of water bodies. It is reported that the nitrogen and phosphorus in the TS are also indispensable elements for the growth of seaweed. This suggested that TS has the potential to promote seaweed production by acting as a nutrient source[5-7]. A trial to use TS as a nutrient source for seaweed cultivation attracted attention worldwide, especially in Japan, where there are a lot of sewage-treatment plants adjacent to the coast[8]. The use of TS in seaweed farming could lead not only to reduced pollution of coastal environment[9], but also to a potential increase in seaweed production by utilization of TS, which would be expected to have a beneficial economic effect[10]. However, there is little information on the conditions for seaweed growth using TS as a nutrient source.

The use of seaweed includes bioremediation of the maricultural environment in terms of a nutrient-removal process[11-16]. At present, seaweed cultivation using nutrients discharged from fish is used in integrated multitrophic aquaculture farms. When using TS for seaweed cultivation by mixing it with seawater, the greatest barrier will be salinity, which is a fundamental requirement for algal growth. It has been reported that reduced salinity and sewage effluent affect developing *Hormosira banksii* (Phaeophyceae)[17], which has almost none of the commercial value. However, there are few information of optimum condition for valuable seaweed using TS as a fertilizer. There is a trade-off between lowering salinity and increasing nutrients for seaweed growth to utilize the TS.

The objective of this study was the estimation of the optimum condition for growth of the high-value seaweed *Porphyra yezoensis* (Phaeophyceae), which is the major seaweed species cultivated for production of seaweed laver "nori" in Japan, Korea and China, to utilize the TS as a nutrient resource. To estimate the critical salinity condition and the macronutrient requirements (for N and P) of *P. yezoensis* in the early developmental stages, a concept of effluent toxicity test for giant kelp[18] was applied. Furthermore, the algal bioassay using a micro-well plate[19-21] was used for seaweed growth test to examine a large number of conditions.

MATERIALS AND METHODS

Pre-culture of conchocelis: Natural seawater was collected from the Aoshima coast, Kyushu District, Japan. The natural seawater was filtered under vacuum using a membrane filter (0.45 μm HA type, Millipore, Merck KGaA, Darmstadt, Germany) and sterilized by a high-pressure steam sterilizer (121°C, 15 min, BS-325, Tomy Seiko, Tokyo, Japan). The enrichment of the Provasoli's enriched natural seawater (PES) was prepared as described by Provasoli[22]. The PES was made by mixing natural filtered seawater (1000 mL) with the enrichment known as PES medium (20 mL) (Table 1). The culture conditions for maturation of *P. yezoensis* conchocelis were as follows[23]: Aeration, 20°C, 7 μmol m^{-2} sec^{-1}, light:dark = 14:10 h. The PES was changed once every 2 weeks. The weak light condition is suitable for conchocelis maturity.

Preparation of conchospores: *Pyropia yezoensis* conchospores were released from the mature conchocelis in the filtered and sterilized natural seawater under the following culture conditions: Aeration, 20°C, 95 μmol m^{-2} sec^{-1}, light:dark = 10:14 h. At this stage, the PES was changed every day. The presence of spores was checked daily using a light microscope (×100). When a large number of spores were released into the seawater, the spore-containing seawater was passed through a mesh sheet (mesh size 100 μm) to remove impurities and was concentrated by centrifugal separation

Table 1: Preparation of Provasoli's enriched natural seawater (PES)[22]

