Test Report

Project name	Purimar™
Study number	TW-Land(2)
Test number	LET-10-002
Title	Efficacy test of Purimar™ Ballast Water Management System (land scale)
Prepared by	Byong-Jun. Ko (Sign)

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Marine Bio-industry Development Center

Statement of Quality Assurance

Data were reviewed by Quality Assurance Unit of DAU to assure that the study was performed in accordance with protocol and standard operating procedures (SOP) of Marine Bio-industry Development Center (MBDC). The report was an accurate reflection of the raw data generated at the MBDC. Inspection of the routine and repeated procedures that constitute the study was carried out as a continuous major phase at or about the time this study was in progress.

Inspection Phase		Date		
		Inspection	Report to Study Director	Report to Project manager
Test plan		April, 09, 2010	April, 09, 2010	April, 09, 2010
Sampling	1st	April, 14, 2010	April, 14, 2010	April, 14, 2010
	2nd	April, 19, 2010	April, 19, 2010	April, 19, 2010
Test substance	1st	April, 14, 2010	April, 14, 2010	April, 14, 2010
	2nd	April, 19, 2010	April, 19, 2010	April, 19, 2010
Test procedures (Bacteria test)	1st	April, 14, 2010	April, 14, 2010	April, 14, 2010
	2nd	April, 19, 2010	April, 19, 2010	April, 19, 2010
Observation and counting (≥50㎛, 10㎛-50㎛)	1st	April, 14, 2010	April, 14, 2010	April, 14, 2010
	2nd	April, 19, 2010	April, 19, 2010	April, 19, 2010
Observation and counting (Bacteria test)	1st	April, 15, 2010	April, 15, 2010	April, 15, 2010
	2nd	April, 16, 2010	April, 16, 2010	April, 16, 2010
	3rd	April, 20, 2010	April, 20, 2010	April, 20, 2010
	4th	April, 21, 2010	April, 26, 2010	April, 26, 2010
Raw data	1st	April, 15, 2010	April, 15, 2010	April, 15, 2010
	2nd	April, 20, 2010	April, 20, 2010	April, 20, 2010
Test report		April, 28-30, 2010	May, 03, 2010	May, 03, 2010

Nam Kyu Lee (Sign)

Nam-Kyu, Lee / Quality Assurance

May, 07, 2010

Date

Study Personnel and Participants

The test participants recognized the study plan, manual, procedure, guide of Marine Bio-industry Development Center in performing the test.

	Name	Date
Study Personnel	Jae Young. Baek (sign)	May 07, 2010
	Jae Woo, Lee (sign)	May 07, 2010
	Sun Ju. Choi (sign)	May. 07, 2010
	Eun-Jung. Jung (sign)	May. 07, 2010
	Hyna. Song (sign)	May. 07. 2010
QA/QC	Eun-young, Kang (sign)	May, 07, 2010
Efficacy Testing Manager	Byong-Jin Ku (sign)	May. 07. 2010

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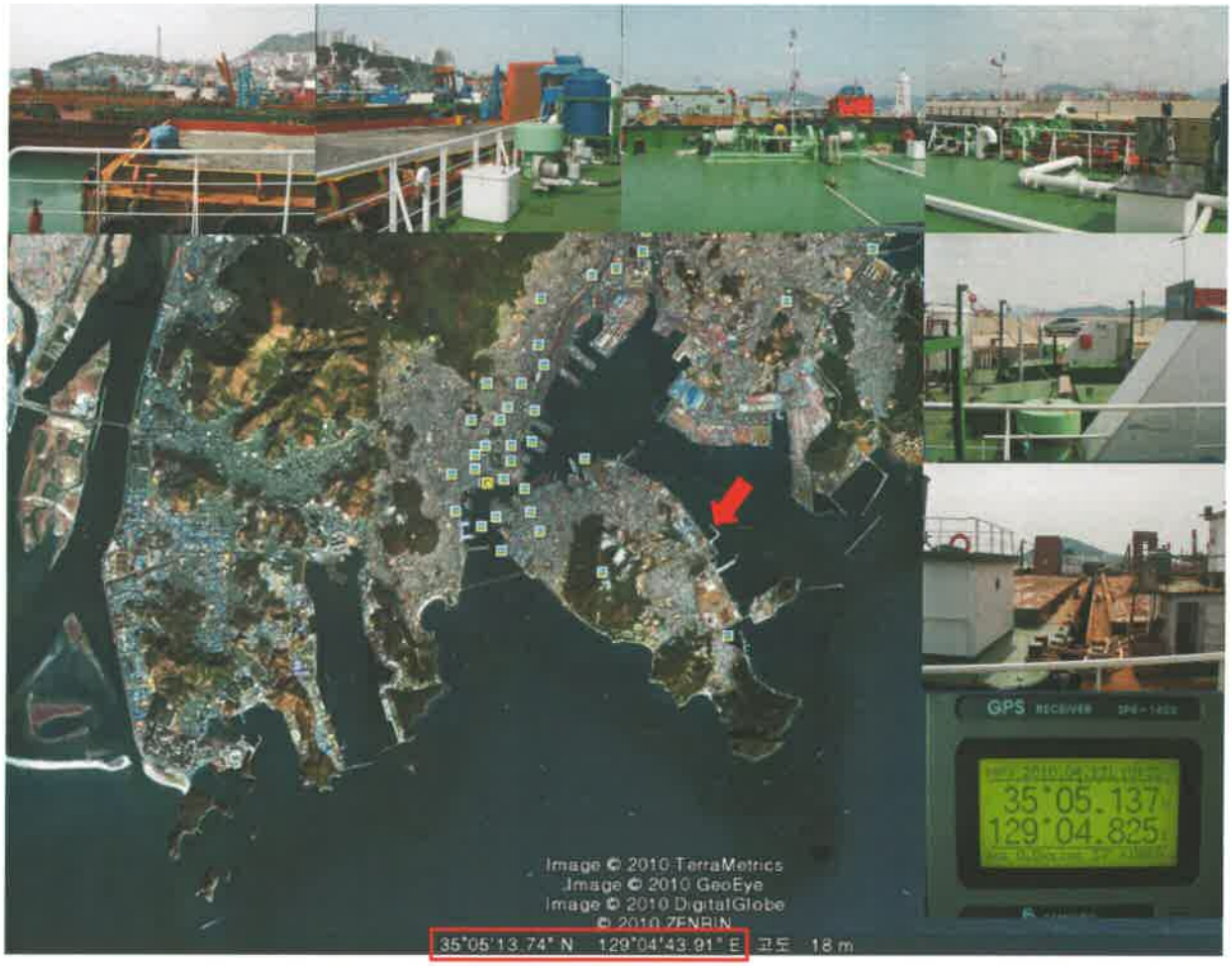
1. Results

1.1 Sampling point

GPS coordinate	35°05.137N 129°04.825E



1.2 Water parameters

Table 1. Results of DOC, POC, TSS

Date	Sample ID & lot No.	TOC [a)] (mg/L)	DOC [b)] (mg/L)	POC [c)] (mg/L)	TSS [d)] (mg/L)
Day 0	OwT-0414	4.61±0.38	3.37±0.23	1.24	5.3±1.2
	OwM-0414	4.67±0.47	3.64±0.18	1.03	11.3±2.1
	OwB-0414	5.12±0.19	3.09±0.31	2.03	14.7±2.1
Acceptability (Salinity : > 32 PSU)		-	Acceptable (> 1 mg/L)	Acceptable (> 1 mg/L)	Acceptable (> 1 mg/L)

Data were presented as mean±S.D of three repeated measurement.
a) TOC: Total Organic Carbon
b) DOC: Dissolved Organic Carbon
c) POC: Particulate Organic Carbon (POC=TOC-DOC)
d) TSS: Total Suspended Solids

Table 2. Water parameters at field

Date	Sample ID & lot No.	pH	Temp (°C)	Salinity (‰)	DO (mg/L)	Turbidity (NTU)	Chlorophyl a
Day 0	OwT-0414	8.06±0.00	12.60±0.00	33.95±0.00	9.09±0.01	5.5±0.0	0.667±0.001
	OwM-0414	8.07±0.00	12.60±0.00	33.96±0.00	9.20±0.01	4.6±0.0	0.684±0.002
	OwB-0414	8.05±0.00	12.59±0.00	33.96±0.00	9.21±0.01	4.9±0.1	0.700±0.001
	C0T-0414	8.07±0.00	12.76±0.01	32.86±0.00	8.91±0.01	3.4±0.1	0.711±0.000
	C0M-0414	8.06±0.01	12.90±0.00	33.79±0.04	8.69±0.01	3.3±0.0	0.696±0.002
	C0B-0414	8.06±0.00	13.19±0.01	33.90±0.01	8.83±0.02	3.5±0.1	0.708±0.001
	T0T-0414	8.07±0.01	13.24±0.00	33.94±0.00	8.82±0.01	3.5±0.1	0.005±0.000
	T0M-0414	8.07±0.01	13.17±0.00	33.98±0.01	8.78±0.01	3.2±0.1	0.003±0.003
	T0B-0414	8.07±0.01	13.36±0.01	33.92±0.00	8.80±0.00	3.9±0.0	0.007±0.002
Day 5	C5T-0419	7.82±0.01	13.47±0.01	33.93±0.00	8.19±0.01	0.9±0.0	0.480±0.001
	C5M-0419	7.83±0.01	13.47±0.01	33.95±0.01	8.23±0.01	0.3±0.0	0.482±0.001
	C5B-0419	7.84±0.01	13.45±0.01	33.99±0.01	8.25±0.01	0.2±0.0	0.470±0.001
	TnT-0419	8.01±0.01	13.42±0.08	33.85±0.00	8.59±0.01	1.8±0.0	0.000±0.000
	TnM-0419	8.00±0.00	13.42±0.01	33.92±0.00	8.57±0.00	0.7±0.0	0.000±0.000
	TnB-0419	8.02±0.00	13.45±0.00	34.01±0.01	8.63±0.01	1.3±0.1	0.000±0.000

Data were presented as mean±S.D of three repeated measurement.

