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EDITED BY
Jeng-Wei Tsai,
China Medical University, Taiwan

REVIEWED BY
Zhanhui Qi,
Key Laboratory of South China Sea
Fishery Resources Exploitation and
Utilization (CAFS), China
Zengjie Jiang,
Yellow Sea Fisheries Research Institute
(CAFS), China

*CORRESPONDENCE
Yuanqing Ma
mayuanqing@shandong.cn

[†]These authors have contributed
equally to this work and share
first authorship

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The interaction between DOC released by cultured kelp (*Saccharina japonica*) and the bacterial community reveals the potential for increasing marine carbon sequestration by macroalgae culture

Mingliang Zhang^{1†}, Huawei Qin^{1†}, Zhidong Wang²,
Bin Li¹ and Yuanqing Ma^{1*}

¹Marine Carbon Sink Research Center, Shandong Marine Resource and Environment Research Institute, Yantai, China, ²College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China

Macroalgae culture as a marine carbon sequestration strategy has been recently proposed. To understand the potential for carbon sequestration by macroalgae culture, we studied the interaction of dissolved organic carbon (DOC) released by *Saccharina japonica*, an important cultured kelp species in China, and the bacterial community. We found that *S. japonica* effectively released DOC containing both labile and refractory components, and the DOC released by *S. japonica* into the water decreased average molecular weight ($Mass_{wa}$) by 18 Da, meaning it was rich in low molecular weight matter. Degradation of labile DOC components occurred during the initial 2 months of the experiment. The $Mass_{wa}$ and molecular diversity of the DOC was increased in turn by bacterial degradation, and the variation in the DOC composition resulted in the succession of the bacterial community. During labile DOC degradation, anaerobic Gammaproteobacteria, Bacteroidia and Campylobacteria were the dominant microbial classes in the water, and aerobic Iodidimonadaceae became the dominant family during the final stage of the experiment. Finally, 5% of the DOC was transformed into refractory DOC. Although the sinking biomass was not involved in the study, the produced refractory DOC through the interaction between releasing DOC and bacterial degradation indicates a potential of carbon sequestration by macroalgae culture.

KEYWORDS

macroalgae culture, carbon sequestration, bacterial community, refractory dissolved organic carbon, *Saccharina japonica*

Highlights

- The DOC released by *S. japonica* contained both labile and refractory components.
- Anaerobic and sulfidic bacteria dominated degradation of labile components in the DOC.
- 5% DOC released by *S. japonica* was transformed to refractory DOC.
- Degradation increased the $Mass_{wa}$ and molecular diversity of the DOC.

1 Introduction

The ocean accounts for 71% of Earth's area and is the largest active carbon pool in the world, with 20 and 50 times the carbon stored in either the terrestrial or atmosphere carbon pool, respectively (Post et al., 1990). The ocean also significantly buffers against global warming, by absorbing 23% CO₂ released by human activities through industrial activities (Sabine et al., 2004; DeVries et al., 2017). Removing atmospheric CO₂ by increasing ocean carbon sequestration is an effective strategy for mitigating global climate change. The most important ocean carbon sequestration strategy is the 'blue carbon' project. By protecting and restoring the blue carbon ecosystem (i.e., mangroves, salt marshes, and seagrass beds) particulate organic carbon can be sequestered in the sediments (NASEM, 2019). Although macroalgae is the most productive marine macrophyte, it has been excluded from the 'blue carbon' strategic framework initially, mainly because most macroalgae grow on rocks, precluding the burial of organic carbon in sediment (Hill et al., 2015; Dorte and Carlos, 2016). Different to forest in land, organic carbon in macroalgae biomass can be degraded in short time-scale also limited the carbon sequestration of macroalgae. However, macroalgae can indirectly sequester large amounts of carbon in seawater in the form of dissolved organic carbon (DOC) through bacterial degradation of biomass and the extracellular exudate from macroalgae (Chen et al., 2020; Watanabe et al., 2020). A portion of this final product of this bacterial degradation is refractory to further biodegradation. According to the microbial carbon pump theoretical framework, the products of bacterial degradation comprise a refractory dissolved organic carbon (RDOC) pool in the ocean carbon cycle, which can sequester carbon for 5000–6000 years (Jiao et al., 2010; Ortega et al., 2019). Therefore, the indirect production of RDOC by macroalgae provides a positive buffering effect against global warming. For the reason above, macroalgae was proposed as the fourth species of blue carbon in recent years (IPCC, 2020).

Releasing DOC is a basic physiological function for macroalgae. To protect their tissues, more than 20% of the net primary production of macroalgae is released in the form of DOC (Abdullah and Fredriksen, 2004; Wada et al., 2007; Reed et al., 2015; Gao et al., 2021). A large amount of biomass exists in the coastal ocean, and macroalgae DOC is an important component of the coastal DOC pool (Reed et al., 2015; Mahmood et al., 2017; Ortega et al., 2019). For example, in Oura Bay, Japan, 20%–34% of the coastal DOC originates from the *Ecklonia cava* Kjellman release (Wada and Hama, 2013). Macroalgae DOC provides an important metabolic substrate for bacteria along the coast, and some of the RDOC produced by bacteria indirectly originates from the macroalgae. Around the world, macroalgae indirectly sequester roughly 0.12 Pg C/yr in the form of RDOC, which is 12% of the global annual blue carbon sequestration (Krause-Jensen and Duarte, 2016; NASEM, 2019). The indirect production of RDOC by macroalgae should not be ignored in the global carbon cycle.