PES medium	Tris hydroxymethyl aminomethane	5.0 g
	$NaNO_3$	3.5 g
	Na2-glycerophosphate	500 mg
	Fe stock solution	250 mL
	P-2 metal mix	250 mL
	Vitamin B12 stock solution (0.1 mg mL^{-1})	1.0 mL
	Thiamine-HCl stock solution (1.0 mg mL^{-1})	5.0 mL
	Biotine stock solution (0.1 mg mL^{-1})	0.5 mL
	Distilled water	
	Total	1000 mL
	pH 7.8	
Fe stock solution	Na_2-EDTA.$2H_2O$	330 mg
	$Fe(NH_4)_2(SO_4)_2.6H_2O$	351 mg
	Distilled water	
	Total	500 mL
P-2 metal mix	Na_2-EDTA.$2H_2O$	500 mg
	H_3BO_3	570 mg
	$FeCl_3.6H_2O$	24.5 mg
	$MnSO_4.4H_2O$	82.0 mg
	$CoSO_4.7H_2O$ stock solution (4.8 mg mL^{-1})	0.5 mL
	$ZnSO_4.7H_2O$	11.0 mg
	Distilled water	
	Total	500 mL

PES: Dissolve 20 mL of PES medium into 1000 mL of sterilized seawater

Table 2: Concentration of nitrogen and phosphorus in treated sewage, seawater and PES

Samples	Ammonium (mg N L^{-1})	Nitrate (mg N L^{-1})	Total nitrogen (mg N L^{-1})	Total phosphorus (mg P L^{-1})
Treated sewage (plant A)	4.3	2.2	6.8	2.1
Treated sewage (plant B)	16.8	2.5	20.4	1.3
Seawater (Nori farm)	0.6	0.8	3.0	0.1
PES	0.3	11.5	11.8	1.4

(600×g, 5 min, Suprema 21, Tomy Seiko). The concentrated spore suspension was used for subsequent culture and bioassay.

Pre-culture of juvenile sporophytes: To carry out a bioassay using juvenile sporophytes, the spores were cultivated in a micro-well plate (Nunclon 96 wells, 300 µL/well; Thermo Fisher Scientific, Waltham, MA, USA). The concentrated spore suspension (20 µL, containing 2000-3000 spores), 10×PES (20 µL) i.e., 10×concentrate of the PES medium and sterilized artificial seawater (160 µL) were added to each well. The total volume in the well was 200 µL. The composition of the artificial seawater (g L^{-1}, salinity 30%) was as follows: NaCl, 21.03; Na_2SO_4, 3.52; KCl, 0.61 g; KBr, 0.088; $Na_2B_4O_7 \cdot 10H_2O$, 0.034 g; $MgCl_2 \cdot 6H_2O$, 9.5 g; $CaCl_2 \cdot 2H_2O$, 1.32 g; $CrCl_2 \cdot 6H_2O$, 0.02 g and $NaHCO_3$, 0.17 g, using reagent-grade chemicals in deionized-distilled water[24]. The culture conditions for spore germination and sporophyte growth were as follows: 15°C, 95 µmol m^{-2} sec^{-1}, light:dark = 10 h: 14 h. After 14 day incubation, the medium in the well was discharged and washed three times with artificial seawater using a micropipette. The adhesion of growing juvenile sporophytes to the well bottom was checked for using a microscope (DIAPHOT TMD300; Nikon, Tokyo, Japan).

Water sampling and analysis: The TS samples were collected from the effluent drainage at two sewage-treatment plants (Plant A, 11th Dec. 2015; Plant B, 28th Nov. 2016). Plant A (Miyazaki Prefecture, Kyushu District, Japan) employed a conventional treatment system, using the oxidation ditch method. While Plant B (Saga Prefecture, Kyushu District, Japan) had a system installed to inhibit nitrification in order to discharge the nutrients to the nori farm area. In addition, surface seawater was also collected from the nori farm area. Water samples were stored in sterile 1-L polyethylene bottles and immediately transported to the laboratory, where they were tested for water quality and used for the bioassay. The TS concentrations of ammonium (NH_4-N, indophenol blue absorptiometry), nitrate (NO_3-N, cadmium reduction method, HACH DR-2800: Hach Company, Loveland, CO, USA), total nitrogen (T-N) (TOC Analyzer, Wet Oxidation/NDIR Method Model; Shimadzu, Kyoto, Japan) and total phosphorus (T-P, peroxo-sulfuric acid decomposition/ascorbic acid method) were analyzed as described in the Japanese Industrial Standards (JIS K 0102 (Japanese Industrial Standards Committee))[25] or the HACH DR-2800 procedures manual (Catalog Number DOC022.53.00725). The salinity of seawater was determined using an electric conductivity meter (ES-51; Horiba, Kyoto, Japan) and it was found to be 20%. The water quality parameters of the TS, seawater and PES used in this study were shown in Table 2. Before preparation of the test medium for the bioassay, the TS was dechlorinated by adding sodium thiosulfate.

