Test Report

Project name	Purimar™
Study number	TW-Land(8)
Test number	LET-10-015
Title	Efficacy test of Treated Ballast Seawater by Purimar™ Ballast Water Management System (Land scale)
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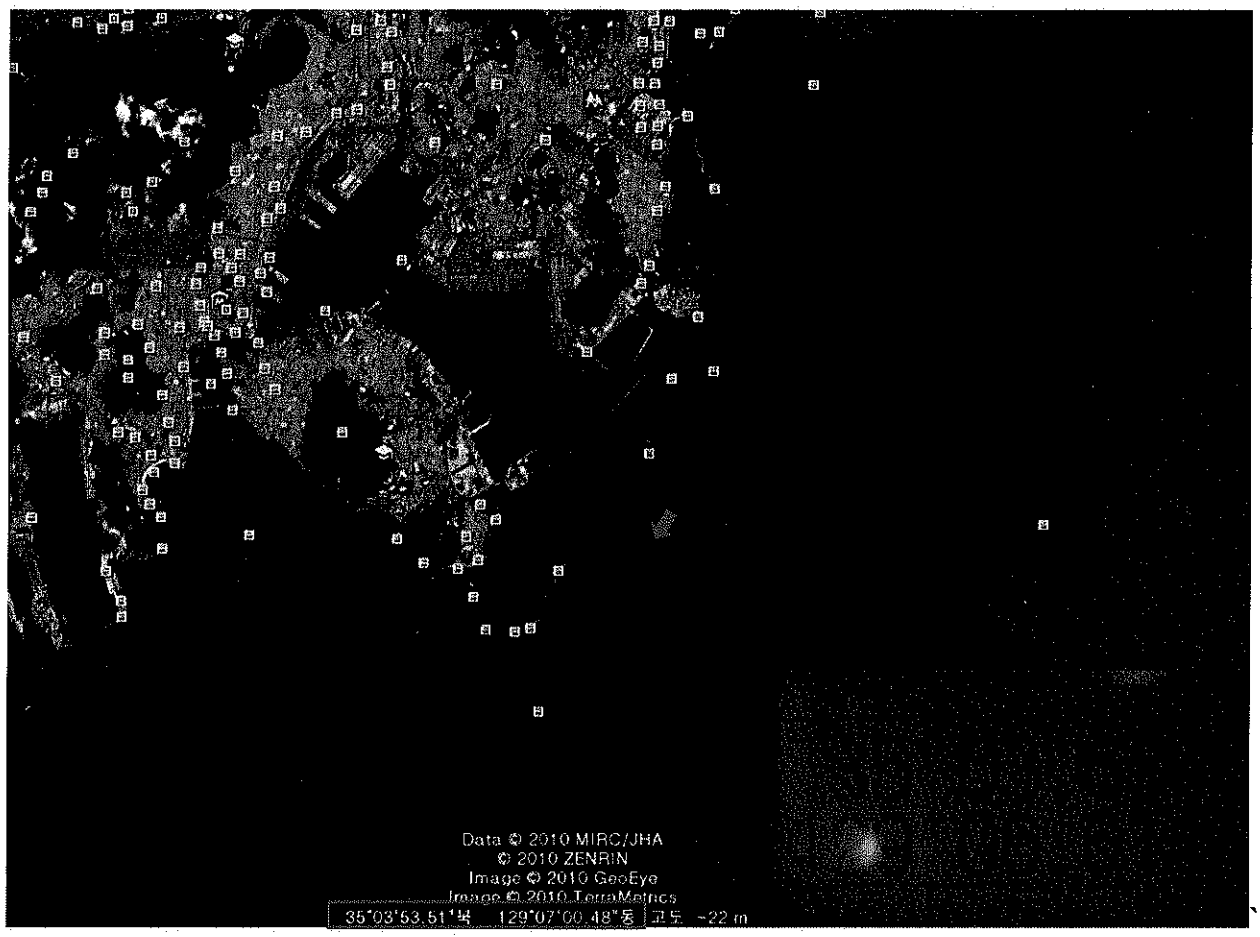
1. Results

1. 1 Sampling point

GPS coordinate	35°03.684N 129°06.614E
Salinity	32.4 ‰



1.2 Water parameters

Table 1. Results of TOC, 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-0929	4.82±0.38	3.52±0.46	1.30	10.4±0.2
	OwM-0929	5.25±0.37	3.82±0.80	1.43	10.9±0.3
	OwB-0929	5.06±0.35	3.92±0.13	1.13	11.7±0.3
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-0929	8.11±0.00	21.58±0.00	32.24±0.00	7.57±0.00	-	3.62±0.00
	OwM-0929	8.11±0.00	21.58±0.01	32.26±0.01	7.58±0.01	-	3.42±0.00
	OwB-0929	8.10±0.01	21.42±0.03	32.24±0.01	7.58±0.01	-	3.45±0.00
	C0T-0929	8.04±0.00	22.00±0.01	32.16±0.01	7.53±0.00	-	7.85±0.00
	C0M-0929	8.03±0.00	22.02±0.01	32.11±0.02	7.52±0.01	-	7.70±0.00
	C0B-0929	8.03±0.00	22.50±0.02	32.06±0.00	7.48±0.01	-	6.90±0.00
	T0T-0929	8.06±0.01	22.20±0.00	32.28±0.00	7.30±0.01	-	0.00±0.00
	T0M-0929	7.98±0.01	22.15±0.04	32.19±0.01	7.23±0.01	-	0.00±0.00
	T0B-0929	8.00±0.02	22.35±0.02	32.16±0.01	7.19±0.00	-	0.00±0.00
Day 2	C2M-1001	7.74±0.01	21.95±0.00	32.36±0.00	4.45±0.03	-	5.00±0.00
	T2M-1001	8.02±0.01	21.89±0.01	32.47±0.01	7.28±0.01	-	0.00±0.00
Day 5	C5T-1004	7.44±0.02	22.66±0.02	32.15±0.01	6.88±0.02	-	2.08±0.00
	C5M-1004	7.26±0.03	22.52±0.01	32.09±0.02	6.87±0.02	-	2.10±0.01
	C5B-1004	7.33±0.00	22.59±0.01	32.14±0.01	6.88±0.01	-	2.08±0.00
	T5T-1004	7.77±0.00	22.35±0.00	32.23±0.00	6.98±0.00	-	0.00±0.00
	T5M-1004	7.69±0.01	22.24±0.01	32.17±0.03	7.04±0.00	-	0.00±0.00
	T5B-1004	7.71±0.00	22.24±0.01	32.21±0.01	7.02±0.01	-	0.00±0.00