Macroalgae culture is a rapid developing industry, producing 34.7 million t/y (FAO, 2021). Besides providing seafood and raw material for industry, macroalgae culture is one of the most effective carbon dioxide removal approaches developed in recent years (NASEM, 2021). The production of macroalgae in China accounts for 58.2% of the global supply (FAO, 2021). Among the species cultured in China, the kelp *Saccharina japonica* is the most important. Many studies have been conducted on the interaction between natural macroalgae DOC and the bacterial community, but none have focused on cultured macroalgae DOC (Wada et al., 2008; Zhang and Wang, 2017; Chen et al., 2020). Bacterial communities are sensitive to environmental variation. Physical, chemical, and biological factors can all disturb the interaction between macroalgae DOC and bacteria in seawater, and these uncontrollable factors make *in situ* studies difficult. Nearly all DOC degradation experiments have been performed under laboratory conditions, even though degradation processes in the laboratory can be different from those in the ocean (Wada et al., 2008; Chen et al., 2020; Watanabe et al., 2020; Gao et al., 2021). In this study, the interaction and dynamics of DOC release by the cultured kelp *S. japonica* and the bacterial community succession is studied in the laboratory.

2 Material and methods

2.1 Sample collection and pretreatment

S. japonica were collected in November 2021 from Chudao (122° 35' E, 37° 09' N), Sangou Bay, the most productive kelp bay in northern China. Kelp with intact leaves (each with length 1.2–1.5 m and fresh weight 20–40 g) were picked from culture

ropes. Surface water was collected from 1 m below sea level. All samples were transported into laboratory in 4 h. Seawater was immediately filtered through a 0.7 μm -pore Whatman glass fiber filter to eliminate phytoplankton and particulate matter. Kelp leaves were washed five times with filtered seawater to remove particulate matter and any fouling organisms attached to the kelp (Zhang and Wang, 2017).

2.2 Experimental process

To gather the DOC released by *S. japonica*, 800 g fresh kelp biomass was placed into a 100 L glass container. To simulate the actual kelp culture, the biomass density in the collecting process was consistent with the culture density in natural water. After 48 h, when the color of water turned yellowish brown, 30 L water was transferred into degradation containers as the treatment group for the 120 d degradation experiment. Simultaneously, 30 L filtered seawater without enrichment of DOC released by *S. japonica* was established as the control group. Both groups had three replicates. All degradation containers were covered loosely to avoid contact with the atmosphere. The experiment was kept in dark conditions at room temperature (20–26°C) to reduce the impact of photodegradation and possible growth of picophytoplankton, which may have passed through the filtering process and added DOC to the experiment. During the 120 d degradation process, water samples were collected at different time points for analysis of the concentration and composition of DOC and for determination of dynamic variations in abundance and community structure of planktonic bacteria.

Samples for measuring the concentration of DOC, fluorescent dissolved organic matter (FDOM), bacterial abundance, and community structure were collected on days 0, 2, 4, 8, 16, 24, 30, 45, 60, 75, 90, and 120. Samples for DOC molecular composition were collected on days 0, 60, and 120. Samples (50 mL and 500 mL) were collected from each replicate into brown glass bottles for the analysis of DOC concentration and molecular composition, respectively. The concentration samples were also used for the analysis of FDOM. Meanwhile, 4.5 mL samples with added 5% glutaraldehyde (final concentration) were collected for the analysis of bacterial abundance. All water samples were filtered through 0.22 μm -pore polycarbonate membranes before analysis. Lastly, 500 mL water samples were filtered, and the filters were collected for the analysis of the bacterial community structure. The glass containers in all experiments were acid-washed, rinsed with Milli-Q water, and combusted at 450°C for 8 h.

2.3 Sample analysis

2.3.1 Concentrations of DOC

Concentrations of DOC were measured by the high-temperature catalytic oxidation method using a TOC-VCPH analyzer (Shimadzu, Kyoto, Japan). Potassium hydrogen

diphosphate and high-carbon deep sea water produced by Hansell's laboratory were used as DOC standards. Milli-Q water was used as a blank and was analyzed every ten samples.

2.3.2 Bacterial abundance

Bacterial abundance samples were stained with SYBR Gold Nucleic Acid Gel Stain then measured with a FACS Aria flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Bacterial abundance was enumerated with Cellquest software.

2.3.3 Components of FDOM

Components of FDOM were analyzed with an excitation-emission matrix (EEM) using parallel factor (PARAFAC) analysis. EEM was measured with a fluorescence spectrophotometer (Hitachi F-4500, Tokyo, Japan) with scanning speed 1200 nm/min at 950 V. In the scanning process, the excitation (Ex) wavelength ranged from 200 to 500 nm with 5 nm increments, and the emission (Em) wavelength ranged from 250 to 550 nm with 5 nm increments. The raster for both Ex and Em was 5 nm. PARAFAC analysis of the FDOM components was performed with the drEEM toolbox (<http://dreem.openfluor.org/>) and MATLAB (MathWorks, Natick, MA, USA).