Table 3. Water parameters at MBDC lab

Date	Sample ID & lot No.	pH	Temp (°C)	Salinity (‰)	DO (mg/L)	Turbidity (NTU)
Day 0	OwT-0414	8.08±0.01	14.9±0.1	33.2±0.1	10.35±0.01	4.32±0.02
	OwM-0414	8.02±0.00	14.6±0.1	33.5±0.1	10.44±0.02	3.37±0.04
	OwB-0414	8.06±0.00	14.7±0.0	34.5±0.0	10.16±0.00	4.31±0.07
	C0T-0414	7.99±0.01	15.9±0.0	34.2±0.2	9.98±0.01	4.39±0.02
	C0M-0414	8.03±0.00	15.6±0.1	34.4±0.0	9.86±0.01	4.33±0.09
	C0B-0414	8.09±0.00	15.3±0.1	34.4±0.0	9.95±0.01	3.94±0.07
	T0T-0414	7.79±0.01	13.0±0.0	33.0±0.1	10.11±0.35	3.10±0.08
	T0M-0414	8.04±0.01	12.5±0.1	32.9±0.1	10.39±0.01	2.74±0.03
	T0B-0414	8.10±0.00	12.9±0.0	33.5±0.0	10.29±0.02	2.90±0.04
Day 5	C5T-0419	7.48±0.01	11.6±0.2	33.1±0.1	10.29±0.31	0.84±0.02
	C5M-0419	7.65±0.02	9.7±0.1	33.5±0.0	10.58±0.06	0.83±0.04
	C5B-0419	7.77±0.01	10.0±0.1	33.5±0.0	10.52±0.08	0.92±0.04
	TnT-0419	7.98±0.01	8.4±0.1	33.5±0.0	10.71±0.18	1.90±0.08
	TnM-0419	8.12±0.01	8.1±0.1	33.5±0.0	10.57±0.07	1.66±0.08
	TnB-0419	8.14±0.01	11.2±0.1	33.8±0.1	10.66±0.19	1.54±0.01

Data were presented as mean±S.D of three repeated measurement.

1.3 Influent water : Original water

1) ≥ 50 μm organism

Table 4. Survival number of the organism larger than 50 μm

Sampling	Species	Survival number/m^3	Minimum Dimension (μm)
Top	*Artemia salina*	84750 ± 7529	150 ~ 200
	Acartia sp.	333 ± 144	100 ~ 150
	Calanus sp.	83 ± 144	160
	Paracalanus sp.	167 ± 144	90 ~ 110
	Crustacean naupli	167 ± 144	150
	Brachionus plicatilis	408333 ± 15275	70 ~ 150
	Ditylum sp.	466667 ± 20817	60 ~ 80
	Coscinodiscus sp.	10000 ± 5000	65 ~ 110
	Odontella sp.	3333 ± 2887	108
Middle	*Artemia salina*	100750 ± 4828	150 ~ 200
	Acartia sp.	167 ± 144	100 ~ 150
	Calanus sp.	250 ± 250	160
	Paracalanus sp.	167 ± 144	90
	Crustacean naupli	250 ± 0	150
	Brachionus plicatilis	485000 ± 37749	70 ~ 150
	Ditylum sp.	525000 ± 35000	60 ~ 80
	Coscinodiscus sp.	23333 ± 2887	65 ~ 210
	Odontella sp.	3333 ± 2887	110
	Pleurosigma sp.	3333 ± 2887	59
Bottom	*Artemia salina*	93750 ± 2462	150 ~ 200
	Acartia sp.	417 ± 144	100 ~ 150
	Calanus sp.	167 ± 144	160
	Paracalanus sp.	83 ± 144	90
	Crustacean naupli	83 ± 144	150
	Brachionus plicatilis	488333 ± 37528	70 ~ 150
	Ditylum sp.	425000 ± 15000	60 ~ 80
	Coscinodiscus sp.	21667 ± 7638	65 ~ 110
	Odontella sp.	5000 ± 5000	110 ~ 130
	Pleurosigma sp.	1667 ± 2887	59
Total	10 species 3 phyla/divisions	1050528 ± 84792	-
Acceptability	Influent condition: Acceptable (≥ 5 species from 3 different phyla/divisions)	Influent condition: Acceptable (≥10^5 ind./ m^3)	-

Data were presented as mean±S.D of three repeated measurement.

2) 10 μm - 50 μm organism

Table 5. Survival number of the organism between 10 to 50 μm

Sampling	Species	Survival number/mL (Mean)	Minimum Dimension (μm)
Top	*Tetraselmis suecica*	1066.67 ± 51.32	11 ~ 12
	Protoperidinium sp.	1.67 ± 1.15	30 ~ 45
	Thalassiosira sp.	0.67 ± 0.58	20 ~ 40
Middle	*Tetraselmis suecica*	1240.00 ± 82.85	11 ~ 12
	Chaetoceros sp.	2.33 ± 1.15	11 ~ 18
	Thalassiosira sp.	2.33 ± 1.53	20 ~ 40
	Melosira sp.	1.33 ± 0.58	10 ~ 13
Bottom	*Tetraselmis suecica*	1136.67 ± 64.29	11 ~ 12
	Thalassiosira sp.	2.00 ± 1.00	20 ~ 40
	Protoperidinium sp.	1.00 ± 1.00	30 ~ 45
	Melosira sp.	1.33 ± 0.58	10 ~ 13
Total	5 species 3 phyla/divisions	1152.00 ± 89.01	-
Acceptability	Influent condition: Acceptable (≥ 5 species from 3 different phyla/divisions)	Influent condition: Acceptable (≥ 10^3 ind./mL)	-

Data were presented as mean±S.D of three repeated measurement.

1.4 Elimination efficacy

1) ≥ 50 μm organism

Table 6. Survival number of the organism larger than 50 μm

Test substance	Sampling		Survival number/m^3	Acceptibility	Elimination efficacy (%)
Original water	Day 0 (Ow)	T [a)]	973833 ± 20738	Influent condition: Acceptable ($\geq 10^5$ ind./m^3)	-
		M [b)]	1141583 ± 64933		
		B [c)]	1036167 ± 17766		
Control (untreated) seawater	Day 0 (C0)	T	1022167 ± 29267	-	-
		M	926250 ± 23813		
		B	898167 ± 22329		
	Day 5 (C5)	T	37000 ± 7612	Effluent condition: Acceptable ($\geq 10^2$ ind./m^3)	
		M	82250 ± 11476		
		B	56667 ± 11547		
Treated ballast seawater	Day 0 (T0)	T	0.0 ± 0.0	Effluent condition : Acceptable (<10 ind./m^3)	100.0 [d)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
Neutralized water	Day 5 (Tn)	T	0.0 ± 0.0	Effluent condition : Acceptable (<10 ind./m^3)	100.0 [e)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		

a) T: Top, b) M: Middle, c) B: Bottom, d) Calculation = (C0-T0) /C0*100, e) Calculation = (C0-Tn) /C0*100

Data were presented as mean±S.D of three repeated measurement.

2) 10 µm - 50 µm organism

Table 7. Survival number of the organism between 10 and 50 µm

Test substance	Sampling		Survival number/mL	Acceptibility	Elimination efficacy (%)
Original water	Day 0 (Ow)	T	1069.00 ± 52.14	Influent condition: Acceptable ($\geq 10^3$ ind./mL)	-
		M	1246.00 ± 80.21		
		B	1141.00 ± 64.09		
Control (untreated) seawater	Day 0 (C0)	T [a)]	1243.33 ± 65.12	-	-
		M[b)]	1348.33 ± 61.44		
		B[c)]	1218.67 ± 84.36		
	Day 5 (C5)	T	291.33 ± 30.50	Effluent condition: Acceptable ($\geq 10^2$ ind./ ml)	
		M	352.00 ± 59.92		
		B	308.00 ± 35.17		
Treated ballast seawater	Day 0 (T0)	T	0.00 ± 0.00	Effluent condition : Acceptable (<10 ind./ ml)	100.0 [d)]
		M	0.00 ± 0.00		
		B	0.00 ± 0.00		
Neutralized water	Day 5 (Tn)	T	0.00 ± 0.00	Effluent condition : Acceptable (<10 ind./ ml)	100.0 [e)]
		M	0.00 ± 0.00		
		B	0.00 ± 0.00		

a) T: Top, b) M: Middle, c) B: Bottom, d) Calculation = (C0-T0) /C0*100, e) Calculation = (C0-Tn) /C0*100

Data were presented as mean±S.D of three repeated measurement.

3) Heterotrophic bacteria

Table 8. Survival number of heterotrophic bacteria

Test substance	Sampling		CFU / mL	Acceptibility	Elimination efficacy (%)
Original water	Day 0 (Ow)	T	26600 ± 10566	Influent condition: Acceptable ($\geq 10^4$ CFU/mL)	-
		M	34000 ± 2778		
		B	28633± 4398		
Control (untreated) seawater	Day 0 (C0)	T [a)]	9567 ± 5454	-	-
		M [b)]	21033 ± 3029		
		B [c)]	16333 ± 2122		
	Day 5 (C5)	T	11367 ± 1450	-	
		M	14200 ± 2707		
		B	16267 ± 1365		
Treated ballast seawater	Day 0 (T0)	T	0.0 ± 0.0	-	100.0 [d)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
Neutralized water	Day 5 (Tn)	T	413 ± 85.0	-	97.2 [e)]
		M	477 ± 83.3		
		B	407 ± 55.1		

a) T: Top, b) M: Middle, c) B: Bottom, d) Calculation = (C0-T0) /C0*100, e) Calculation = (C0-Tn) /C0*100

Data were presented as mean±S.D of three repeated measurement.

