Seasonal changes of fouling-forming microalgae on lithium manganese oxide disks in seawater, East Sea, Republic of Korea

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ABSTRACT

For investigation of biofouling characteristics of marine microalgae on lithium manganese oxide disk (LD), which is manufactured for recovery of mineral resources from seawater, we carried out an exposing test for LD at Okgye, East Sea, Korea. The amount and species composition of the attached microalgae on the LD surfaces were determined based on the exposing period and season. The immersion test was conducted for 7 d, 14 d and 21 d for each of the three different seasons of May, August and October. The number of microalgae attached to the LD is dependent in season and exposing duration. In May and October, the microalgae adhered approximately 4,000 cells/cm² after 7 d and after 14 d, more than 2-fold were attached that exceeding 10,000 cells/cm². In spring and summer, centric diatom, *Guinardia* sp. and *Cyclotella* sp. were dominated in seawater, and they predominantly adherent in short exposure periods of within 7 d. However, dominant adherent species were succeeded to *Cylindrotheca* sp., pennate diatom, as longer exposure times. The microalgae morphology, which favored attachment was also a major cause of prolonged fouling upon exposure of LD surface.

Keywords: Biofouling, *Cylindrotheca*, *Closterium*, Diatom, *Guinardia* sp., lithium manganese oxide, *Navicular* sp.

1. Introduction

Marine biofouling is a serious concern to offshore installations, which are constructed for various purposes such as marine vessels, the leisure industry, antifouling development, and resource recovery [1-3]. Biofouling increases water flow resistance and corrosion of artificial surfaces [4, 5] and results in serious economic losses [6-8]. The biofouling proceeds through several stages: formation of an organic film that biochemically conditions the surface, bacteria adherence and primary colonization, secondary colonization by unicellular eukaryotes, and tertiary colonization, which defines the macro-fouling stage [2]. In the early fouling process, the microorganisms rapidly form a biofilm on the submerged surface; the biofilm is a major factor affecting the attachment and growth of diatoms.

Diatom species have been reported to be the major constituents of biofilms in marine environment and due to the EPS (extracellular polymeric substances) secreted from the raphes or cell walls of diatom, it becomes easy to adhere to sediment particles or artificial

surfaces. Therefore, diatom which have high adhesion capacity, have a significant effect on the function of submerged surfaces in marine environments. Studies regarding diatom species in biofouling investigation are exceptionally important because diatom plays a significant role in the settlement of macro organisms such as barnacles [9-11]. Numerous studies have been conducted regarding initial biofouling by microorganisms such as bacteria and algae, which have defined cell morphology, adhesion mechanisms, physical and chemical properties of the adhesive itself, as well as interactions with various artificial surfaces. Shim et al. [12] evaluated the species of diatoms attached to several types of artificial substrates exposed at Incheon Port such as glass, acrylic, titanium, copper, and anti-fouling (AF) coated slide glass. Patil and Anil [13] assessed biofouling by diatoms exposing the soil-simulated fiber and coupon-shaped glass at Dona Paula Bay, India, and Yang et al. [10] studied the characteristics of seasonal fouling on the glass panels. In the case of Munda [14], the concrete plate was exposed to predict the attachment of diatom species in the rock environment and Mitlbavkar and Anil [5] evaluated seasonal changes of diatoms

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species on stainless steel and polystyrene surfaces. Hunsucker *et al.* [15] studied the fouling characteristics according to the position (head and side) of an in-service ship and anti-fouling types of paints. Based on these studies, it can be observed that specific diatoms differ in terms of season, exposure location, duration, and materials of the artificial substrates. Therefore, to control biofouling of various types of exposed materials in seawater, area-material-specific fouling species studies should be conducted.