Effect of salinity on conchospores and juvenile sporophytes: The maximum fluorescence intensity of chlorophyll was determined to reflect the growth response in the spore bioassay using the micro-well plate. The effect of salinity on spore germination was tested over a range of salinity concentrations (0, 1, 3, 5, 7, 10, 15, 20, 30 and 60%). The nutrient concentrations were fixed at those of the conventional PES. An aliquot of the concentrated spore suspension (20 µL, containing 2000-3000 spores), the 10×PES (20 µL) and each salinity test solution (160 µL), based on the composition of artificial seawater were prepared in deionized-distilled water and were added to each well. The culture conditions for the spore bioassay were as follows: 15°C, 95 µmol m^{-2} sec^{-1}, light:dark = 10:14 h, test period 14 days. The test medium in the well was replaced after 7 days. The fluorescence intensity (excitation 430 nm, emission 680 nm) was determined in each well using a plate reader (Infinite M200; Tecan, Männedorf, Switzerland) after 14 days. The spore growth on the well bottom was also observed under a microscope. Six replicates were set-up for each salinity treatment section. The series of each treatment was repeated three times (Test 1, 2 and 3) using independent spore suspensions which varied with respect to the collection date.

The effect of salinity on juvenile sporophyte growth was examined using the same set-up and conditions as for the salinity effect test on the conchospores. The 10×PES (20 µL) and each salinity test medium (180 µL) were added to each well to which the growing juvenile sporophytes adhered to the well bottom. Sporophyte growth was observed under a microscope (×100). Preparation of the test medium for each salinity concentration was shown in Table 3.

Table 3: Preparation of media for each test

Test		Treatments									
salinity effect test	Salinity (%)	0	1	3	5	7	10	15	20	30	60
	Nutrient concentration	PES	PES	PES	PES	PES	PES	PES	PES	PES	PES
Mixing test of TS and seawater	TS	0	16	33	50	66	76	83	90	96	100
(TS collected from Plant A)	Salinity	30	25	20	15	10	7	5	3	1	0
	Nutrient concentration	No addition of PES medium									
Nutritional requirement test	Salinity	Fixed at 30%**									
	Nutrient concentration	0	1/40PES	1/20PES	1/10PES	1/5PES	1/2PES	PES	3PES	5PES	10PES
TS requirement test	TS	0	0.8	1.6	3.1	6.3	13	25	50	100	
(TS collected from Plant B)	Salinity	Fixed at 30%									
Actual seawater effect test	Seawater	0	0.8	1.6	3.1	6.3	13	25	50	100	
(seawater collected from Nori farm)	Salinity	Fixed at 30%									

TS: Treated sewage. **Addition of the chemicals to prepare the artificial seawater

Effect of mixing treated sewage and seawater on spore germination: The effect of TS addition on spore growth was examined using the spore bioassay, varying the mix ratio of TS and artificial seawater. The TS (%) were set as follows: 0% (salinity 30%, seawater only), 16% (25%), 33% (20%), 50% (15%), 66% (10%), 76% (7%), 83% (5%), 90% (3%), 96% (1%) and 100% (1%, TS only).