4) *Escherichia coli*

Table 9. Survival number of *Escherichia coli*

Test substance	Sampling		CFU / 100 mL	Acceptibility	Elimination efficacy (%)
Original water	Day 0 (Ow)	T	5.7 ± 3.2	-	-
		M	5.3 ± 3.8		
		B	8.3 ± 0.6		
Control (untreated) seawater	Day 0 (C0)	T [a)]	8.7 ± 3.1	-	-
		M [b)]	7.3 ± 4.6		
		B [c)]	5.7 ± 0.6		
	Day 5 (C5)	T	0.0 ± 0.0	-	
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
Treated ballast seawater	Day 0 (T0)	T	0.0 ± 0.0	Effluent condition: Acceptable (<250 CFU/ 100ml)	100.0 [d)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
Neutralized water	Day 5 (Tn)	T	0.0 ± 0.0	Effluent condition: Acceptable (<250 CFU/ 100ml)	100.0 [e)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		

a) T: Top, b) M: Middle, c) B: Bottom, d) Calculation = (C0-T0) /C0*100, e) Calculation = (C0-Tn) /C0*100

Data were presented as mean±S.D of three repeated measurement.

5) *Enterococcus faecalis*

Table 10. Survival number of *Enterococcus faecalis*

Test substance	Sampling		CFU / 100 mL	Acceptability	Elimination efficacy (%)
Original water	Day 0 (Ow)	T	20.3 ± 5.5	-	-
		M	16.3 ± 7.5		
		B	24.7 ± 3.1		
Control (untreated) seawater	Day 0 (C0)	T [a)]	22.0 ± 6.2	-	-
		M [b)]	24.0 ± 11.1		
		B [c)]	15.3 ± 3.5		
	Day 5 (C5)	T	0.0 ± 0.0	-	
		M	0.3 ± 0.6		
		B	0.0 ± 0.0		
Treated ballast seawater	Day 0 (T0)	T	0.0 ± 0.0	Effluent condition: Acceptable (<100 CFU/ 100ml)	100.0 [d)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
Neutralized water	Day 5 (Tn)	T	0.0 ± 0.0	Effluent condition: Acceptable (<100 CFU/ 100ml)	100.0 [e)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		

a) T: Top, b) M: Middle, c) B: Bottom, d) Calculation = (C0-T0) /C0*100, e) Calculation = (C0-Tn) /C0*100

Data were presented as mean±S.D of three repeated measurement.

6) *Vibrio cholera* O1, O139

Table 11. Survival number of *Vibrio cholera* O1, O139

Test substance	Sampling		CFU / 100 mL	Acceptability	Elimination efficacy (%)
Original water	Day 0 (Ow)	T	0.0 ± 0.0	-	-
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
Control (untreated) seawater	Day 0 (C0)	T [a)]	0.0 ± 0.0	-	-
		M [b)]	0.0 ± 0.0		
		B [c)]	0.0 ± 0.0		
	Day 5 (C5)	T	0.0 ± 0.0	-	
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
Treated ballast seawater	Day 0 (T0)	T	0.0 ± 0.0	Effluent condition: Acceptable (<1 CFU/ 100ml)	N.D[d)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
Neutralized water	Day 5 (Tn)	T	0.0 ± 0.0	Effluent condition: Acceptable (<1 CFU/ 100ml)	N.D[d)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		

a) T: Top, b) M: Middle c) B: Bottom, d) N.D: Not detected

Data were presented as mean±S.D of three repeated measurement.

2. Validity

2.1 Water parameters measurement

- Influent condition in DOC, POC, TSS was acceptable for IMO standards.
  - Salinity: > 32 PSU
  - DOC: > 1 mg/L
  - POC: > 1 mg/L
  - TSS: > 1 mg/L

Date	Sample ID & lot No.	Salinity [a)] (‰)	DOC (mg/L)	POC (mg/L)	TSS (mg/L)
Day 0	OwT-0414	33.95±0.00	3.31±0.25	1.47	5.3±1.2
	OwM-0414	33.96±0.00	3.62±0.12	1.35	11.3±2.1
	OwB-0414	33.96±0.00	3.18±0.18	1.52	14.7±2.1

a) Salinity: data at field

- All measurements were tested more than three times.
- Sample was analyzed as soon as possible after arrival at MBDC lab.

2.2 Biological efficacy test

- Influent condition in all tests was acceptable for IMO standards (MEPC58/2/4).
  - The organism larger than 50 μm: $\geq 10^5$ individuals/m^3
  - The organism between 10 μm and 50 μm: $\geq 10^3$ individuals / mL
  - Heterotrophic bacteria: $\geq 10^4$ CFU/ mL

Sample		≥ 50 μm (individuals/m^3)	10 μm - 50 μm (individuals/mL)	Heterotrophic bacteria (CFU/mL)
Field	OwT	159420	1704	-
Lab	OwT	973833 ± 20738	1069.00 ± 52.14	26600 ± 10566
	OwM	1141583 ± 64933	1246.00 ± 80.21	34000 ± 2778
	OwB	1036167 ± 17766	1141.00 ± 64.09	28633± 4398

- The organism larger than 50 μm: ≥ 5 species from 3 different phyla/divisions
- The organism between 10 μm and 50 μm: ≥ 5 species from 3 different phyla/divisions

Sample	≥ 50 μm	10 μm - 50 μm
Ow	10 species 3 phyla/divisions	5 species 3 phyla/divisions

• All samples were tested within 6 hours after sampling

Date	Sample	Sampling time	Test ending time
Day 0	OwT	11:08~11:11	17:00
	OwM	11:04~11:07	17:00
	OwB	10:58~11:02	17:00
	C0T	11:22~11:27	17:00
	C0M	11:31~11:36	17:00
	C0B	11:40~11:44	17:00
	T0T	14:31~15:07	18:00
	T0M	13:33~15:06	18:00
	T0B	13:29~15:05	18:00
Day 5	C5T	13:48~14:01	17:30
	C5M	13:47~13:59	17:30
	C5B	13:46~13:58	17:30
	TnT	14:37~14:52	18:00
	TnM	13:42~14:43	18:00
	TnB	13:45~14:42	18:00
Acceptability		Acceptable : analyzed live within 6 hours	

• Discharge results from the control water were a concentration more than 10 times the values in regulation D2.1.

- The organism larger than 50 μm: ≥ 100 individuals/m^3
- The organism between 10 μm and 50 μm: ≥ 100 individuals/mL

Sample	≥ 50 µm organism	10 µm - 50 µm organism
Top	7400 ± 1522	1243.33 ± 65.12
Middle	16450 ± 2295	1348.33 ± 61.44
Bottom	11333 ± 2309	1218.67 ± 84.36

3. Conclusion

3.1 Biological efficacy in treated ballast seawater by Purimar™ BWMS

- Elimination efficacy of organism larger than 50 μm in treated ballast seawater and neutralized water by Purimar™ BWMS was 100%.
- Elimination efficacy of organism between 10 and 50 μm in treated ballast seawater and neutralized water by Purimar™ BWMS was 100%.
- Elimination efficacy of heterotrophic bacteria in treated ballast seawater by Purimar™ BWMS was 100%
- Elimination efficacy of heterotrophic bacteria in neutralized water by Purimar™ BWMS was 97.2%. Therefore, elimination efficacy of heterotrophic bacteria after neutralization was reduced.
- Elimination efficacy of *Escherichia coli* in treated ballast seawater and neutralized water by Purimar™ BWMS was 100%.
- Elimination efficacy of *Enterococcus faecalis* in treated ballast seawater and neutralized water by Purimar™ BWMS was 100%.
- *Vibrio cholera* O1, O139 were not detected.
- Therefore, zooplankton, phytoplankton, bacteria which exist in seawater was removed in treated ballast seawater by Purimar™ BWMS.

4. Appendix

Appendix 1. Water parameters (TOC, DOC, TSS) of original water (Ow)

Date	Sample ID & lot No.	R [a)]	TOC [b)] (mg/L)	DOC [c)] (mg/L)	TSS [d)] (mg/L)
Day 0	OwT-0414	R1	4.92	3.11	4.0
		R2	4.73	3.45	6.0
		R3	4.19	3.55	6.0
	OwM-0414	R1	4.46	3.43	12.0
		R2	5.21	3.76	9.0
		R3	4.34	3.73	13.0
	OwB-0414	R1	5.00	2.75	14.0
		R2	5.34	3.18	13.0
		R3	5.01	3.34	17.0

a) R: Replicate
b) TOC: Tptal Organic Carbon (TOC=DOC+POC)
c) DOC: Dissolved Organic Carbon
d) TSS: Total Suspended Solids