The LD (disk type of lithium adsorbents) used in the experiment is a lithium manganese oxide (LMO) material developed by the Korea Institute of Geoscience and Mineral Resources for lithium recovery from seawater. The Korea Institute of Geoscience and Mineral Resources has developed and evaluated various types of adsorption materials for the recovery of dissolved resources in seawater during a long period of time. In particular, a lithium adsorption material based on LMO was developed and patented by the U.S. Patent [16, 17] and published several papers related to the developed material properties [18-21]. For lithium recovery in seawater, a pilot study was produced and in parallel, a biofouling evaluation study was conducted. As first, started with a study of fouling by bacteria, an early stage of biological fouling. Powder type adsorbents were exposed in Sacheon, East Sea for 30 d to evaluate the control of fouling species by zinc oxide antifouling agents [3]. A surface observation method was applied [22] to find out the actual fouling shape on the surface of the adsorbent material, and a study was conducted to evaluate the adhesion to the shape and surface area, such as circular, powder, and rod shapes [23]. The study of contamination by plankton, which could lead to the next major contamination from bacteria, was started [24]. The adsorption material used in this study is made by molding lithium manganese oxide particles into a disk shape.

We evaluated the biofouling characteristics of microalgae communities due to seasonal changes and exposure duration on the surface of lithium manganese oxide disk (LD) exposed in the Okgye Sea in Korea. To investigate the seasonal and temporal distributions of the fouling microalgae, the LD were exposed to dynamic seawater for a certain period and collected after 7 d, 14 d, and 21 d in May, August, and November 2013, respectively, in the Okgye Sea in Korea. Cell counting and identification of LD surfaces were conducted using an optical microscope and a scanning electron microscope (SEM).

2. Experiments

2.1. Study Area

The investigations were conducted near the Okgye Sea (37.37° N, 129.04° E), Gangneung, in the mid-latitude of the east coast of Korea. This installation is located at a 1 km distance from the Seawater Lithium Research Center and 750 m from the coast. Seawater depth was observed between 12 and 15 m. During the experimental periods, the average values of surface temperature was $13.4 \pm 0.7^\circ\text{C}$, $26.4 \pm 0.4^\circ\text{C}$, and $17.6 \pm 1.3^\circ\text{C}$ and salinity was 33.9 ± 0.1 psu, 31.4 ± 0.1 psu, and 33.5 ± 0.2 psu in May, August, and November, respectively. Usually in this area, concentration of nutrients such as nitrogen (N) and phosphorus (P) com-

pounds were high in winter and low in summer. The value of dissolved inorganic nutrients at the surface layer is less than the value at the bottom (Fig. S1). These data were obtained from the marine environmental monitoring network by the Ministry of Oceans and Fisheries [25].

2.2. Seawater Sampling and Microalgae Identification

Seawater samples were collected from surfaces at depths of 5 m and 10 m near the pilot plant of lithium recovery field near the Okgye Harbor. The seawater was sampled once a month except March during Feb - Oct 2013. Three points of vertical seawater samples (0 m, 5 m, and 10 m) were collected at the pilot plant station. Three 1-liter samples for each depth were immediately preserved on board with Lugol's solution and subsamples of 200 ml were obtained after natural concentration of 48 h for cell counts. All samples were thoroughly shaken and aliquots of 1 ml of subsamples were allowed to settle in the counting chambers. Microalgae was identified and counted by light microscope (LM) (Axioplan; Carl Zeiss, Oberkochen, Germany) using the Utermöhl settling technique [26]. The cells were counted three times at x400 magnification of microscope and then converted to cell/ml. The majority of the cells were identified up to the level of species.

2.3. Exposure of Lithium Manganese Oxide Disks to Seawater

The lithium adsorbent used in this study was a granular form of porous lithium manganese oxide (LMO) cast in the form of a disk (pellet, diameter 30 mm, thickness 4 mm). The granular form of manganese oxide was made from powdered manganese oxide through simple mixing, forming and calcination processes. The process is to obtain a precursor mixture suitable for molding using an LMO precursor composed by lithium carbonate and manganese carbonate and a binder (water glass, sodium silicate solution), then form it into a sphere using extrusion equipment, heat-treated to form solid. The shape of the spherical adsorbent has the advantage of increasing packing density and improving stability to the polishing environment. The cast disc-shaped adsorbent has a porous structure to facilitate the passage of seawater (Fig. 1).