2.3.4 Molecular composition of DOC

The molecular composition of DOC was analyzed by Fourier transform-ion cyclotron resonance-mass spectrometry (FT-ICR-MS) using a Bruker ultra-high definition FT-ICR mass spectrometer Solarix XR 7.0T (Bruker Daltonics, Bremen, Germany) at China National Analytical Center, Guangzhou. Before the analysis, DOC was solid-phase extracted and eluted using a previously described method (Dittmar et al., 2008). Data analysis and chemical formula assignment were performed with the TRFu algorithm based on the formula criteria of $5 \leq C \leq 50$, $1 \leq O$, $N \leq 4$, $S \leq 2$, $P \leq 2$, $0 < O/C \leq 1.2$, and $0.3 \leq H/C \leq 2.5$, within 1.0 ppm (Fu et al., 2020). Once formula assignment for all peaks was accomplished, parameters such as modified aromaticity index (AImod) and double-bond equivalents (DBE) were calculated for each formula. AImod and DBE were calculated according to equations (1) and (2) (Koch and Dittmar, 2006):

$$\text{AImod} = (1 + C - 0.5O - S - 0.5H - 0.5N - 0.5P) / (C - 0.5O - S - N - P) \quad (1)$$

$$\text{DBE} = 1 + C - O - S - 0.5N - 0.5P - 0.5H \quad (2)$$

where C, H, O, N, S, and P are the numbers of carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus atoms in a molecular formula, respectively. All formulas were divided into six groups according to the values of AImod and H/C. The group with $\text{AImod} > 0.66$ represented polycyclic aromatic compounds. The group with $0.66 \geq \text{AImod} > 0.50$ represented highly aromatics compounds. The group with $\text{AImod} \leq 0.50$ and $H/C < 1.5$ represented highly unsaturated compounds. The group with $\text{AImod} \leq 0.5$, $2.0 > H/C \geq 1.5$ and without nitrogen atoms in their molecular formula represented unsaturated aliphatic compounds

($N = 0$). The group with $\text{Almod} \leq 0.5$, $2.0 > \text{H/C} \geq 1.5$, and with nitrogen atoms in their molecular formula represented nitrogenous unsaturated aliphatic compounds ($N > 0$). Lastly, the group with $\text{Almod} \leq 0.5$ and $\text{H/C} \geq 2.0$ represented saturated fatty and sulfonic acids. To compare the overall characteristics of the samples, the weight average of the molecular weight (Mass_{wa}), weight average of oxygen to carbon ratios (O/C_{wa}), weight average of hydrogen to carbon ratios (H/C_{wa}), weight average of double-bond equivalents (DBE_{wa}), weight average of carbon to nitrogen ratios (C/N_{wa}), and weight average of carbon to sulfur ratios (C/S_{wa}) were calculated using a method previously described (Koch et al., 2014).

2.3.5 Bacterial community structure analysis

Three parallel samples at each sampling time point were combined before DNA extraction as per Zhao et al. (2019). Bacterial genomic DNA was extracted using the HiPure Water DNA Kit (D3145-02, MGBio, China). The DNA concentration was verified with a NanoDrop 2000 (Thermo-Fisher, United States) and 1% agarose gel electrophoresis. The 16S rRNA gene was amplified with primers targeting the V3–V4 hypervariable regions: 343F-5'-TACGGRAGGCAGCAG-3' and 798R-5'-AGGGTATCTAATCCT-3'. Library sequencing and data processing were conducted by OE Biotech Co., Ltd. (Shanghai, China). The raw sequencing data were pre-processed by Cutadapt (version 3.4) to remove the primer sequence (Martin, 2011). The abundance of the amplicon sequence variants (ASV) was obtained by de-noising with Dada 2 (Version 1.8) (Callahan et al., 2016). All representative reads were annotated and BLAST searched against the Silva database (version 138) using QIIME 2 (version 2020.11) (Bolyen et al., 2019).

2.4 Statistical analysis

Statistical significance of differences in the DOC concentration and bacterial abundance were analyzed by one-way analysis of variance (ANOVA). The difference of bacterial communities in ASV's level was measured by analysis of similarity (ANOSIM). To test the bacterial community succession in the experiment, β -diversity was analyzed using Bray-Curtis distances based on the relative abundance matrices of the ASVs. Hierarchical cluster analysis (HCA) and nonmetric multidimensional scaling (NMDS) were calculated based on the Bray-Curtis distances.

3 Results

3.1 Variations in DOC concentration and bacterial abundance during the 120-day degradation experiment

The dynamic variations in DOC concentration and bacterial abundance had different characteristics during the 120-day

degradation process. The DOC concentration reached a peak of 65.29 ± 9.58 mg C/L at the start of experiment (day 0) and decreased sharply to 10.36 ± 0.57 mg C/L on day 24 (Figure 1). Over the following 46 d, the DOC concentration declined slowly, reaching 9.46 ± 0.46 mg C/L on day 60. During the last 2 months of the experiment, the DOC concentration remained relatively stable. Based on the variation in the DOC concentration, the entire degradation process was divided into three stages: DOC rapid decline (RD) stage (day 0–24), DOC slow decline (SD) stage (day 24–60), and DOC relatively stable (RS) stage (day 60–120). At the end of the experiment, the DOC concentration in the treatment group was significantly higher than in the control ($p < 0.05$), and the DOC concentration in the control did not significantly change during the experiment. Similar to the changes in DOC concentration, the bacterial abundance was initially the highest on day 0 at $1.96 \pm 0.07 \times 10^8$ cells/mL, remained stable on day 1, then smoothly decreased to $2.03 \pm 1.2 \times 10^7$ cells/mL by day 60. During the next 2 months, bacterial abundance remained relatively stable, similar to the trend in the DOC concentration. Also similar to the variation of DOC in the control, the bacterial abundance in the control did not change significantly during the experiment. The final bacterial abundance in the treatment group was significantly higher ($p < 0.05$) than in the control.