Appendix 2. Water parameters at field

Date	Sample ID & lot No.	R [a)]	pH	Temp (°C)	Salinity (‰)	DO (mg/L)	Turbidity (NTU)	Chlorophyla
Day 0	OwT-0414	R1	8.06	12.60	33.95	9.09	5.5	0.667
		R2	8.06	12.60	33.95	9.10	5.5	0.666
		R3	8.06	12.60	33.95	9.09	5.5	0.668
	OwM-0414	R1	8.07	12.60	33.96	9.20	4.6	0.682
		R2	8.07	12.60	33.96	9.19	4.6	0.684
		R3	8.07	12.60	33.96	9.21	4.6	0.685
	OwB-0414	R1	8.05	12.59	33.96	9.20	4.8	0.701
		R2	8.05	12.59	33.96	9.22	4.9	0.699
		R3	8.05	12.59	33.96	9.22	4.9	0.699
	C0T-0414	R1	8.07	12.75	32.86	8.92	3.4	0.711
		R2	8.07	12.76	32.86	8.91	3.4	0.711
		R3	8.07	12.77	32.86	8.91	3.3	0.711
	C0M-0414	R1	8.05	12.90	33.82	8.68	3.3	0.697
		R2	8.06	12.90	33.81	8.69	3.3	0.694
		R3	8.06	12.90	33.75	8.70	3.3	0.697
	C0B-0414	R1	8.06	13.19	33.89	8.81	3.5	0.709
		R2	8.06	13.19	33.90	8.84	3.5	0.708
		R3	8.06	13.18	33.91	8.85	3.6	0.708
	T0T-0414	R1	8.07	13.24	33.94	8.82	3.4	0.005
		R2	8.07	13.24	33.94	8.81	3.5	0.005
		R3	8.06	13.24	33.94	8.82	3.6	0.005
	T0M-0414	R1	8.08	13.17	33.99	8.78	3.2	0.000
		R2	8.07	13.17	33.98	8.78	3.2	0.005
		R3	8.07	13.17	33.98	8.79	3.3	0.003
	T0B-0414	R1	8.08	13.36	33.92	8.80	3.9	0.005
		R2	8.07	13.36	33.92	8.80	3.9	0.008
		R3	8.06	13.37	33.92	8.80	3.9	0.008
Day 5	C5T-0419	R1	7.83	13.46	33.93	8.20	0.9	0.479
		R2	7.82	13.48	33.93	8.19	0.9	0.480
		R3	7.82	13.48	33.93	8.19	0.9	0.480
	C5M-0419	R1	7.84	13.47	33.96	8.23	0.3	0.483
		R2	7.83	13.47	33.95	8.23	0.3	0.481
		R3	7.83	13.48	33.95	8.22	0.3	0.481
	C5B-0419	R1	7.85	13.44	33.99	8.26	0.2	0.469
		R2	7.84	13.45	33.99	8.25	0.2	0.471
		R3	7.84	13.45	33.98	8.24	0.2	0.471
	TnT-0419	R1	8.02	13.47	33.85	8.60	1.8	0.000
		R2	8.01	13.45	33.85	8.59	1.8	0.000
		R3	8.01	13.33	33.85	8.59	1.8	0.000
	TnM-0419	R1	8.00	13.41	33.92	8.57	0.7	0.000
		R2	8.00	13.42	33.92	8.57	0.7	0.000
		R3	8.00	13.42	33.92	8.57	0.7	0.000
	TnB-0419	R1	8.02	13.45	34.00	8.64	1.3	0.000
		R2	8.02	13.45	34.01	8.63	1.4	0.000
		R3	8.02	13.45	34.01	8.63	1.3	0.000

a) R: Replicate

Appendix 3. Water parameters at MBDC lab.

Date	Sample ID & lot No.	R [a)]	pH	Temp (°C)	Salinity (‰)	DO (mg/L)	Turbidity (NTU)
Day 0	OwT-0414	R1	8.08	14.8	33.2	10.36	4.34
		R2	8.08	15.0	33.1	10.35	4.30
		R3	8.09	14.9	33.2	10.34	4.32
	OwM-0414	R1	8.02	14.6	33.5	10.42	3.40
		R2	8.02	14.6	33.5	10.44	3.38
		R3	8.02	14.5	33.6	10.46	3.32
	OwB-0414	R1	8.06	14.7	34.5	10.16	4.26
		R2	8.06	14.7	34.5	10.16	4.39
		R3	8.06	14.7	34.5	10.16	4.28
	C0T-0414	R1	7.99	15.9	34.0	9.99	4.38
		R2	7.98	15.9	34.3	9.98	4.41
		R3	7.99	15.9	34.3	9.98	4.37
	C0M-0414	R1	8.03	15.7	34.4	9.85	4.43
		R2	8.03	15.6	34.4	9.86	4.25
		R3	8.03	15.6	34.4	9.87	4.31
	C0B-0414	R1	8.09	15.3	34.4	9.95	3.97
		R2	8.09	15.3	34.4	9.96	3.86
		R3	8.09	15.4	34.4	9.95	3.99
	T0T-0414	R1	7.79	13.0	33.0	9.71	3.19
		R2	7.79	13.0	33.0	10.31	3.05
		R3	7.80	13.0	33.1	10.31	3.07
	T0M-0414	R1	8.04	12.4	32.9	10.38	2.73
		R2	8.04	12.6	32.9	10.40	2.72
		R3	8.05	12.6	33.0	10.39	2.77
	T0B-0414	R1	8.10	12.9	33.5	10.31	2.94
		R2	8.10	12.9	33.5	10.28	2.90
		R3	8.10	12.9	33.5	10.27	2.87
Day 5	C5T-0419	R1	7.47	11.4	33.0	9.93	0.86
		R2	7.48	11.7	33.1	10.48	0.82
		R3	7.48	11.8	33.1	10.47	0.83
	C5M-0419	R1	7.64	9.6	33.5	10.52	0.88
		R2	7.64	9.6	33.5	10.59	0.81
		R3	7.67	9.8	33.5	10.64	0.81
	C5B-0419	R1	7.76	9.9	33.5	10.61	0.88
		R2	7.77	10.0	33.5	10.50	0.92
		R3	7.77	10.1	33.5	10.45	0.95
	TnT-0419	R1	7.97	8.4	33.5	10.50	1.98
		R2	7.98	8.4	33.5	10.81	1.82
		R3	7.98	8.5	33.5	10.81	1.89
	TnM-0419	R1	8.11	8.0	33.5	10.64	1.74
		R2	8.12	8.1	33.5	10.55	1.58
		R3	8.13	8.2	33.5	10.51	1.66
	TnB-0419	R1	8.14	11.1	33.8	10.87	1.55
		R2	8.14	11.2	33.8	10.60	1.54
		R3	8.15	11.3	33.7	10.51	1.54

a) R: Replicate

Appendix 4. Survival number of ≥ 50㎛ organism in original water(Influent water)

Date	Sample ID	Classification	Survival number /m³			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	OwT-0414	*Artemia salina*	91250	86500	76500	84750	7529
		Acartia sp.	500	250	250	333	144
		Calanus sp.	250	0	0	83	144
		Paracalanus sp.	250	250	0	167	144
		Crustacean nauplii	250	0	250	167	144
		Brachionus plicatilis	395000	405000	425000	408333	15275
		Ditylum sp.	460000	490000	450000	466667	20817
		Coscinodiscus sp.	15000	10000	5000	10000	5000
		Odontella sp.	5000	5000	0	3333	2887
	OwM-0414	*Artemia salina*	106000	99750	96500	100750	4828
		Acartia sp.	250	250	0	167	144
		Calanus sp.	500	0	250	250	250
		Paracalanus sp.	250	0	250	167	144
		Crustacean nauplii	250	250	250	250	0
		Brachionus plicatilis	525000	480000	450000	485000	37749
		Ditylum sp.	525000	560000	490000	525000	35000
		Coscinodiscus sp.	25000	25000	20000	23333	2887
		Odontella sp.	0	5000	5000	3333	2887
		Pleurosigma sp.	5000	0	5000	3333	2887
	OwB-0414	*Artemia salina*	91750	96500	93000	93750	2462
		Acartia sp.	500	500	250	417	144
		Calanus sp.	0	250	250	167	144
		Paracalanus sp.	250	0	0	83	144
		Crustacean nauplii	0	0	250	83	144
		Brachionus plicatilis	525000	450000	490000	488333	37528
		Ditylum sp.	410000	425000	440000	425000	15000
		Coscinodiscus sp.	20000	30000	15000	21667	7638
		Odontella sp.	5000	10000	0	5000	5000
		Pleurosigma sp.	0	5000	0	1667	2887

a) R: Replicate
b) S.D: Standard deviation

Appendix 5. Survival number of ≥ 50㎛ organism in control (untreated) seawater

Date	Sample ID	Classification	Survival number /m³			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	C0T - 0414	*Artemia salina*	13000	12000	10750	11917	1127
		Acartia sp.	250	0	0	83	144
		Ditylum sp.	250	0	250	167	144
		Coscinodiscus sp.	535000	560000	515000	536667	22546
		Odontella sp.	440000	460000	475000	458333	17559
		Pleurosigma sp.	0	15000	5000	6667	7638
	C0M- 0414	*Artemia salina*	5000	5000	10000	6667	2887
		Acartia sp.	0	0	5000	1667	2887
		Brachionus plicatilis	11750	11500	10250	11167	804
		Ditylum sp.	250	0	0	83	144
		Coscinodiscus sp.	475000	445000	465000	461667	15275
	C0B- 0414	*Artemia salina*	460000	470000	420000	450000	26458
		Podon polyphemoides	0	5000	5000	3333	2887
		Brachionus plicatilis	6750	6500	5750	6333	520
		Ditylum sp.	250	0	250	167	144
		Coscinodiscus sp.	455000	470000	450000	458333	10408
		Odontella sp.	405000	445000	435000	428333	20817
Day 5	C5T - 0419	*Artemia salina*	250	250	500	333	144
		Brachionus plicatilis	10000	15000	25000	16667	7638
		Ditylum sp.	20000	15000	10000	15000	5000
		Coscinodiscus sp.	0	15000	0	5000	8660
	C5M- 0419	*Artemia salina*	500	500	500	500	0
		Crustacean nauplii	250	0	0	83	144
		Brachionus plicatilis	25000	25000	25000	25000	0
		Ditylum sp.	45000	50000	55000	50000	5000
		Coscinodiscus sp.	5000	0	15000	6667	7638
	C5B- 0419	*Brachionus plicatilis*	10000	10000	5000	8333	2887
		Ditylum sp.	35000	40000	50000	41667	7638
		Coscinodiscus sp.	5000	0	15000	6667	7638

a) R: Replicate
b) S.D: Standard deviation

Appendix 6. Survival number of ≥ 50㎛ organism in treated ballast seawater and neutralized water by treatment of Purimar™ BWMS

Date	Sample ID	Classification	Survival number /m³			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	T0T - 0414	-	0	0	0	0.0	0.0
	T0M- 0414	-	0	0	0	0.0	0.0
	T0B- 0414	-	0	0	0	0.0	0.0
Day 5	TnT - 0419	-	0	0	0	0.0	0.0
	TnM- 0419	-	0	0	0	0.0	0.0
	TnB- 0419	-	0	0	0	0.0	0.0

a) R: Replicate
b) S.D: Standard deviation

Appendix 7. Survival number of 10㎛ - 50㎛ organism in original water(Influent water)