For the periodical and seasonal characteristics of biofouling by microalgae on the lithium adsorbents, test samples were immersed in the pilot plant in the seawater near Okgye harbor. Test samples, LD were packed in nylon fabric and installed into a PVC cage. This cage hung on the pilot plant construction and was immersed in seawater up to a depth of 7 m. These adsorbents were retrieved after 7 d, 14 d, and 21 d and investigated regarding the abundance of species of attached microalgae. For detaching the micro algae samples from the adsorbent's surfaces, half of the adsorbents are immersed in 15 ml sterilized seawater using a vibrator with 90 s. Lugol's solution is added and aliquots of 1 ml of subsamples were counted and identified using an optical microscope. The changing morphology of porous pellet surfaces after exposure is analyzed using SEM (S-3500N; Japan, KBSI Chuncheon Center). Samples were prepared for electron microscopy by drying and coating them with gold and palladium under a 2-kV extra high-tension condition. This experiment was conducted three times: May (20th May - 10th June, spring), August (13th August - 3rd September, summer), and November (24th October - 15 November, fall) in 2013.

Fig. 1. SEM image of LD surface after 7 d exposing to seawater (3.00 kV, 200x).

3. Results

3.1. Abundance and Composition of Microalgae in Seawater

Abundance and species composition of microalgae are determined by biological factors owing to ingestion of high-level consumers and nonbiological factors such as nutrients, water temperature, and light intensity [27, 28]. Generally, phytoplankton bloom occurs primarily in spring and autumn in Korea due to biological and environmental factors [29, 30]. Total microalgal abundance was the highest in May and lowest in February in the Okgye area of Gangneung during February to October 2013 (Table 1). These values represented the average number of microalgae cells collected from sea water at 0 m (surface), 5 m, and 10 m depth, respectively. The density of microalgae increased from April (438×10^3 cell/L) and peaked in May and lowered in June and August due to frequent precipitation, and subsequently increased again in September. In February, microalgae are rarely present, suggesting that there is an exceptionally rare occurrence at 10 m depth, because strong winds and active vertical mixing in winter causes concentration of microalgae to be below the critical depth [28].

During the investigation periods, a total of 99 species of microalgae were identified. Seventy-one species belonged to Heterokontophyta (41 genera, 2 phyla) and 28 species belonged to Dinophyta (15 genera, 2 phyla). In this area, Bacillariophyceae were predominant at 97%, Dinophyta were 2.6%, and Dictyochophyceae were 0.4%. Especially, centric diatoms were 85.7% and pennate diatoms were 11.3%. Monthly dominant species were changed. In April, *Leptocylindrus danicus* (38.7%) and *Nitzschia*

spp. (23.1%) were the dominant species. *L. danicus* continuously increased by 80.1% in May. In June, July, and September, *Guinardia* sp. were predominant with concentrations of 75.3%, 68.8%, and 39.9%, respectively. In August and October, the *Cyclotella* sp. were the most abundant with concentrations of 29.0% and 24.7%, respectively. The number of identified species was more diverse from July to October. Moreover, after August, there was a decrease the dominance rate for specific species but various species were present this area. The monthly dominant microalgae are listed in Table S1. All dominant species were widely distributed species that exist in the temperate coastal area of the northern hemisphere. *L. danicus* has been recorded to be the most dominant species in spring [31-33] and *Chaetoceros* spp. is the most frequently observed species and they have steady appearance regardless of the season in the coastal area of East Sea [31-35]. *G. cylindrus* was predominant in June, July, and September in this study. *Dictyococha* sp. was relatively rich in February. However, the total number of cells counted in February was very rare, and the largest number was identified in August, which is supported by other studies that *Dictyococha* sp enriches at high water temperatures [36].

As shown in results above, the composition of plankton species in seawater of the Okgye Sea was changed by season. This is due to the complex effect of temperature and water mass characteristics. The composition of these seasonal planktons is not different from the results of the long-term studies of the Japan sea (far East Sea) in the past [37]. Differences in the community structure of phytoplankton are apparent in summer and winter when the large differences of temperature. In winter, diatoms were dominate, such as *Skeletonema* spp., which has withstand low temperatures and low light condition. And in the summer, the abundances of Dinophyta were increases. As a whole area of the Korean peninsula, the species composition was similar, but the predominant species varied from season. In Asan Bay, western estuary of Korean Peninsula, *Skeletonema* spp. or *Thalassiosira* spp. predominates in winter and *Rizosolenia* spp. and *Pseudo-nitzschia* spp. were dominated in spring [28]. In the Yellow Sea, changes in plankton species are more diverse due to freshwater inflows and silty (clay) environment [38]. *Leptocylindrus* spp. and *Guinardia* spp. dominated in May and June in the Uljin Sea of the East Sea located south from this study area, but *Thalassiosira* spp. were extremely dominated in April, unlike the results of this study [39]. Therefore, predominant species may appear differently depending on the seasons and regions of the Korean Peninsula, so it is essential to investigate the ambient seawater when evaluating exposure to biofouling.