3.2 Variation in DOC components during the 120-day degradation experiment

PARAFAC analysis of FDOM and FT-ICR-MS results were used to track the variation in DOC composition during the study. Four FDOM components (i.e., C1–C4) were identified during the 120-day degradation, with Ex|Em wavelengths of 250 (310)|400 nm, 260|465 nm, 280|340 nm, and 355|420 nm respectively (Figure S1). According to the Ex|Em wavelengths, C1 and C2 represent humic-like fluorescent components originating from autochthonous production (Gao and Guéguen, 2018; Kulkarni et al., 2018). C3 represents an autochthonous protein-like component (Bittar et al., 2015), and C4 represents humic-like fluorescent components originating from microbial degradation (Amaral et al., 2021). All components had different dynamic variations in fluorescence intensity during the 120-day degradation process (Figure 2). The fluorescence intensity of both C1 and C2 decreased slightly until the end of the experiment. Different from the other humic-like fluorescence components, the fluorescence intensity of C4 increased slowly over the entire degradation process. The fluorescence intensity of C3 declined sharply during the initial 24 days then changed little until the end of experiment. In contrast, the fluorescence intensity of all components in the control group did not change significantly during the experiment.

FT-ICR-MS analysis revealed the variation in DOC composition at the molecular level during the 120-day degradation. Throughout the experiment, a total of 11376 different molecular formulas (MFs) covering a mass range of 109–999 Da (including ^{13}C isotopologs) were identified. Based on the elemental compositions, MFs were classified into five groups, i.e., CHO-, CHON-, CHOS-, CHONS-, and CHOSP-, which represented 95% of the MFs identified in the experiment (Table 1). The MFs changed significantly during the initial 2 months of treatment. In the day 0 treatment (T0) sample, the total number of MFs in the DOC was 32% higher than in the day 0 control (C0) sample. Meanwhile, the number of MFs in the CHON-, CHOS-, CHONS-, and CHOSP- groups were 17%, 75%, 347%, and 58% higher, respectively, in T0 than C0. The relative abundance of unsaturated fatty and sulfonic acids, unsaturated aliphatic compounds ($N > 0$), and unsaturated aliphatic compounds ($N = 0$) were 2.4%, 5%, and 15% higher, respectively, in T0 than in C0 (Figure 3). After 60 days degradation, the MFs in the treatment group (T60) increased by 15% compared with T0, and the number of MFs in the CHO-, CHON-, and CHOSP- groups increased by 10%, 28%, and 116%, respectively, between T0 and T60. In contrast, the relative abundance of unsaturated fatty and sulfonic acids, unsaturated aliphatic compounds ($N > 0$), and unsaturated aliphatic compounds ($N = 0$) decreased by 3.5%, 4.5%, and 18%, respectively, between T0 and T60. During the last 2 months of the experiment, the MFs and molecular composition changed little in the treatment. In contrast, the variation in the MFs and molecular composition in the control samples was negligible during the entire experiment.

The average chemical characteristics for the treatment samples also changed significantly during the degradation process (Table 2). The Mass_{wa} at T0 was 18 Da lower than in C0 because of the DOC released by *S. japonica*. DBE_{wa} and Almod_{wa} were 13% and 15% lower, respectively, in T0 than in C0. In contrast, $\text{N}/\text{C}_{\text{wa}}$ and $\text{S}/\text{C}_{\text{wa}}$ were 75% and 142% higher, respectively, in T0 than in C0. After 60 days degradation, Mass_{wa} in T60 increased by 26 Da compared with T0, and $\text{O}/\text{C}_{\text{wa}}$, DBE_{wa} , and Almod_{wa} increased by 20%, 22%, and 28%, respectively, between T0 and T60. However, $\text{H}/\text{C}_{\text{wa}}$ and $\text{N}/\text{C}_{\text{wa}}$ decreased by 15% and 18%, respectively, between T0 and T60. There was very little variation in the average chemical characteristics between T60 and T120, except for an increase in Mass_{wa} of 10 Da. During the entire degradation process, the changes in average chemical characteristics in the control group were relatively small.

3.3 Variation in the bacterial community during the 120-day degradation experiment

During the experiment, 2228 ASVs were identified. The raw data of ASVs were uploaded into national center for biotechnology information (NCBI) sequence read archive (SRA) database (accession number: PRJNA855139). Only 16% were present in both the treatment and control (Figure S2). The bacterial community in the treatment group was significantly different from the control based on the ANOSIM analysis of ASVs (Table S1). Based on the ASVs, 28 phylum, 56 classes, 147

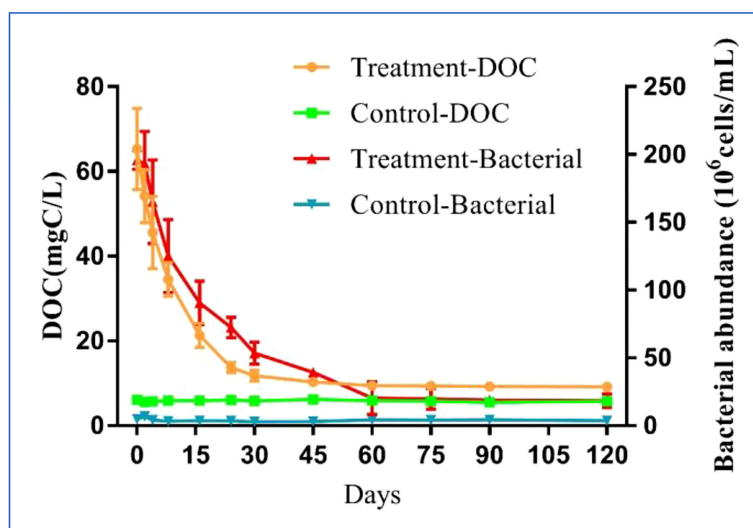


FIGURE 1

Variations of DOC concentration and bacterial abundance in the 120-day degradation of DOC released by *Saccharina japonica* and that in natural seawater.