Date	Sample ID	Classification	Survival number / ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	OwT-0414	*Tetraselmis suecica*	1080	1010	1110	1066.67	51.32
		Protoperidinium sp.	3	1	1	1.67	1.15
		Thalassiosira sp.	1	0	1	0.67	0.58
	OwM-0414	*Tetraselmis suecica*	1260	1150	1310	1240.00	81.85
		Chaetoceros sp.	1	3	3	2.33	1.15
		Thalassiosira sp.	2	4	1	2.33	1.53
		Melosira sp.	2	1	1	1.33	0.58
	OwB-0414	*Tetraselmis suecica*	1090	1110	1210	1136.67	64.29
		Thalassiosira sp.	2	3	1	2.00	1.00
		Protoperidinium sp.	1	0	2	1.00	1.00
		Melosira sp.	1	2	1	1.33	0.58

a) R: Replicate
b) S.D: Standard deviation

Appendix 8. Survival number of 10㎛ - 50㎛ organism in control (untreated) seawater

Date	Sample ID	Classification	Survival number / ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	C0T - 0414	*Tetraselmis suecica*	1230	1310	1180	1240.00	65.57
		Diploneis sp.	1	0	3	1.33	1.53
		Thalassiosira sp.	2	3	1	2.00	1.00
	C0M-0414	*Tetraselmis suecica*	1290	1330	1410	1343.33	61.10
		Diploneis sp.	2	4	2	2.67	1.15
		Protoperidinium sp.	1	0	2	1.00	1.00
		Melosira sp.	1	2	1	1.33	0.58
	C0B-0414	*Tetraselmis suecica*	1180	1150	1310	1213.33	85.05
		Thalassiosira sp.	1	3	1	1.67	1.15
		Protoperidinium sp.	2	5	4	3.67	1.53
Day 5	C5T-0419	*Tetraselmis suecica*	290	320	260	290.00	30.00
		Thalassiosira sp.	1	2	1	1.33	0.58
	C5M-0419	*Tetraselmis suecica*	380	390	280	350.00	60.83
		Thalassiosira sp.	1	0	1	0.67	0.58
		Protoperidinium sp.	1	0	0	0.33	0.58
		Melosira sp.	0	1	2	1.00	1.00
	C5B-0419	*Tetraselmis suecica*	310	340	270	306.67	35.12
		Melosira sp.	1	0	1	0.67	0.58
		Protoperidinium sp.	1	1	0	0.67	0.58

a) R: Replicate
b) S.D: Standard deviation

Appendix 9. Survival number of 10㎛ - 50㎛ organism in treated ballast seawater and neutralized water by treatment of Purimar™ BWMS

Date	Sample ID	Classification	Survival number / ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	T0T-0414	-	0	0	0	-	-
	T0M-0414	-	0	0	0	-	-
	T0B-0414	-	0	0	0	-	-
Day 5	TnT-0419	-	0	0	0	-	-
	TnM-0419	-	0	0	0	-	-
	TnB-0419	-	0	0	0	-	-

a) R: Replicate
b) S.D: Standard deviation

Appendix 10. Number of heterotrophic bacteria in original water, control (untreated) seawater, treated ballast seawater and neutralized water by treatment of Purimar™ BWMS

Date	Sample ID		CFU/mL			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (04/14)	Ow-0414	Top	31300	14500	34000	26600	10566
		Middle	35800	30800	35400	34000	2778
		Bottom	26400	33700	25800	28633	4398
	C0-0414	Top	9800	4000	14900	9567	5454
		Middle	24500	19700	18900	21033	3029
		Bottom	14600	18700	15700	16333	2122
	T0-0414	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
Day5 (04/19)	C5-0419	Top	12800	9900	11400	11367	1450
		Middle	11100	15400	16100	14200	2707
		Bottom	14700	17200	16900	16267	1365
	Tn-0419	Top	350	380	510	413	85.0
		Middle	450	410	570	477	83.3
		Bottom	460	410	350	407	55.1

a) R: Replicate
b) S.D: Standard deviation

Appendix 11. Number of *Escherichia coli* in original water, control (untreated) seawater, treated ballast seawater and neutralized water by treatment of Purimar™ BWMS

Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (04/14)	Ow-0414	Top	2	8	7	5.7	3.2
		Middle	1	8	7	5.3	3.8
		Bottom	9	8	8	8.3	0.6
	C0-0414	Top	6	12	8	8.7	3.1
		Middle	2	10	10	7.3	4.6
		Bottom	6	6	5	5.7	0.6
	T0-0414	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
Day5 (04/19)	C5-0419	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
	Tn-0419	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0

a) R: Replicate
b) S.D: Standard deviation

Appendix 12. Number of *Enterococcus faecalis* in original water, control (untreated) seawater, treated ballast seawater and neutralized water by treatment of Purimar™ BWMS

Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (04/14)	Ow-0414	Top	20	26	15	20.3	5.5
		Middle	12	25	12	16.3	7.5
		Bottom	22	28	24	24.7	3.1
	C0-0414	Top	27	24	15	22.0	6.2
		Middle	12	34	26	24.0	11.1
		Bottom	19	12	15	15.3	3.5
	T0-0414	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
Day5 (04/19)	C5-0419	Top	0	0	0	0.0	0.0
		Middle	1	0	0	0.3	0.6
		Bottom	0	0	0	0.0	0.0
	Tn-0419	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0

a) R: Replicate
b) S.D: Standard deviation

Appendix 13. Number of *Vibrio cholera* O1, O139 in original water, control (untreated) seawater, treated ballast seawater and neutralized water by treatment of Purimar™ BWMS

Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (04/14)	Ow-0414	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
	C0-0414	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
	T0-0414	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
Day5 (04/19)	C5-0419	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
	Tn-0419	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0

a) R: Replicate
b) S.D: Standard deviation

Appendix 14. The microscope image of ≥ 50㎛ in original water (Day0)

Specific Name	Original water
Artemia salina *Acrtia* sp. *Ditylum* sp.	
Brachionus plicatilis *Ditylum* sp.	
Calanus sp. *Ditylum* sp.	

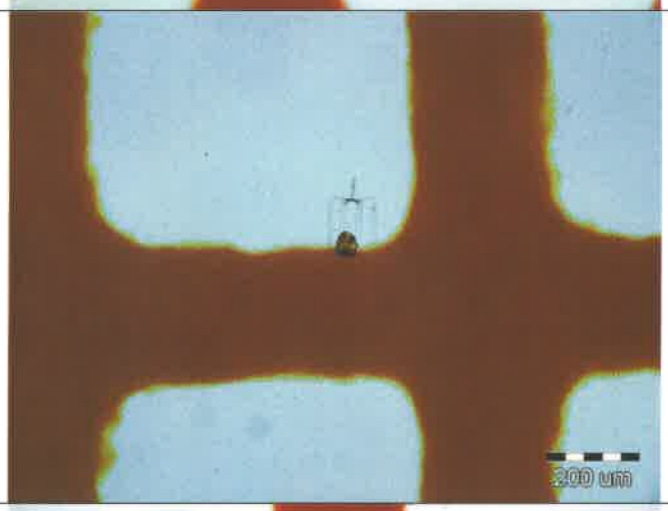
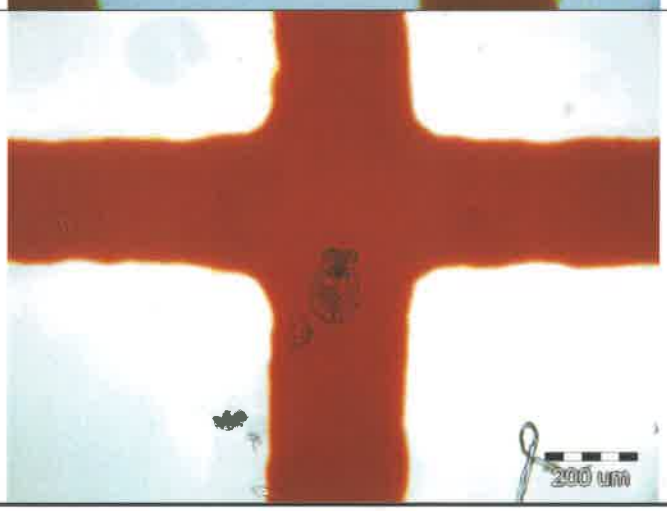
Magnification : 10

Appendix 15. The microscope image of ≥ 50㎛ in control (untreated) seawater by treatment of Purimar™ BWMS (Day0)

Specific Name	Control (untreated) seawater
Artemia salina ***Paracalanus* sp.** ***Brachionus plicatilis***	
Brachionus plicatilis ***Ditylum* sp.**	
Crustacean nauplii	

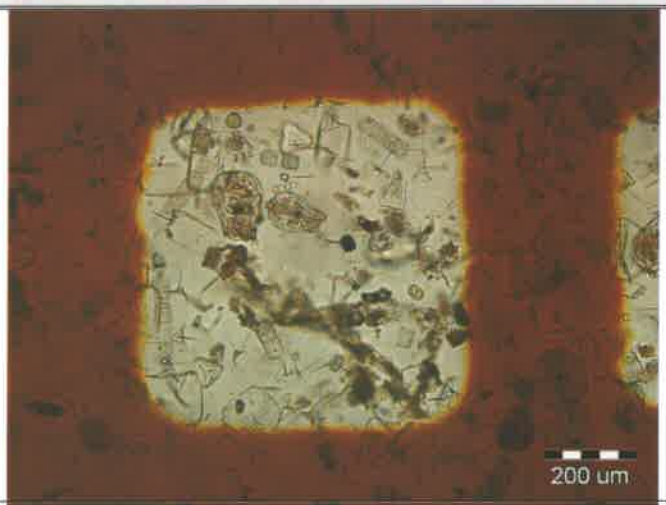
Magnification : 10

Appendix 16. The microscope image of ≥ 50㎛ in control (untreated) seawater by treatment of Purimar™ BWMS (Day5)

Specific Name	Control (untreated) seawater
Artemia salina	
Ditylum sp.	
Brachionus plicatilis	

Magnification : 10

Appendix 17. The microscope image of ≥ 50㎛ in treated ballast seawater by treatment of Purimar™ BWMS (Day0)