3.2. Seasonal Changes in Species and Quantity of Microalgae Attached to the Lithium Manganese Oxide Disk

3.2.1. Concentration of attached microalgae on LD surface

Attached microalgae on the surface of LD are represented by the

Table 1. Monthly Abundances of Phytoplankton by Depth (cell/mL)

	Feb	Apr	May	Jun	Jul	Aug	Sep	Oct
Surface	3	438	585	10	212	28	105	20
5 m	2	510	526	27	339	54	151	33
10 m	3	277	632	182	215	58	157	41
No. of species	5	23	15	11	51	45	51	56

number of cells per unit area (cm^2). The overall average adherent cells were 5,830 cells/ cm^2 . The mean number of adherent cells was 7,060 cells/ cm^2 in May, 2,099 cells/ cm^2 in August, and 8,332 cells/ cm^2 in October (Fig. 2). Total adhesion of cells were highest in October; the maximum adherence of cells was observed to be 11,746 cells/ cm^2 on the LD surface that was exposed for 21 d in May. In May and October, the adhered number of microalgae increased with longer exposure period. In May, the increase in the amount of adherence between 7 and 14 d relatively small increases (about 25%) compared to the increase from rapidly between the 14 d and 21d exposure periods. The adherence amount for the 21 d exposure was more than two times that for the 14 d exposure. In October, the attached microalgae density was increased with exposure time and rapidly accreted during more earlier period of 7 d - 14 d. In August, the adherence of microalgae was the highest at 4,706 cells/ cm^2 during a 14 d exposure period and decreased after 14 d. Overall, the amounts of adhering microalgae to LD were higher in May and October, and lower in August. The high attachment rate of microalgae in May is related to the high density of ambient sea water in Okgye. However, in October, there is no effect owing to microalgae abundances in ambient seawater. The increased abundance of the standing crops of microalgae in the spring and less in the summer is affected by the biomass of microalgae in the ambient seawater. Despite the lower abundance of microalgae in ambient water column in October than in August, the amount of adhesion microalgae increased to the level of May.

Comparing the identified species with ambient seawater, the pennate diatom was observed to be the most abundant, and several species were not observed in seawater (Table S2). *Odontella longicrusis*, *Bacillaria paxillifer*, *Mastogloia* sp., *Pleurosigma angulatum*, *Pleurosigma decorum*, *Pseudo-nitzschia delicatissima*, and *Pseudo-nitzschia* sp. were not observed in seawater at the genus level. The proportion of diatom present in all identified microalgae is about 97% in both seawater and LD surface. However, the composition of diatoms was different in seawater and LD. The diatom of centric was 85.7% and that of pennate was 11.3% in ambient seawater but, the LD surface has exceeded the pennate diatom by 67% while the centric diatom was 29.5%. Consequently, the morphological characteristics such as the raphes that made EPS were favorable to adhesion dominate the adhesion efficiency.

Fig. 2. Seasonal distributions of attachment microalgae on the absorbent's surface according to exposing periods.

3.2.2. Seasonal succession of diatom species on LD surface

The dominant biofilm forming the microalgae species on the LD surface demonstrated either similarities or differences according to exposure time and season. The *Cylindrotheca* sp., *Cyclotella* sp., *Guinardia* sp., *Licmophora* sp., *Navicula* sp., *Thalassionema* sp., and *Prorocentrum* sp. were the most frequently observed diatom genera in this study. Interestingly, in this study, centric diatoms such as *Cyclotella* sp. and *Guinardia* sp. were primarily identified in spring time. As shown in Table S2, fouling diatoms for artificial submerged surfaces were primarily related to pennate diatoms (benthic). The adhering of microalgae to the LD surfaces was largely affected by environmental changes due to seasonal causes and the differences of the substrates. The results of this study also indicate that the attached diatoms were related to the season and the phytoplankton composition of ambient seawater. The dominant species of marine phytoplankton are the *L. danicus* (April and May), the *Guinardia* spp. (June and July), *Cyclotella* spp. (August and October), and *Guinardia* spp. (September) in seawater.