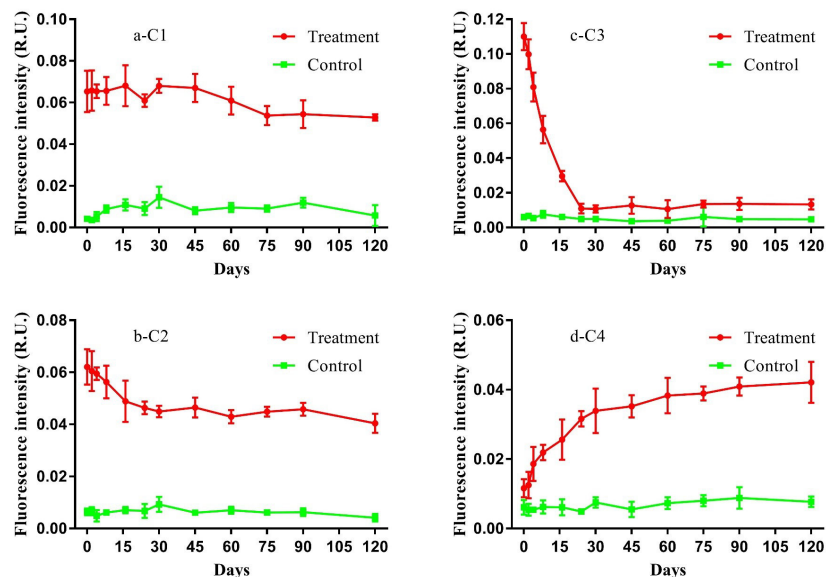


FIGURE 2

Variations of components (i.e., C1, C2, C3, and C4) of fluorescent dissolved organic matter (FDOM) in the 120-day degradation of DOC released by *Saccharina japonica* and that in natural seawater.

orders, 241 families, and 419 genera were identified. HCA and NMDS analysis showed significant bacterial community succession during the 120-day degradation. In the treatment group, the bacterial community was divided into three clusters: T-I (T0, T2, T4, and T8), T-II (T16, T24, T30, and T45), and T-III (T60, T75, T90, and T120) (Figure 4; Figure S4). In the control group, the bacterial community was divided into two clusters: C-I (C0, C2 and C4) and C-II (C8, C16, C24, C30, C45, C60, C75, C90, and C120). In addition, T-I and C-I, T-III, and C-II clustered together. The analysis of AONSIM also revealed the bacterial community succession in treatment and control (Table S1). The dominant classes in cluster T-I were Gammaproteobacteria, Bacteroidia, and Campylobacteria, with relative abundances of each one nearly 30% (Figure 5). Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia were the dominant groups in cluster T-II. The relative abundance of Gammaproteobacteria and Bacteroidia were the same in clusters T-I and cluster T-II. In contrast, the relative abundance of Alphaproteobacteria was 20% higher in cluster T-II than in T-I. Cluster T-III had only two dominant classes: Alphaproteobacteria and Gammaproteobacteria, and the relative abundance of Alphaproteobacteria was nearly 70%. In the control group, Gammaproteobacteria and Alphaproteobacteria were the dominant classes in C-I and C-II respectively, each with a relative abundance of nearly 70%.

4 Discussion

Macroalgae culture has been recently proposed as a marine negative emission technique (NASEM, 2021). However, there are no marine carbon sequestration projects using macroalgae culture in operation, mainly because the carbon sequestration ability of macroalgae is controversial (Hill et al., 2015). This study may increase the understanding of carbon sequestration through the release of DOC by macroalgae culture.

After 48 h DOC collection (day 0), the DOC concentration in the treatment group was nearly ten times that of the control, indicating that DOC is effectively released by *S. japonica*. Bacterial abundance peaked at the same time, indicating that DOC released by *S. japonica* is strongly favored by bacteria (Wada et al., 2008; Gao et al., 2021). Nearly 90% of the DOC was degraded during the DOC RD stage, and the rest of the decomposition occurred during the DOC SD stage. Bacterial abundance decreased by an order of magnitude in the DOC RD and SD stages, and then remained stable during the DOC RS stage (Figure 1). The variation in the DOC concentration and bacterial abundance indicated that most of the DOC released by *S. japonica* was decomposed during the initial 2 months of the experiment, similar to observations of previous studies (Wada et al., 2008; Watanabe et al., 2020; Gao et al., 2021; Manikandan et al., 2021). However, the DOC released by phytoplankton can

TABLE 1 The numbers of molecular formulas (MFs) contained in the DOC at different sampling time points during the 120-day degradation.

	T0	T60	T120	C0	C60	C120
total	7536	8650	8579	5710	5703	5851
CHO	2330	2573	2724	2752	2678	2653
CHON	2157	2756	2864	1842	1831	1966
CHOS	1149	1119	989	655	643	596
CHONS	1054	674	566	236	177	273
CHOSP	436	940	893	276	275	231

T0, T60 and T120 represent the samples collected in treatment group on the 0, 60th and 120th day; and C0, C60 and C120 represent the samples collected in control group on the 0, 60th and 120th day.

be decomposed within days to weeks (Hama et al., 2004). This indirectly indicates that not only labile DOC components, but also some refractory DOC components are released by kelp (Wada et al., 2008). Comparing the variation in the DOC concentration between treatment and control, 5% of the DOC released by *S. japonica* was estimated to have remained as RDOC. Considering 20% or more net primary production is released as DOC by kelp (Wada et al., 2007; Gao et al., 2021), we

propose that carbon sequestration by macroalgae culture through the interaction between DOC release and bacterial degradation is not negligible.