Specific Name	Control (untreated) seawater
-	
-	
-	

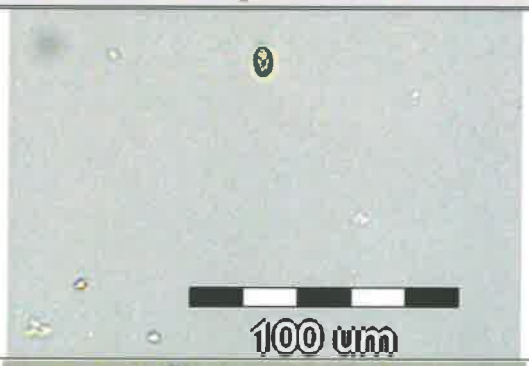
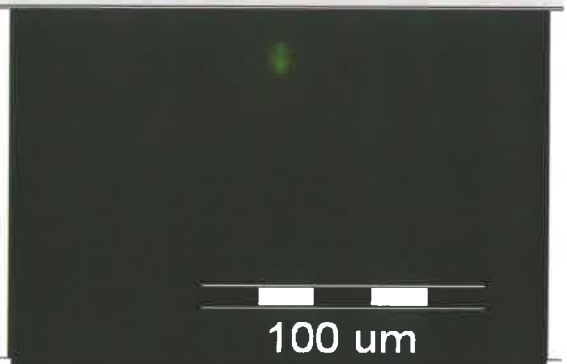
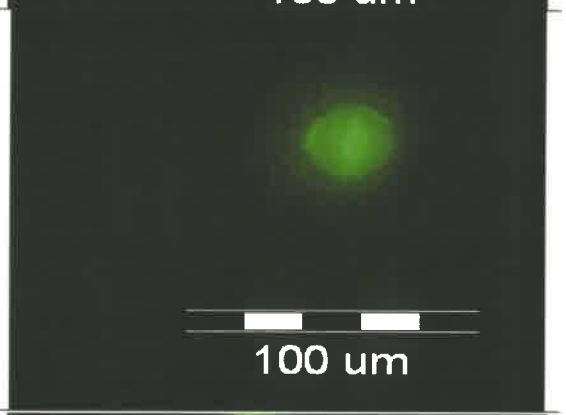
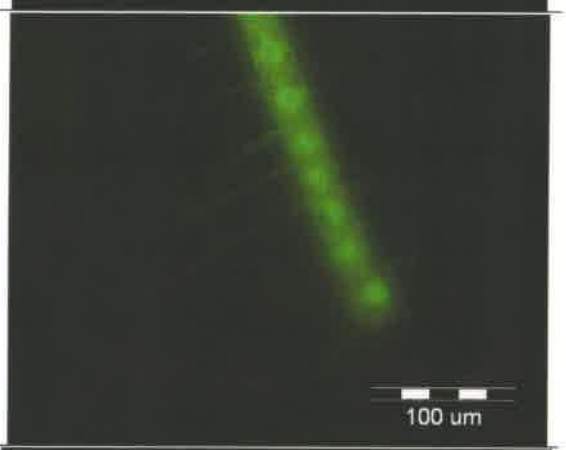
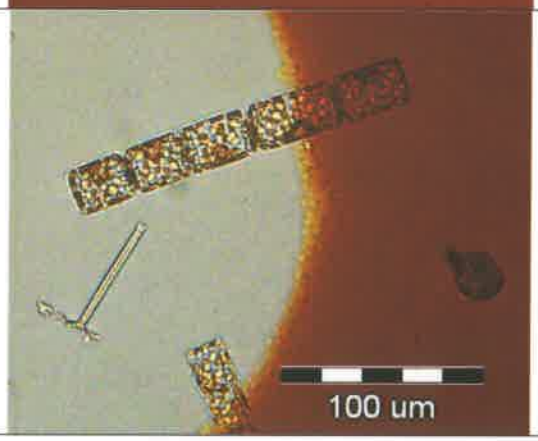
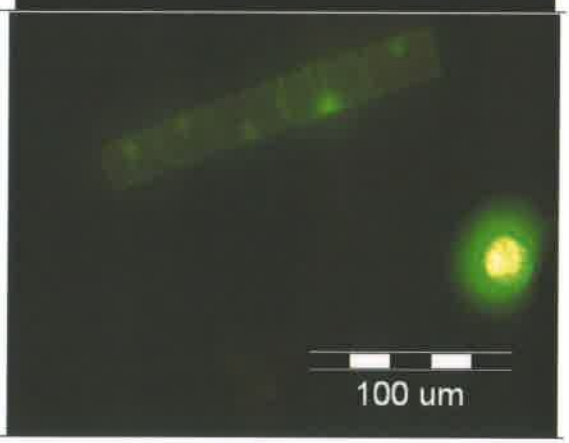
Magnification : 10

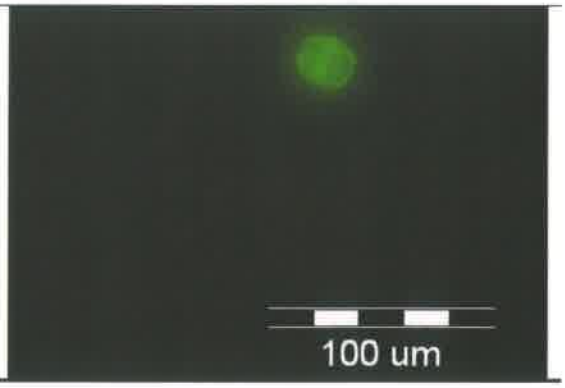
Appendix 18. The microscope image of ≥ 50㎛ in neutralized water by treatment of Purimar™ BWMS (Day5)

Specific Name	Control (untreated) seawater
-	
-	
-	

Magnification : 10

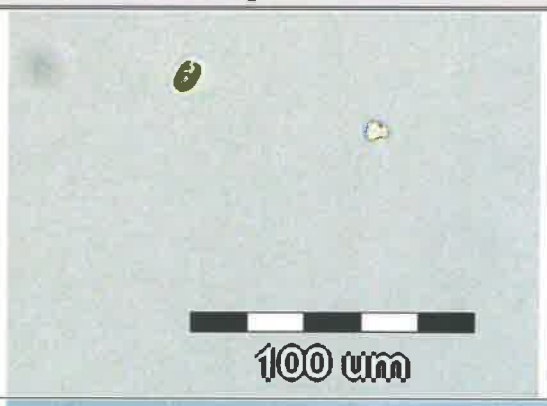
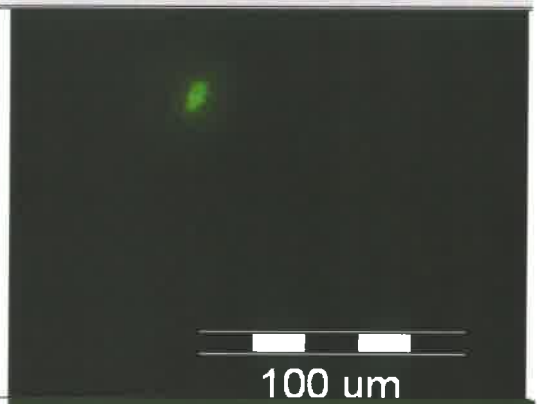
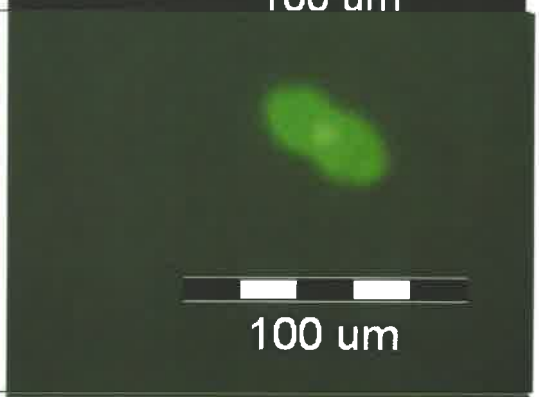
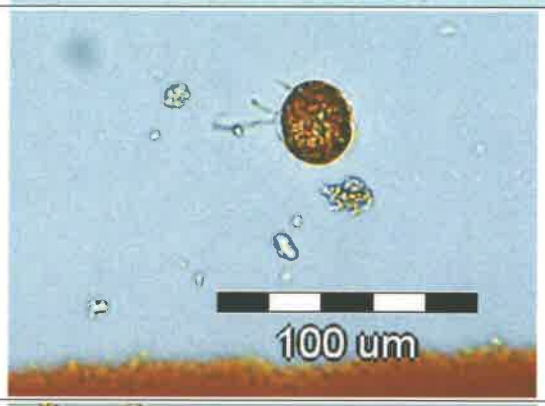
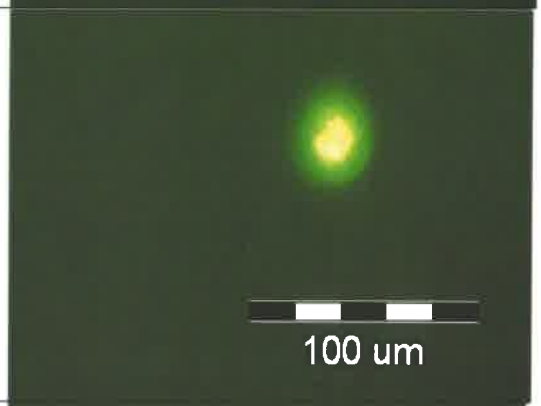
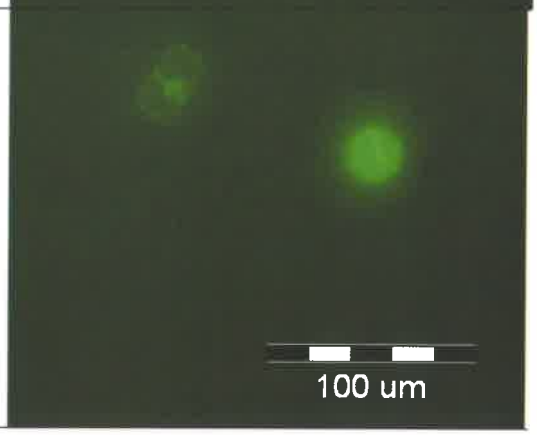
Appendix 19. The microscope image of 10㎛−50㎛ in original water (Day0)