On the LD surface, *Guinardia* sp. were predominant in spring (May) during the initial 7 d immersion, which is the second dominant species in May and predominant in June. Although *Guinardia* sp. is not known to be a major species for biofilm formation, it affects adhesion to LD surfaces when the microalgae abundance increases during phytoplankton bloom in spring. However the dominant species was succeeded by *C. closterium* after 21 d. For the seven day exposure, *Guinardia* spp. accounted for 86%, but after the 14 d exposure, the dominance rate was reduced to 62% and the minimum relative abundance after 21 d was observed to be 29%. The relative abundance of *C. closterium* increased from 7.9% to 62.3%; a few exist in sea water in May and June. Yang et al., [10] also demonstrated that the number of diatoms and EPS significantly increased after two weeks of exposure. In August, fouling is dominated by *Cyclotella* spp. at 28.3% during the 7 d immersion period. *Cyclotella* sp. was the most abundant diatom in Okgye seawater in this period. *C. closterium* was not dominant in the early stage of summer but it increased to 75% during the 14 d exposure period and decrease to 26% after 21 d. The percentage of *C. closterium* decreased as the total fouling amount was lower at 21 d of exposure, but the fouling species increased, such as *Cyclotella* spp. (19%), *Nitzschia* spp. (10%) and *Thalassionema* spp. (12%). This is also related to the increase in species present in the seawater since summer (Table 1). Especially in August, species belonging to Dinoflagellates were additionally observed in seawater, and thus found *Protoperidinium* sp. and *Dinophysis* sp. on the LD surface (included in Others in Table S2). The appearance of various species seems to have interfered with the dominance of one species. The *Navicula* sp. is a ubiquitous biofilm forming diatom. In this study, *Navicular* sp. were predominant for 21 d in fall. In spring and summer, biofouling diatoms of early stages were significantly affected by ambient water column. However, over a longer exposure time and as biofilm accumulates, it remains greater attachment species such as *C. closterium* after the 7 d exposure in fall, *L. paradoxa* was the abundant species with 31.1% after *Navicula* sp. After the 14 d and 21 d exposure periods, *C. closterium* also increased with exposure time. The biofilm composition of phytoplankton was changed in May and August as the immersion time increased

Table 2. Relative Abundances of Dominant Attachment Diatoms

	May			Aug			Oct		
	7-d	14-d	21-d	7-d	14-d	21-d	7-d	14-d	21-d
<i>Cylindrotheca closterium</i>	7.90	23.7	62.3	6.60	74.8	25.9	9.80	15.9	20.6
<i>Cyclotella</i> spp.				28.3	7.90	19.0			
<i>Guinardia</i> spp.	86.5	62.7	28.5		0.8		0.40	0.20	
<i>Licmophora</i> spp.							31.1	3.40	16.8
<i>Leptocylindrus danicus</i>	2.30	1.80	0.40						
<i>Navicula</i> spp.							41.7	60.7	46.0
<i>Nitzschia palea</i>	2.20	5.50	4.80	4.70	4.90	10.2	8.10	4.60	2.60
<i>Thalassionema</i>					3.40	12.2	3.00	0.50	0.30
<i>Prorocentrum</i> spp.				11.3				0.40	
Others	1.10	6.30	4.00	49.1	8.20	32.7	5.90	14.3	13.7
sum	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

(Table 2). The relative abundance of pennate diatoms was increased over time in spring. Compared with fouling amounts, the relative abundances of pennate diatom were positively related with number of attachment cells. Especially, *C. closterium* was most related to biofilm of lithium adsorbents in this area.

4. Discussion

It is known that the concentration and species distribution of microalgae causing biofouling on the inorganic surface depends on the seasonal effects (temperature, salinity etc.), the attachment ability of the microorganism and the properties of the substrates. Here, the characteristics of attachment microalgae to the LD surface are discussed through changes in the diatom community according to seasons and exposure periods.