The variation in the DOC composition revealed the details of the transformation of DOC released by *S. japonica* to RDOC. Generally, protein-like components are considered an indicator of labile composition in DOC (Yang et al., 2015) and humic-like components are considered a potential tracer of RDOC (Lønborg

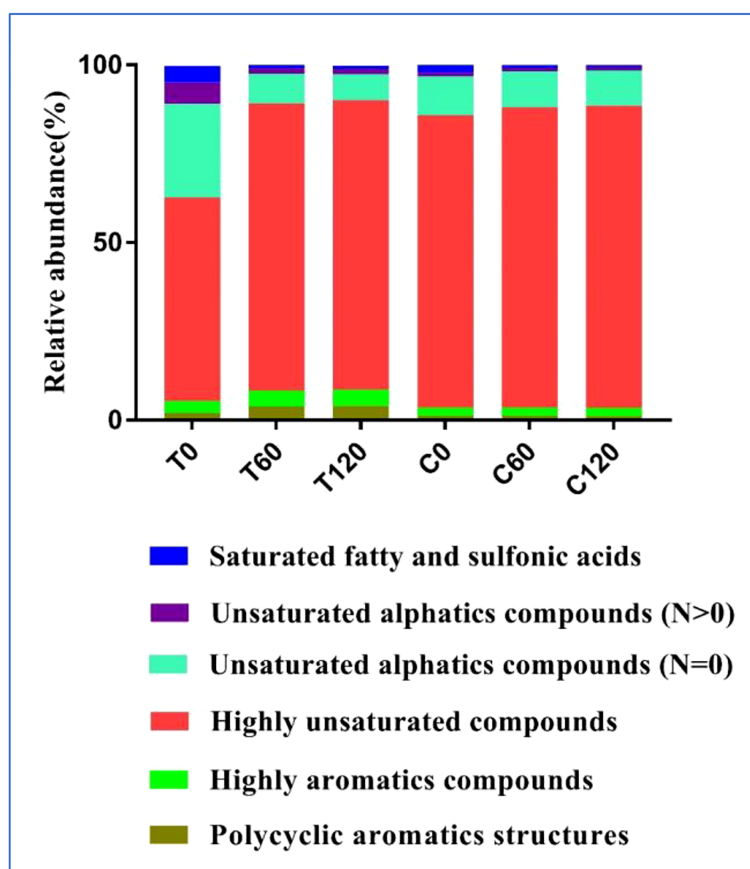


FIGURE 3

The relative abundance of DOC components identified with threshold criteria of the modified aromaticity index (Almod) values and H/C ratios in the 120-day degradation of DOC released by *Saccharina japonica* and that in natural seawater.

TABLE 2 The average chemical characteristics at different sampling points in 120-day degradation.

	T0	T60	T120	C0	C60	C120
Mass _{wa}	402.08	428.37	438.52	420.1	413.7	417.56
O/C _{wa}	0.422	0.505	0.495	0.44	0.438	0.439
H/C _{wa}	1.380	1.201	1.221	1.299	1.288	1.286
DBE _{wa}	7.311	8.952	8.965	8.276	8.238	8.352
N/C _{wa}	0.028	0.023	0.022	0.016	0.016	0.016
S/C _{wa}	0.017	0.017	0.016	0.007	0.005	0.005
Almod _{wa}	0.201	0.259	0.253	0.232	0.238	0.237

T0, T60 and T120 represent the samples collected in treatment group on the 0, 60th and 120th day; and C0, C60 and C120 represent the samples collected in control group on the 0, 60th and 120th day.

et al., 2013; Chen et al., 2020). Fluorescence analysis revealed that the labile components (C3) were rapidly exhausted during the DOC RD stage. Interestingly, humic-like components (C1 and C2) were slowly degraded during the entire experiment and did not significantly change the DOC concentration during the last 2 months of the experiment (DOC RS stage). This might represent a small percentage of the refractory components in the DOC released by *S. japonica*. Meanwhile, the RDOC component (C4) was produced gradually during the degradation process (Figure 2) (Chen et al., 2020). FT-ICR-MS analysis also revealed that both labile and refractory components were present in the DOC released by *S. japonica*. Generally, the degree of recalcitrance to biodegradation is in the order of polycyclic aromatic compounds > highly aromatics compounds > highly unsaturated compounds > unsaturated aliphatic compounds (N = 0) > unsaturated aliphatic compounds (N > 0) > saturated fatty and sulfonic acids (Schmidt et al., 2011; Cai et al., 2019). By comparing the molecular composition of T0 and C0, we found that labile components such as saturated fatty and sulfonic acids and unsaturated aliphatic compounds (N > 0) increased in relative abundance by 2.4% and 5%, respectively. Meanwhile, refractory components, such as polycyclic aromatic compounds and highly aromatics compounds, increased in relative abundance by 0.8% and 1%, respectively. This indicates that these labile components, and a small amount of refractory components, were present in the DOC released by kelp (Figure 3) (Wada et al., 2008; Watanabe et al., 2020). The relative abundance of labile and refractory components changed little between T60 and T120, suggesting that most degradation was completed during the DOC RD and SD stages.