Effect of nutrient concentration on spores: The nutrient requirement for the early development stage of *P. yezoensis* was investigated using the spore bioassay. The concentration of the nutrients in the enriched seawater was prepared by adjusting the concentration of PES medium. The 10 concentrations of nutrients in the media were as follows: seawater only (nutrient-free), 1/40PES (the nutrient concentration is one-forth that of standard PES), 1/20PES, 1/10PES, 1/5PES, 1/2PES, PES, 3PES (the nutrient concentration is three times that of standard PES), 5PES and 10PES.

Effect of treated sewage on spores and sporophytes: The effect of TS concentration, as the nutrient source on spores and sporophytes was investigated using the bioassay method. The salinity was fixed at 30% in the test media, the chemicals used to prepare the artificial seawater were added to the TS and mixed with artificial seawater to set the percentage of TS. The reason of fixed at 30% was to make no influence of salinity in each treatment. The percentages of TS in seawater were set as follows: 0% (seawater only), 0.8, 1., 3.1, 6.3, 13, 25, 50 and 100% (TS only). The concentrated spore suspension (20 µL) and the test medium (180 µL) were added to each well. For the sporophyte bioassay, the test medium (200 µL) was added to each well to which were adhered the growing juvenile sporophytes. In addition, the effect of actual seawater collected from the nori farm on the spores and sporophytes was investigated to compare with the effect of the TS. The artificial seawater chemicals were added to the actual seawater to set the salinity at 30% and mixed with artificial seawater. The percentage of actual seawater was set as the same as in the spore TS requirement test.

Statistical analysis: The effects of salinity and nutritional requirement on spores or sporophytes were analyzed using only four replicates by omitting the maximum and the minimum from the six replicates of each treatment. The differences among the treatments in each bioassay were analyzed using a one-way analysis of variance (ANOVA). When a significant difference (α 5%) was detected based on ANOVA, a *post-hoc* multiple comparisons (Dunnett's test) was performed to identify significant differences between the treatments. Each variable was presented as mean and ±standard deviation (SD).

RESULTS AND DISCUSSION

Effect of salinity on spore germination and sporophyte growth: Spore germination in the 0-7% salinity treatments was significantly lower ($p<0.05$) compared to the standard treatment (30%) (Fig. 1). Microscopic observations revealed that there were no spores on the bottom of the wells at salinity levels of 7% or lower, with the spores disappearing at these concentrations during the test period. In two of the three replicates, the germination (measured as fluorescence intensity) at salinity levels of 10 and 15% was significantly lower than that at 30% salinity. In contrast, spore germination was markedly reduced at a high-salinity concentration of 60% (Fig. 1).

Sporophyte growth in the 0-20% salinity treatment range was significantly lower ($p<0.05$) than that in standard seawater (30% salinity) (Fig. 2). In particular, sporophyte growth was markedly reduced in the 0-7% treatment and the pigment was observed to have disappeared from sporophytes when viewed under the microscope. However, there was no significant difference in sporophyte growth between 10 and 20% salinity and no damaged sporophytes were observed at these rates.

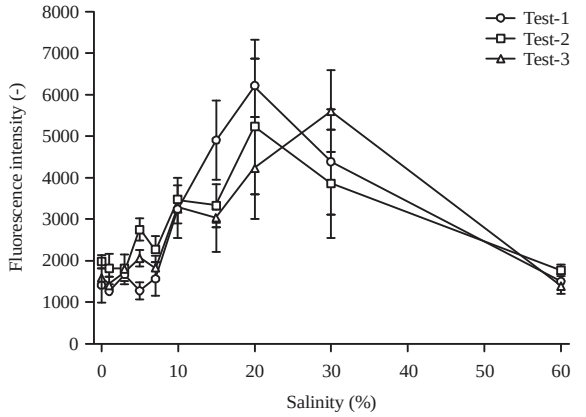

Fig. 1: Effect of salinity on spore germination. Three times of tests were carried out using independent spore suspensions which varied with respect to the collection date
The values presented are Mean±SD (n = 4)