From the above results, it is founded that dominant species which were initially adhered was changed by seasons. The centric diatoms *Guinardia* sp. and *Cyclotella* sp. were dominant, in early May and August, respectively unlike pennate diatoms which are known to be dominant adhering species. In particular, *Guinardia* spp. has an initial dominance rate of about 87%. This is due to the abundance of *Guinardia* spp. in seawater due to the plankton bloom resulting from the rises of temperature in spring. The *Guinardia* spp. has never been recorded as a fouling species in previous studies. In this study, *Guinardia*'s occupancy in spring lasted about 2 weeks in spring due to the continuous supply from seawater and LD provided a good habitat. Thus, biofilm composed diatom on lithium adsorbents were abundant with centric diatom in the initial stage owing to the abundance of ambient seawater in the Okgye Sea. This kind of small and centric type of diatoms has a large growth rate in spring [40]. This means that the abundance of microalgae in seawater is also an important factor affecting biofouling as well as the adherence ability of microalgae. Standing crops of phytoplankton in seawater are induced by environmental factors such as seasonal effects. Recent study has shown that the diatom fouling increased in the calm period (pre- and post-monsoon) due to seasonal effects of the Yellow Sea near the Korean peninsula [10]. These seasonal effects can induce changes in the species

composition of diatoms attached to artificial surfaces and it can result in serious fouling problems depending on species [35].

However, if the substrate is exposed for a longer period, it has been found that adhesion abilities of microalgae become more important. Pennate diatoms, as they are known constitute the major portion of the biofilm because they have raphe(s) that enables to attach and glide over the substratum and settle down on the surface of artificial structures through the physical processes [11]. In spite of the abundance of microalgae in seawater being lower in fall than other seasons, the proportion of pennate species increased in fall. At this time, the number of adherent cells increased sharply between 7 d and 14 d because of the high percentage of pennate diatoms in the early biofilm (Fig. 3). This was also the case in spring, where the increase in adhered microalgae was small between 7 d and 14 d, but increased rapidly between 14 d and 21 d, which is attributed to an increase in pennate diatom in the later period. In this study, *Navicula* sp. and *C. closterium* were found to be the most influential pennate diatoms on the LD surface. The *Navicula* sp, which is ubiquitous biofilm forming diatom. *Navicula* sp. has increased adhesion capability as they secrete the mucilaginous at two points and therefore, they can easily attach even to rough substrates [41]. In this study, *Navicula* spp. were not found as an adherent species in spring and summer, but it accounted for more than 40% over the entire period of fall. It is related to the abundance of *Navicula* sp. in seawater in the

Fig. 3. Changes in microalgae composition according to season and exposure period (classification by shape of diatom).

Table 3. Identified Diatom Species on Submerged Artificial Surfaces

Materials	Area	Species (or genera)	References
Glass, Acryl, Titanium, Copper, painted slide glass	Incheon, Yellow Sea, Korea	<i>Amphora coffeaeformis</i> <i>Synedra tabulate</i> , <i>Licomophora paradoxa</i> , <i>Melosira. nummuloides</i>	[12]
Glass (fibred, coupons)	Dona Paula Bay, India	<i>Navicula transitans</i> var. <i>derasa</i> f. <i>delicatula</i> <i>Amphora coffeaeformis</i> , <i>T. nitzschoides</i> , <i>Cylindrotheca. closterium</i> and <i>N. sigma</i>	[13]
Concrete plates 1m, 3m, 7m depth	Punta Madonna Northern Adriatic Sea	<i>Berkeleya</i> , <i>Navicula</i> , <i>Licomopora</i> , <i>Achnanthes</i> , <i>Nitzschia</i>	[14]
Stainless steel (SS), polystyrene (PS) ship hulls	Dona Paula Bay, India	<i>Navicula</i> , <i>Amphora</i>	[5]
- AF (copper SPC) ship - FR ship	Miami, FL, USA	<i>Achnanthes</i> , <i>Amphora</i> , <i>Navicula</i> , <i>Lampriscus</i> , <i>Thalassiosiphysa</i> (new diatom)	[15]
Lithium manganese oxide disk (LD)	Okgye, East Sea, Korea	<i>Cylindrotheca</i> , <i>Cyclotella</i> , <i>Guinardia</i> , <i>Licomophora</i> , <i>Navicula</i>	This study