FT-ICR-MS analysis showed the details of the RDOC production process at the molecular level. The number of MFs was 1826 higher in T0 than in C0, suggesting that DOC released by *S. japonica* contained new organic matter that does not exist in natural water. In addition, the Mass_{wa} was 18 Da lower in T0 than in C0, suggesting that the new matter was mainly low molecular weight organic matter. N/C_{wa} and S/C_{wa} were 75% and 143% higher, respectively, and CHON-, CHOS-, CHOSP-, and CHONS- were 17%, 75%, 58%, 347%, higher, respectively, in T0 than in C0 (Table 1). The variation in the molecular

composition stated above indicates that the DOC released by *S. japonica* provided organic molecules containing nitrogen and sulfur (Chen et al., 2020). In nature, organic molecules containing nitrogen and sulfur mainly originate from proteins, amino acid, and polypeptides, and this may be explained by the molecular composition of C3 in FDOM. The DOC released by *S. japonica* decreased the DBE_{wa} by 18% and Almod_{wa} by 13%; these factors represent the degree of unsaturation and aromaticity, respectively (Table 2). This means that DOC released by *S. japonica* increased the composition of labile DOC (Wada et al., 2008; Gao et al., 2021). During the degradation in the RD and SD stages, DBE_{wa} and Almod_{wa} increased by 22% and 29%, respectively, meaning that degradation produced refractory components. Meanwhile, H/C_{wa} decreased by 15% and O/C_{wa} increased by 20%, which suggested that the degradation process is similar to the chemical reaction of oxidative dehydrogenation. In the initial 2 months of degradation, the number of MFs and Mass_{wa} increased by 1442 and 26 Da, respectively, but changed little during the last 2 months of degradation. This suggests that most of the degradation occurred during the initial 2 months. In addition, degradation produced high molecular weight organic matter and increased the molecular diversity in the treatment.

Van Krevelen diagrams reveal more details of the composition variation during oxidative dehydrogenation. *S. japonica* releasing DOC produced two groups of MFs (O/C > 0.5 and H/C > 1.5), which represented the saturated (refractory component) and reducible (labile component) composition, respectively (Figure 6A) (Cai et al., 2019). As the reducible components were degraded, two more groups of saturated components were produced (O/C > 0.8 and H/C < 1.5) during the initial 2 months (Figure 6B). At the end of the experiment, the increasing peak intensities and declining peak intensities almost overlapped, indicating that degree of saturation in the DOC changed little in the last 2 months of experiment (Figure 6C). Moreover, nearly 80% of the MFs in the DOC were shared between day 60 and day 120 of treatment (Figure S3).

The chemical reaction of oxidative dehydrogenation requires a high temperature, well above 100°C. However, this same reaction

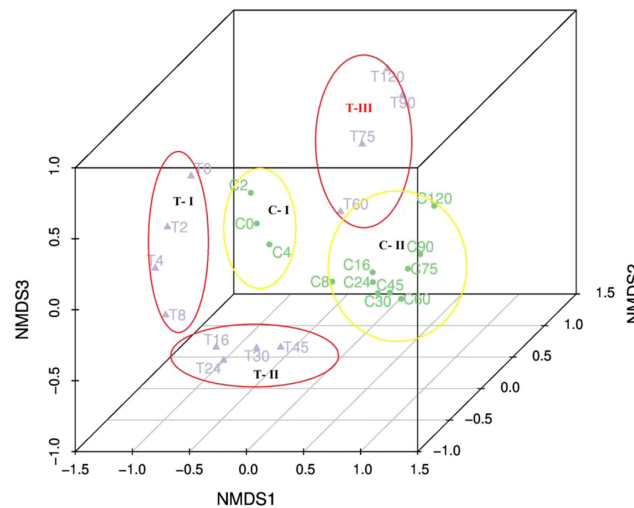


FIGURE 4 Nonmetric multidimensional scaling ordination of the bacterial communities in the 120-day degradation experiment.

can be mediated by bacteria at room temperature and in darkness. The bacterial community succession was divided into 3 groups (T-I, T-II, and T-III) during treatment, but these groups did not completely agree with the stages representing the change in DOC (RD, SD, and RS). The RD stage included all of T-I and a part of T-II. The SD stage included a part of T-II and T-III. Perhaps the bacterial community was extremely sensitive to variations in the environment, such as metabolic substrate and dissolved oxygen (DO) (Sun et al., 2012). At the start of experiment, kelp introduced abundant organic matter containing sulfur (Chen et al., 2020), and maintained 80–100 times more bacterial biomass in treatment compared with the control during the RD stage (Figure 1). The rapid consumption of DOC in the treatment group resulted in depletion of DO (Figure S5). Anaerobic and sulfur-degrading

Bacteroidia and Campylobacteria became the dominant classes in T-I (Voordouw et al., 1996; Hu et al., 2016). Continuous degradation of DOC sustained the anaerobic environment in T-II, and Bacteroidia remained the dominant class in T-II. The Alphaproteobacteria class mainly comprised the Rhodobacteraceae family, which is an important sulfur-degrading family, and this class became dominant in T-II (Figure S6) (Pujalte et al., 2014). In T-III, at the completion of DOC degradation, the aerobic Iodidimonadaceae family belonging to the Alphaproteobacteria class became the dominant family (Lino et al., 2016). Generally, bacterial rapidly consume labile DOC components, resulting in an anoxic environment during treatment in T-I and T-II stages (Figure S5), and the bacterial community changes rapidly with variations in the environment (Figure 4; Figures S4, S6). RDOC is