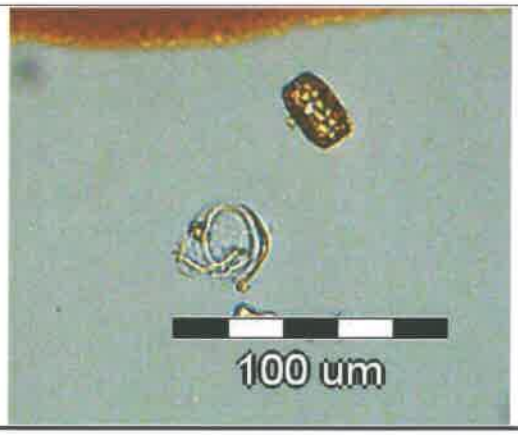
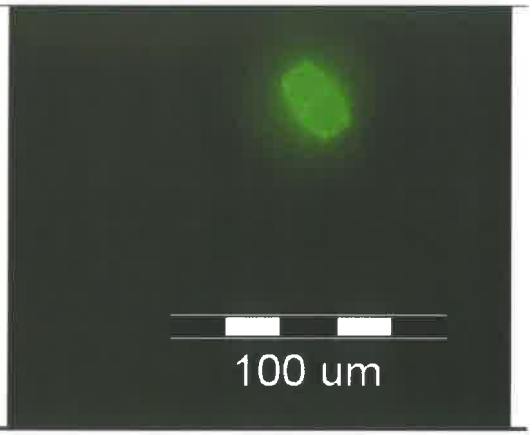
Specific Name	Control (untreated) seawater	
	Optical	Fluorescence
Tetraselmis suecica		
Protoperidinium sp.		
Chaetoceros sp.		
Melosira sp.		

***Thalassiosira* sp.**		

Magnification : 200

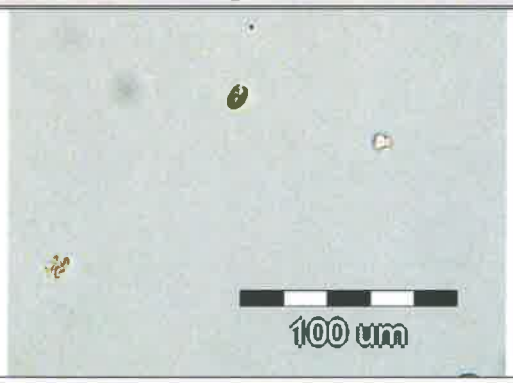
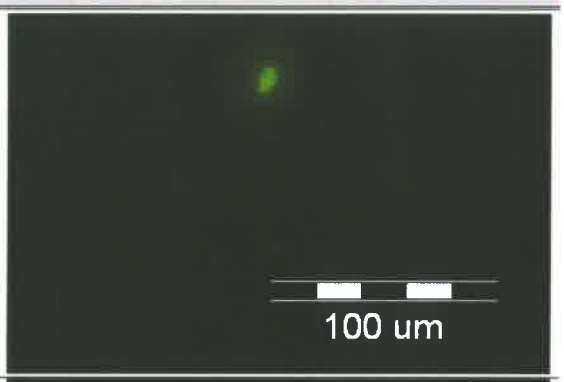
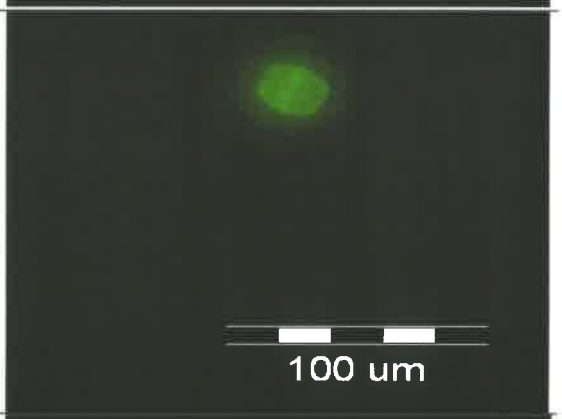
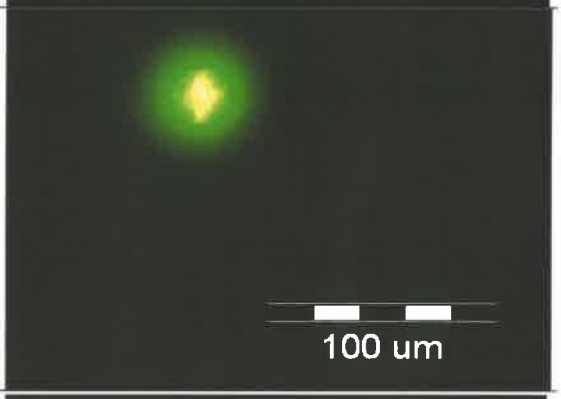
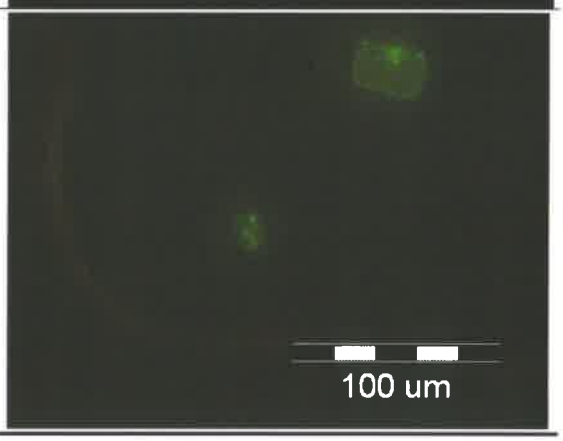
Appendix 20. The microscope image of 10㎛−50㎛ in control (untreated) seawater by treatment of Purimar™ BWMS (Day0)

Specific Name	Control (untreated) seawater	
	Optical	Fluorescence
Tetraselmis suecica		
Diploneis sp.		
Protoperidinium sp.		
Melosira sp.		

***Thalassiosira* sp.**		

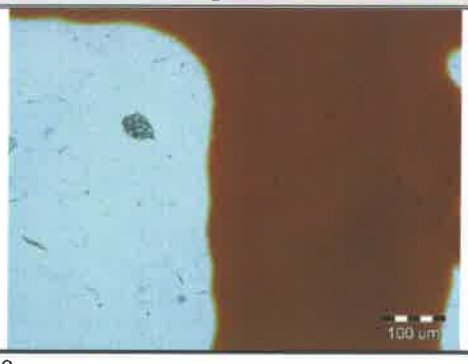
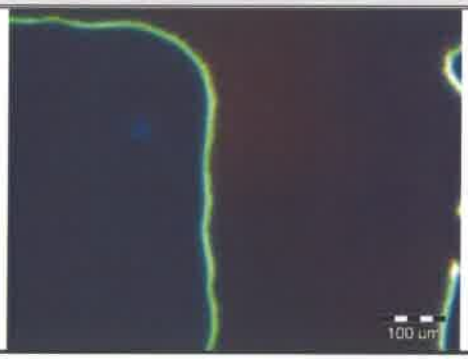
Magnification : 200

Appendix 21. The microscope image of 10㎛−50㎛ in control (untreated) seawater by treatment of Purimar™ BWMS (Day5)

Specific Name	Control (untreated) seawater	
	Optical	Fluorescence
Tetraselmis suecica		
Thalassiosira sp.		
Protoperidinium sp.		
Melosira sp.		

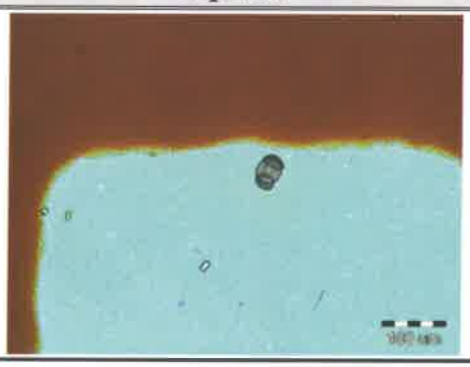
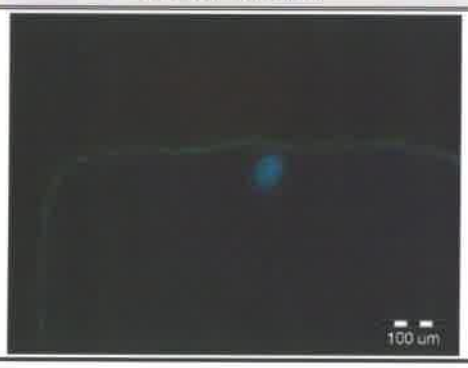
Magnification : 200

Appendix 22. The microscope image of 10㎛−50㎛ in treated ballast seawater by treatment of Purimar™ BWMS (Day0)

Specific Name	Treated ballast seawater	
	Optical	Fluorescence
-		

Magnification : 200

Appendix 23. The microscope image of 10㎛−50㎛ in neutralized water by treatment of Purimar™ BWMS (Day5)