fall. In spring and summer, the centric diatom was dominant in seawater and species composition did not vary, but after July, the apparent frequency of pennate diatom increased with the advent of various species. Hence, *Navicula* sp. can be regarded as a species that reflects seasonal influences during the study period. On the other hand, *C. closterium* and *Nitzschia plea* was attached regardless of season and exposure duration throughout the study periods. *Nitzschia plea* appeared on all LD surface, but the total occurrence was less than 10% of each sample. The longer the exposure time of LD to seawater, the higher the dominance rate of *C. closterium*, and accounted for up to 74% in August. This is related to biofilm thickness and the propagation after attachment of *C. closterium*. According to a study of Daume [42] and Kingston [43], the biofilm of *C. closterium*, which grew for 19 d, increased the settlement by 8 times compared to the biofilm of 3 d, which means that the old non-growth culture of the stationary phase of *C. closterium* could induce settlements rather than the exponentially growing culture. The specific growth rate was also higher than other diatom species [10]. Therefore, mature biofilm of approximately 20 d old has the possibility of including a considerable number of accumulated *C. Closterium* according to a limited habitat of the LD surface.

LD is a substance that can be directly exposed to the sea to recover lithium using ion exchange process. The artificial substrates such as LD exposed to the ocean can act as a major factor in the growth of benthic diatoms with physical and chemical factors that vary with environmental conditions, such as natural substrates such as sediment particles [41, 44, 45]. Several studies have been reported that the adhesion of diatom species on the artificial substrate in seawater (Table 3). Various species such as *A. coffeaeformis*, *S. tabulate*, *L. paradoxa*, and *M. nummuloides* were attached to glass, acrylic, titanium, copper, and anti-fouling (AF) coated slide glass [12]. The adhesion rate of microalgae was higher in glass than other materials. Patil and Anil [13] reported that the *N. transitans* var. *derasa* f. *delicatula*, *A. coffeaeformis*, *T. nitzschoides*, *C. closterium*, and *N. sigma* were the dominant fouler of exposure to two types of glass; fibred and coupons. There was no species specificity between two fibred and coupon types due to of the

same material made by glass. Exposure of the concrete plate from the Northern Adriatic Sea resulted in dominance of *Berkeley* sp., *Navicula* sp., and *Licomopora* sp. [14]. In Florida, US, species such as *Achnanthes* sp., *Amphora* sp., *Navicula* sp., *Lampriscus* sp., *Thalassiosiphysa* sp., and *Achnanthes* sp. were observed in the hull of an in-service ship [15]. Despite reports on the differences in species depending on the type of material [41] as well as geographic location [46]. *Amphora* sp. and *Navicula* sp. were commonly identified. However in this study, *Achnanthes* spp and *Amphora* spp. were rarely identified. *Amphora* spp. also has a characteristic similar to *Navicular* sp. due to its double-raphes structure, and thus appears as a chronic fouling species regardless of latitude and longitude. Exceptionally *Achnanthes* spp. and *Amphora* spp. were not identified as a major species in seawater at the East Sea. One suggestion is that these two species frequently appeared in the soft sedimentary layer in the West Sea and that *Amphora* spp. and *Nitzschia* spp. were reported to increase in the eutrophic environment. These two species with strong adhesion can be considered to inhabit primarily marine sediments, not seawater.

In addition, Attachment of microorganisms during the initial physical process of fouling preferentially induces attachment of benthic diatoms having morphology favorable for adhesion. At this time, recruit rate of microalgae was affected by surface characteristics such as effective size of adhesion or roughness [4, 23]. According to Sweat and Johnsond [47], the pennate diatom had a higher adhesion rate on the smood surface than on the coarse roughness surface. However, centric diatoms such as *L. danicus* and *Cosinodiscus* sp. showed a higher adhesion rate on the surface with fine roughness than the smood surface, which was also consistent with the results of this study. The LD used in this study is a pressed grain with 100 - 200 μ m diameters of LiMnO_4 and has a porous structure to increase the surface area (Fig. 1). Depending on the shape and size of the diatom, the characteristics attached to the LD surface were different. Fig. 4 shows the appearance of the various diatom attached to the LD surface. Smaller centric diatoms, such as *Cyclotella* sp, exist in embedded form between LD particles (Fig. 4 (a)). In case of diatoms with longer shapes