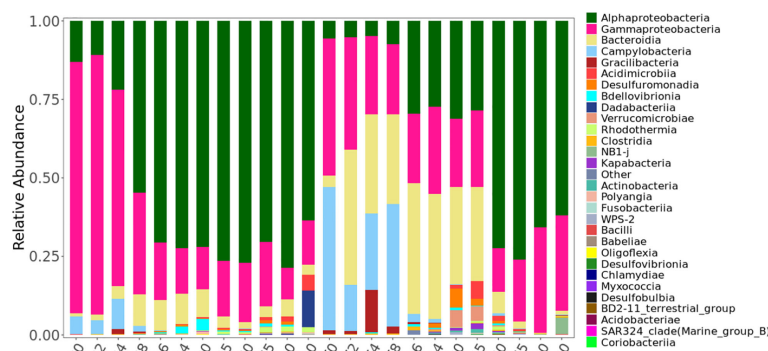


FIGURE 5 Variations in the bacterial community at the class level during the 120-day degradation of DOC released by *Saccharina japonica* and that in natural seawater.

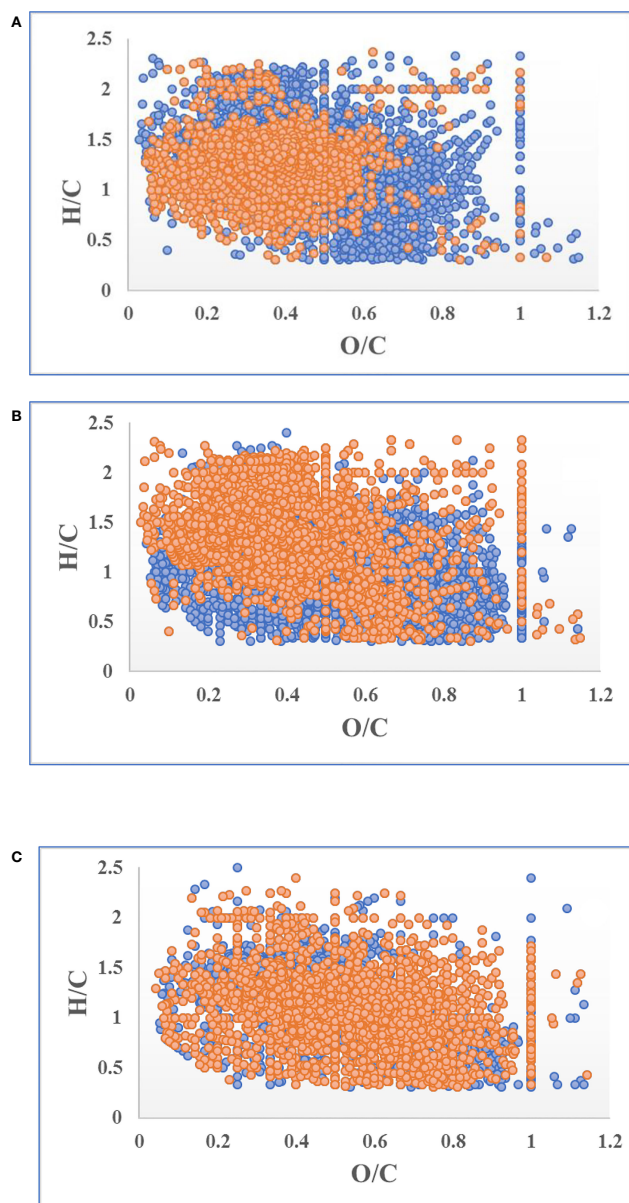


FIGURE 6

Variation in the normal peak intensity of molecular formulas (MFs) during the 120-day degradation. Blue and orange points represent increased and decreased peak intensities respectively. (A) Variation of MF peak intensity in T0 compared to C0; (B) variation of MF peak intensity in T60 compared to T0; (C) variation of MF peak intensity in T120 compared to T60.

the final product of degradation that then remains in the water (Figures 2, 3).

In this experiment, DO was an important factor for the succession of the bacterial community because of the limited air exchange. In contrast, DO may be ample in natural waters as wind and waves increase air exchange. Although the environmental conditions are great different, a reaction similar to oxidative dehydrogenation driven by bacteria was observed in the ocean

(Medeiros et al., 2015). In the deep sea, which is the final reservoir for DOC degraded by bacteria, DOC had higher O/C_{wa} and lower H/C_{wa} than that in the surface DOC. It may indicate that RDOC producing process by interaction between DOC released by cultured kelp (*Saccharina japonica*) and the bacterial community is similar as the RDOC producing process in natural condition. This study may help to reveal the potential to increase marine carbon sequestration using macroalgae culture.

5 Conclusion

The present study shows that DOC released by *S. japonica* was rapidly degraded into RDOC by bacteria. After 2 months, LDOC components in DOC were degraded completely, and 5% of this DOC was transformed into RDOC. Although culture biomass will be harvested during the actual kelp culturing process, an estimated 1–2% of the net primary production can still be sequestered as RDOC. This suggests that sequestering carbon by macroalgae culture is feasible.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/>- PRJNA855139. Further inquiries can be directed to the corresponding authors.

Author contributions

MZ and HQ contributed to data curation, visualization, writing – original draft, validation. ZW and BL performed data curation and visualization. YM contributed to writing – review and editing and resource. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.985548/full#supplementary-material>

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