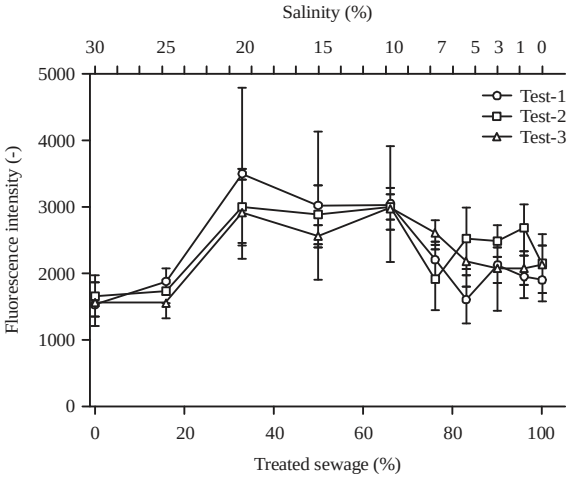

Fig. 3: Relationship between spore germination and the percentage of treated sewage added
The values presented are Mean±SD (n = 4)

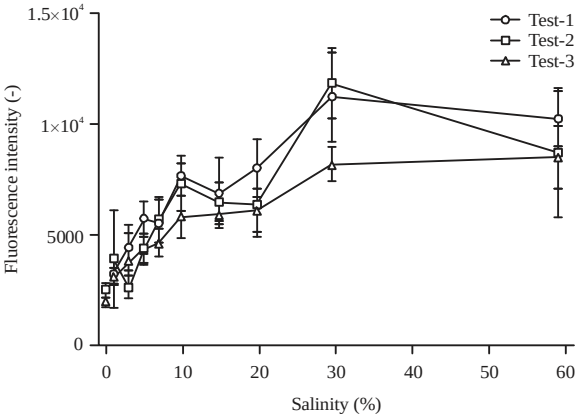

Fig. 2: Effect of salinity on juvenile sporophyte growth
The values presented are Mean±SD (n = 4)

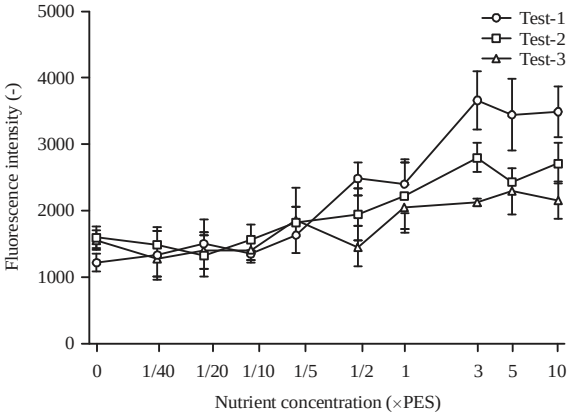

Fig. 4: Relationship between spore germination and the concentration of nutrients by adjusting addition and concentration of the PES medium
The values presented are Mean±SD (n = 4)

Effect of TS addition on spore: When assessing the ability of a mixture of TS and seawater to promote seaweed growth without the need to adjust salinity, the salinity in the mixture decreased with increasing TS percentage in the mixture (Fig. 3). Spore germination in the 33, 50 and 67% TS treatments was significantly higher ($p<0.05$) than that in the TS-free treatment (0%).

Nutrient requirement for maximum germination of spores: The relationship between spore germination and the nutrient concentration in the experimental media shown in Fig. 4. Spore germination in the treatments 1/2PES 1PES 3PES, 5PES and 10PES was significantly higher than that in the nutrient-free treatment used as a control. In addition, the treatments 3PES, 5PES and 10PES showed greater spore germination response than that of 1/2PES and PES. In contrast, there was no significant difference in germination between the nutrient-free treatment and the treatments containing less than 1/5PES.