Fig. 4. Various attachment patterns of diatom attached to LD surface (a) Small microalgae in the stuck between granular adsorption materials, (b) attachment of *Licomophora* (c) *Cylindrotheca* attached to surface (d) Surface fouling appearance of LD exposed 21 d.

such as *Cylindrotheca* sp., *Licomophora* sp., and *Nitzschia* sp., they can be attached using long and flexible cell end even in the curvature surface between LD particles (Fig. 4 (b), (c)). The *Navicula* sp. like species rolled over LD surface and settled in a groove between the particles. The biofilm growth is rapidly accreted when the exposure time is prolonged, the slime builds up and covers pre-existing microbiome (Fig. 4 (d)). At this stage, it would be developed into macro-fouling by large algae such as Rhodophyta or Phaeophyceae, and there is a possibility of directly affecting the lithium recovery rate by ion exchange.

As discussed above, the major chronic species were similar, regardless of the low latitudes of Dona Paula [13] or Florida [48] and the high latitude of Northern Adriatic Sea [14]. However, when an AF (anti-fouling) substance such as a Cu-based substance is treated on the exposed surface, microalgae that can withstand the AF agent adhere and survive. Shim et al. [12] have found *H. virgate*, *L. abbreviata*, and *M. nummuloides* species resistant to toxicity of AF. Hunsucker et al. [15] found attachment microalgae, such as *Thalassiosira* species with AF-resistance by Cu-based AF and silica-based AF. The LD used in this study uses adhesives, when molding lithium manganese oxide powder, but has obtained research results that do not affect the marine ecosystem. In addition, through the seawater leaching test for the prepared LD, it was confirmed that only a small amount of lithium (Li^+) and manganese (Mn^{2+}) ions were eluted from LD. It had not eluted component that affect ecological functions such as growth and reproduction

of the microalgae. Therefore, the species observed on LD surfaces were more sensitive to seasonal changes than LD substances. Compared with the fouling characteristics of the materials used in other studies, the environmental changes due to seasonal reasons rather than specificity due to Li or Mn seemed to have a dominant effect on the microalgae.

It is also noticed a decrease in fouling in August. It can be considered that there was a typhoon before recovering the samples exposed for 21 d and this had an effect on the reduction in the amount of fouling. In other words, the possibility of removing contaminants caused by microalgae in the physical process was observed. In particular, a relative decrease in *C. closterium* was observed, which means that diatoms that are widely attached to the LD surface are also easy to remove by physical process such as waves in shallow seawater layer.

5. Conclusions

The microalgae attached to the surface of lithium manganese oxide disk in the Okgye area is affected by the microalgae species composition of the ambient seawater and by the adhesion properties of microalgae itself. In May, during the spring bloom, the existing phytoplankton in the ambient seawater affected the attached frequency and species. In particular, there was a new discovery that floating species such as *Guinardia* sp. presented as extreme domi-

nant species on the LD surface. In Okgye Sea, biofouling amounts of LD surfaces increased related with pennate (benthic) diatom such as *Cylindrotheca closterium* and *Navicula* sp. Over extended periods exposure to seawater, *Cylindrotheca closterium* were accrued in biofilm and exasperate the biofouling in all experimental seasons. In this study, a micro-succession occurred during the experimental period. The pioneer species can be regarded as the dominant species of the surrounding seawater such as *Guinardia* sp. and the later species were considered as *Cylindrotheca closterium* with a high adherence. In addition, the LD surface structure in the form of particles formed also plays an important role in attachment of the microalgae. According to the results of this study as well as previous studies, benthic diatom affects severe fouling as it is exposed for longer periods. However, if there are numerous floating diatoms in seawater, it is necessary to consider the effect on these species at the early stage of biofouling within 7 d. In addition, as the timing of the rapid increase of the attached species varies depending on the season, it is necessary to vary the exposure period for each season when LD is to be exposed to seawater. The results of this study can provide reliable data in determining the exposure season and duration in order to minimize fouling effects and improve the recovery of seawater mineral resources using lithium manganese oxide.

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Author Contributions

J-A.K. (Ph.D. researcher) conducted all experiments and writing paper. S.J. (Ph.D. senior researcher) performed laboratory experiments. K-S.C. (Ph.D. senior researcher) organized field setting and performed field experiments. H-O.Y. (Ph.D. senior researcher) organized overall experiments and writing paper.

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