Specific Name	Neutralized water	
	Optical	Fluorescence
-		

Magnification : 200

Appendix 24. The image of heterotrophic bacteria: Day 0

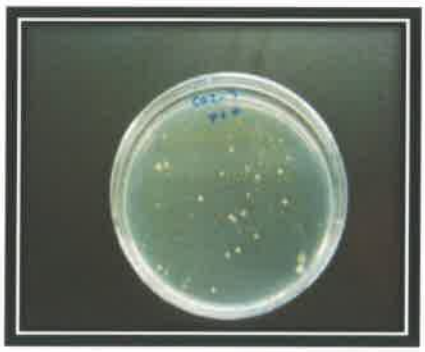
Treated ballast seawater

Top	Middle	Bottom

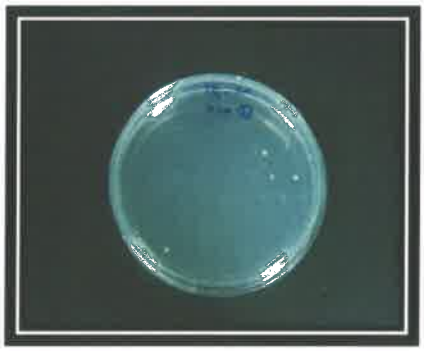
Original water

Top	Middle	Bottom

Control (untreated) seawater

Top	Middle	Bottom
		

Appendix 25. The image of heterotrophic bacteria: Day 5

Neutralized water		
Top	**Middle**	**Bottom**
		
Control (untreated) seawater		
Top	**Middle**	**Bottom**

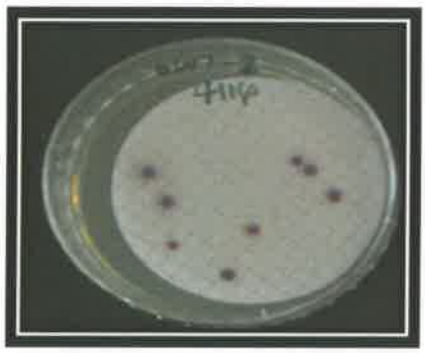
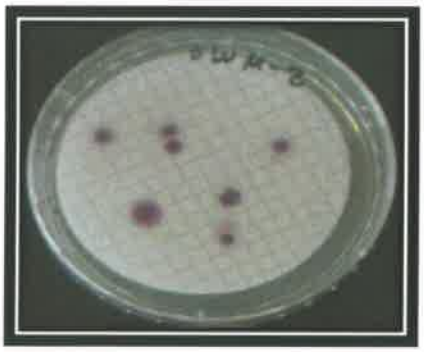
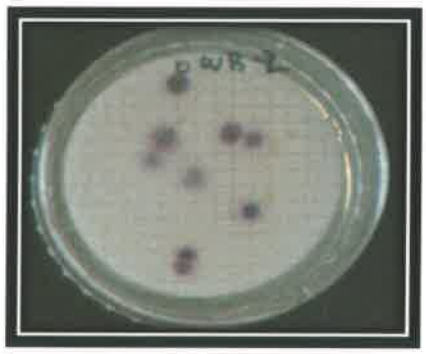
Appendix 26. The image of *Escherichia coli*: Day 0

Treated ballast seawater

Top **Middle** **Bottom**

Original water

Top **Middle** **Bottom**

Control (untreated) seawater

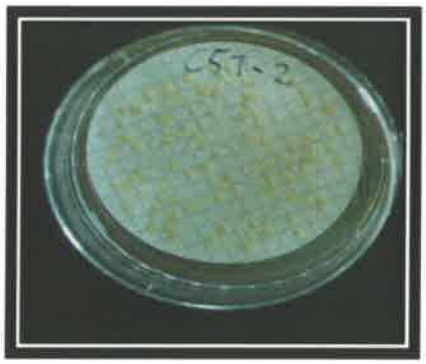
Top **Middle** **Bottom**

Appendix 27. The image of *Escherichia coli*: Day 5

Neutralized water

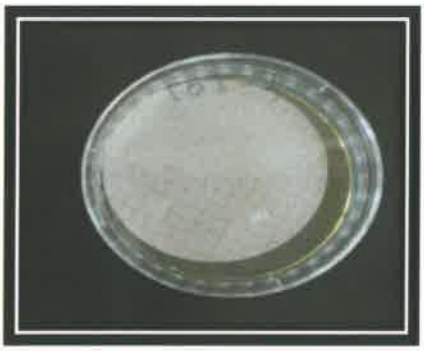
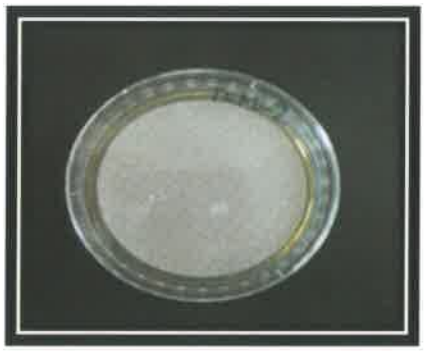
Top	Middle	Bottom

Control (untreated) seawater

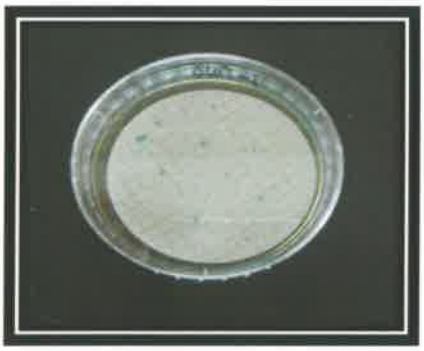
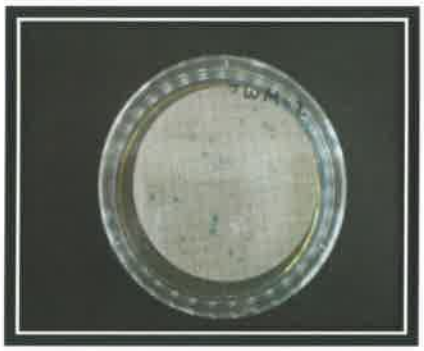
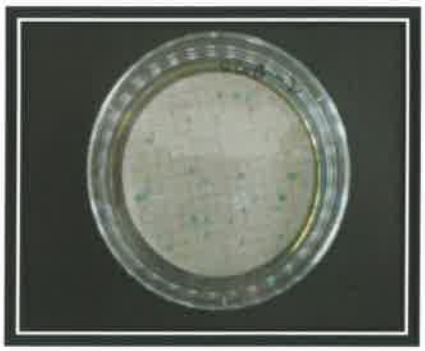
Top	Middle	Bottom
		

Appendix 28. The image of *Enterococcus faecalis*: Day 0

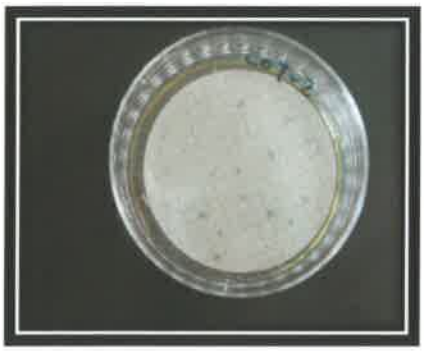
Treated ballast seawater

Top	Middle	Bottom
		

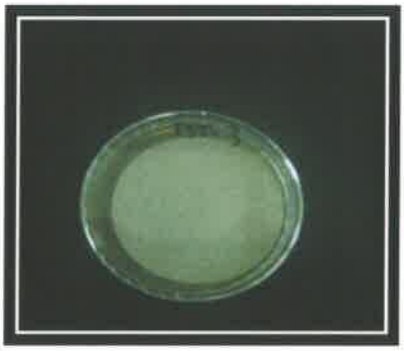
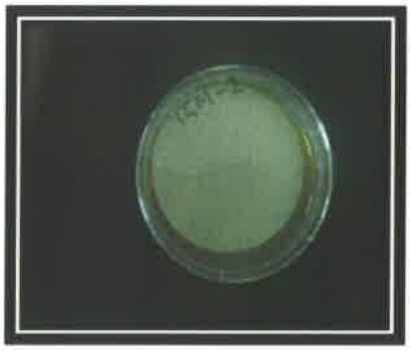
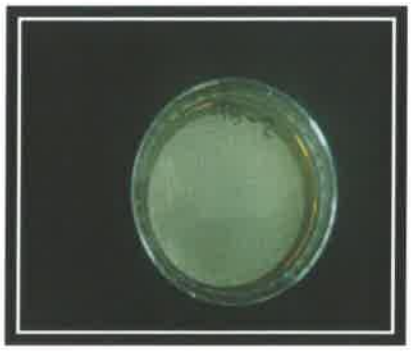
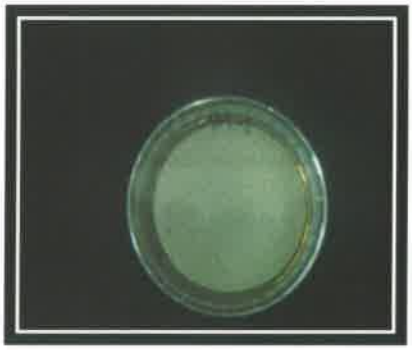
Original water

Top	Middle	Bottom
		

Control (untreated) seawater

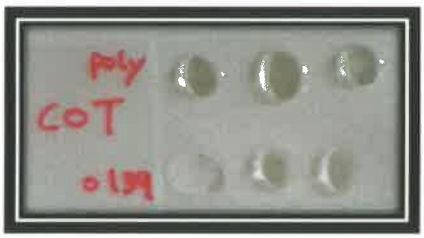
Top	Middle	Bottom
		

Appendix 29. The image of *Enterococcus faecalis*: Day 5

Neutralized water		
Top	**Middle**	**Bottom**
		
Control (untreated) seawater		
Top	**Middle**	**Bottom**
		

Appendix 30. The image of *Vibrio cholera* O1, O139: Day 0

Original water		
Top	**Middle**	**Bottom**

Control (untreated) seawater		
Top	**Middle**	**Bottom**
		

Appendix 31. The image of *Vibrio cholera* O1, O139: Day 5

Neutralized water

Top **Middle** **Bottom**

Control (untreated) seawater

Top **Middle** **Bottom**

5. Attachment

5.1 Filtered natural seawater(FNS) receipt sheet

5.2 Entrance recode sheet

5.3 Flowmeter, seals and power consumption confirming sheet

5.4 Land-based test sheet

5.5 Organism certificate

5.6 Organism check sheet

5.7 Sampling check sheet

5.8 Chain of custody record

5.9 Sample receipt form

5.10 Water parameter measurement sheet (at a field)

5.11 Water parameter measurement sheet (After arrival at the MBDC lab)

5.12 TOC measurement sheet

5.13 TSS(Total suspended solid) measurement sheet

5.14 Microbiology test sheet

5.15 Test results sheet – Classification

5.16 Test result sheet with microbiology