Treated sewage requirement for spore germination and sporophyte growth: The relationship between spore germination and the percentage of TS in the test medium shown in Fig. 5a. The salinity was fixed at 30% to estimate the response to the percentage of TS as the nutrient source. Spore germination in treatments 25-100% TS was significantly higher ($p<0.05$) than that in the 0%

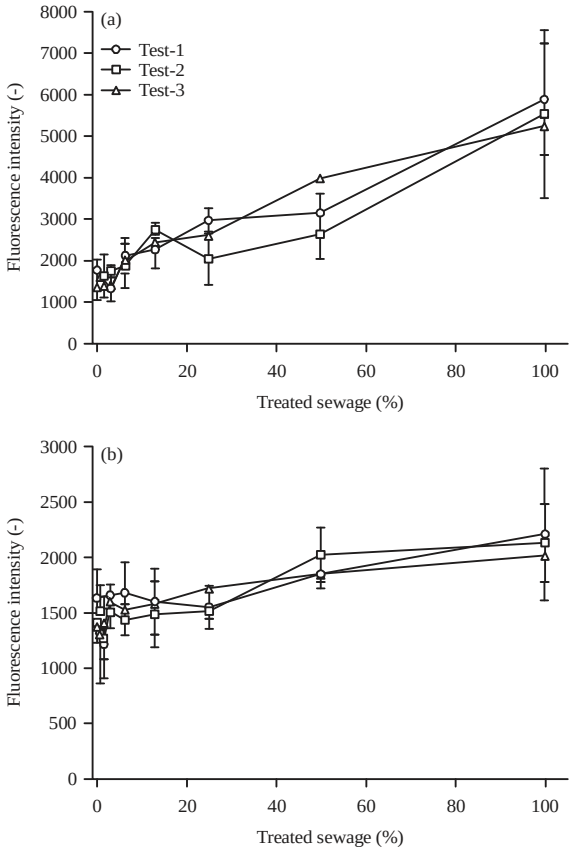

Fig. 5(a-b): Relationship between the spore germination and the added (a) TS (%) collected from the Plant B and (b) Actual seawater collected from the nori farm. There was no relation between the Plant A and Plant B. Salinity was fixed at 30% by addition of chemicals to prepare the seawater in the bioassay using TS
The values presented are Mean±SD (n = 4)

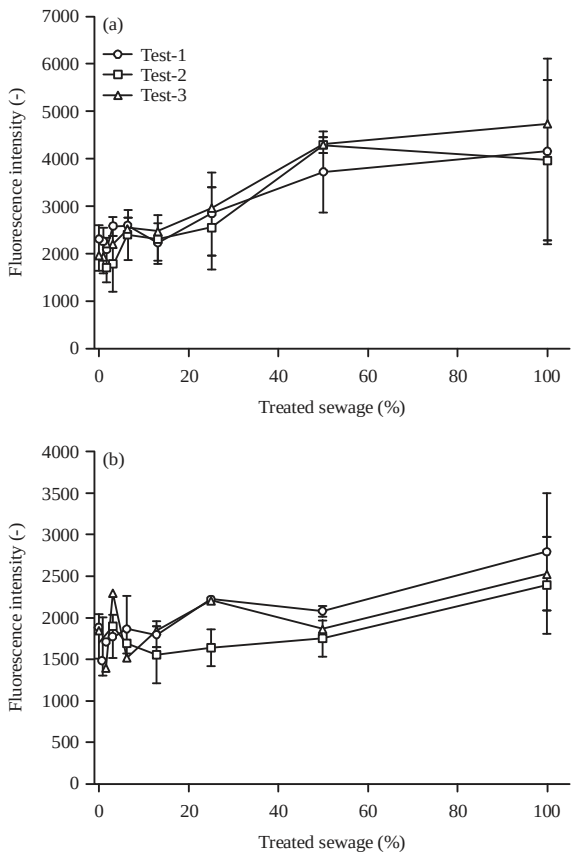

Fig. 6(a-b): Relationship between sporophyte growth and the added (a) TS (%) collected from the Plant B and (b) Actual seawater collected from the nori farm. Salinity was fixed at 30% by addition of chemicals to prepare the seawater in the bioassay using TS
The values presented are Mean±SD (n = 4)

treatment. The germination of spores increased with increasing TS percentage, reaching maximum germination in treatment 100%. Most of the spores developed into sporophytes in treatments 50 and 100%. When the test used the seawater collected from the nori farm, the spore germination at treatments 50 or 100% the actual seawater were significantly higher ($p<0.05$) than that of the actual seawater-free treatment (0% the actual seawater) (Fig. 5b).

The relationship between sporophyte growth and the percentage of TS in the test medium was shown in Fig. 6a. The salinity was fixed at 30%. Sporophyte growth in treatments 50-100% were significantly higher ($p<0.05$) than that in the TS-free treatment. Furthermore, the sporophyte growth was markedly inhibited in the seawater Fig. 6b. There was no difference ($p>0.05$) between sporophyte growth in any of the treatments using seawater.

Critical level of lowering salinity: It was difficult for the spores to adjust to the osmotic pressure under such low-salinity condition less than 7%. However, some spores grew up to form juvenile sporophytes at 10 and 15% salinity, confirming that the spores could survive salinity levels greater than 10% and identifying the critical concentration for spore germination to be 20% salinity. Results from two of the replicate runs showed 20% salinity to be associated with maximum germination and growth of spores, suggesting that a salinity slightly lower than that of seawater was quite suitable for spore germination. The salinity of 10-20% was sufficient for growth of the sporophyte stage. In addition, there was no significant difference between sporophyte growth in the 30 and 60% treatment. The sporophytes

exhibited a greater tolerance of high-salinity conditions than did the spores. The low and high-salinity tolerances of the related macroalga *Pyropia tenera* has been reported to be 19-25% (for low-salinity tolerance) and 40% (for high-salinity tolerance)[26]. In addition, red seaweed (*Acanthophora spicifera*) shows positive growth rates for treatments of 15-35% and negative growth rates[27] for 40-50%. *Pyropia yezoensis* appears to have a greater adaptability to salinity change than *P. tenera* and *A. spicifera*. In contrast, the invasive seaweed *Undaria pinnatifida* in the nori farm sustains a high photosynthetic quantum yield at low salinity[28] 6%. In the nori farm field where *P. yezoensis* compete with the invasive seaweeds, salinity control is the important caution for the nori production.

Trade-off between lowering salinity and increasing nutrients: In general, the lowering salinity with mixing sewage effluent shows a deleterious effect on the seaweeds such as *H. banksii* and *Durvillaea potatorum* lives on several rock platforms[17,29]. The salinity is more important than nutrients for the natural seaweeds. However, there was the trade-off between lowering salinity and increasing nutrients for *P. yezoensis* growth. The 33, 50 and 67% TS conditions had high nutrient concentrations and sufficient salinity to support the spore germination. Although the salinity was appropriate, the nutrient concentration was still too low at 16% TS. In contrast, it was difficult for the spore to germinate under conditions such as the treatments 76-100% TS, as the salinity was too low (at less than 7%), even though there were high nutrient concentrations. A compromise is needed between lowering salinity and increasing nutrients as TS (%) increases, in order to use the TS as an enrichment in seawater to promote seaweed growth. From these results, it proposed that an appropriate ratio of TS to seawater is to keep the salinity at 20%.

Nutrient requirement for maximum growth: The results of nutrient requirement for maximum germination of spores, the greatest number of spores developed to become sporophytes within the test period under higher concentrations than 3PES (T-N, 35.4 mg-N L^{-1}, T-P, 4.2 mg-P L^{-1}). From these results, it was estimated that the lowest concentration of nutrients needed to achieve maximum growth in the early stages of *P. yezoensis* was 3PES. It has been reported that the nitrate uptake of *P. dioica* increases linearly and does not reach an asymptote, atleast up to 500 μM (7 mg-N/L)[30]. The *Pyropia* group has the high nutrient requirement and this characteristic is convenient for utilization of the TS.

In fact in this study, *P. dioica* is able to remove all N available in 500 μM of nitrate concentrations and both N sources (Fig. 4).

Shortage of nutrients in treated sewage for maximum growth: The concentrations of T-N and T-P in the TS were lower than 3PES, which was the lowest concentration of nutrients needed to achieve maximum growth. The nutrient concentration was still short of the maximum required for maximal spore germination, even if the medium contained 100% of the TS collected from Plant B, in which nitrification was inhibited. When the test used the actual seawater collected from the nori farm, depigmentation, attributable to nutritional deficiency[31] was confirmed by observation of the sporophytes growing in treatments 50 and 100% the actual seawater. Nitrogen deficiency causes the most serious influence for depigmentation of *P. yezoensis*[32]. As with spore germination, maximum sporophyte growth was achieved at 100% TS. The nutritional requirement of the sporophyte was higher than the nutrients present in the TS. It was difficult to supply sufficient nutrients for maximal sporophyte growth from just the TS. Furthermore, the actual seawater was insufficient to support sporophyte growth during the test period, because the nutrients had been taken up by the large number of sporophytes. Since the nutrient requirements of both spores and sporophytes were much higher than the nutrient content of the TS, this study do not have to consider the risk of excessive nutrient supply when using TS.

Prospect of exploiting under-used resources for seaweed production: It is clear that TS, which is a major under-used resource, is effective in promoting seaweed growth. However, the use of TS alone for nutrient enrichment of seawater results in limited growth as the TS addition decreases salinity. Based on the results of this study, the maximum percentage of TS which can be added to seawater was 67%. In contrast, the nutritional requirement for maximum *P. yezoensis* sporophyte growth was much higher than the total amount of the nutrients which could be supplied by the TS. To promote further growth, it would be necessary for *P. yezoensis* to be supplied with adequate amounts of nutrients. Alternatively, the use of water more saline than seawater would be able to prevent salinity deficit caused by excessive TS addition. For instance, the processing and disposal of the thick saline waste generated by the desalination plant caused problems in the plant[33,34]. However, it would be easy to increase the TS addition rate by mixing it with the thick saline waste. At present, there is a seawater desalination center (Fukuoka District Waterworks Agency, Japan) that discharges

the saline waste in a mixture with TS to control the salinity in the coastal seawater for consideration to environmental impact. Current findings suggest that there is a link between TS and seaweed cultivation. Furthermore, wastewater which is rich in nutrients, such as the digestive liquor[35] generated from the sewage sludge process and/or wastewater from livestock[36], has potential to further promote the growth of seaweed. To realize the use of these under-utilized resources for seaweed production, there is a need to control the appropriate conditions of salinity and nutrients based on the micro-well plate bioassay used in this study.

CONCLUSION

Porphyra yezoensis spore germination and sporophyte growth needed between 10 and 20% salinity. The nutrients contained in TS were inadequate to achieve maximum spore germination and sporophyte growth, even if 100% of the nutrients in the TS were supplied to the spores and sporophytes. It was proposed that mixing TS with seawater, to keep the salinity at 20% and to maintain as high a nutrient concentration in the culture environment as possible, could promote *P. yezoensis* production. A method to increase the amount of treated sewage, which can be mixed with high saline water more than seawater such as saline waste from a desalination plant.

SIGNIFICANCE STATEMENT

This study discovered the critical salinity condition and the macro-nutrient requirements of valuable seaweed growth that can be beneficial for use treated sewage as a nutrient resource for seaweed cultivation. This study will help the researchers and engineers to set the optimum condition for production of valuable seaweed. Treated sewage showed great potential as a nutrient resource for seaweed.

ACKNOWLEDGMENTS

The authors express gratitude to Saga Ariake Fisheries Promotion Center for kindly supplying *P. yezoensis* conchocelis. This study was supported by JSPS KAKENHI Grant Number 16K00621. The authors would like to thank Enago (www.enago.jp) for the English language review.

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