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-0929	8.10±0.00	19.5±0.1	32.9±0.1	9.04±0.01	3.58±0.02
	OwM-0929	8.18±0.00	14.5±0.1	32.5±0.1	9.59±0.07	4.15±0.02
	OwB-0929	8.18±0.00	13.4±0.1	33.3±0.1	9.79±0.02	3.68±0.19
	C0T-0929	8.20±0.00	13.8±0.0	33.4±0.1	9.37±0.02	4.45±0.13
	C0M-0929	8.21±0.00	13.8±0.1	33.3±0.0	9.25±0.01	4.03±0.02
	C0B-0929	8.23±0.00	11.7±0.0	31.8±0.0	9.38±0.03	4.51±0.06
	T0T-0929	8.18±0.00	14.1±0.1	32.4±0.1	9.57±0.02	4.90±0.07
	T0M-0929	8.24±0.00	10.4±0.1	32.2±0.0	9.66±0.02	3.48±0.04
	T0B-0929	8.24±0.01	11.0±0.1	32.3±0.1	9.58±0.01	3.67±0.04
Day 2	C2M-0908	7.82±0.01	22.3±0.00	32.9±0.0	7.75±0.01	1.93±0.04
	T2M-0908	8.08±0.00	22.4±0.00	33.5±0.0	8.34±0.01	1.46±0.02
Day 5	C5T-1004	8.00±0.01	10.5±0.1	32.2±0.1	9.55±0.03	2.39±0.28
	C5M-1004	7.75±0.00	11.6±0.0	32.2±0.1	9.17±0.01	2.11±0.03
	C5B-1004	7.75±0.00	11.7±0.0	32.1±0.0	9.33±0.01	2.22±0.05
	T5T-1004	7.91±0.00	10.7±0.0	32.2±0.1	9.51±0.02	2.10±0.15
	T5M-1004	7.93±0.00	11.5±0.0	32.3±0.0	9.59±0.02	0.90±0.28
	T5B-1004	7.94±0.00	10.2±0.1	32.4±0.0	9.62±0.01	1.56±0.06

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

Table 4. Change of TRO concentration of test substances

Sample ID [a)]	TRO con. (ppm)	CV(%)
C0	N.D	-
T0	0.72±0.02	2.11
C2	N.D	-
T2	0.18±0.01	3.15
C5	N.D	-
T5	N.D	-

[a)] C0, C2, C5: Control (untreated) seawater at day 0, day 2 and day 5
T0, T2, T5: Treated ballast seawater at day 0, day 2 and day 5

1.3 Influent water : Original water

1) ≥ 50 μm organism

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

Sampling	Phyla/Divisions	Species	Survival number/m^3	Minimum Dimension (μm)
Top	Arthropoda	*Artemia salina*	166000 ± 2646	150 ~ 170
		Copepod nauplii	667 ± 289	80 ~ 130
		Paracalanus sp.	500 ± 0	150 ~ 180
		Canthocalanus sp.	167 ± 289	447
	Rotifera	*Brachionus plicatilis*	470000 ± 20000	68 ~ 130
	Bacillariophyta	*Coscinodiscus* sp.	8333 ± 2887	180 ~ 190
	Dinophyta	*Protoperidinium* sp.	10000 ± 5000	60 ~ 70
Middle	Arthropoda	*Artemia salina*	165000 ± 1500	150 ~ 170
		Oithona sp.	667 ± 289	130 ~ 150
		Acartia sp.	500 ± 0	150 ~ 200
	Rotifera	*Brachionus plicatilis*	571667 ± 16073	68 ~ 130
	Bacillariophyta	*Coscinodiscus* sp.	6667 ± 2887	180 ~ 190
	Dinophyta	*Protoperidinium* sp.	5000 ± 0	60 ~ 70
Bottom	Arthropoda	*Artemia salina*	156833 ± 4252	150 ~ 170
		Copepod nauplii	1000 ± 500	80 ~ 130
		Paracalanus sp.	500 ± 0	150 ~ 180
		Oithona sp.	333 ± 289	130 ~ 150
	Rotifera	*Brachionus plicatilis*	671667 ± 7638	68 ~ 130
	Dinophyta	*Protoperidinium* sp.	3333 ±2887	60 ~ 70
Total	9 species 4 phyla/divisions		746278 ± 89044	-
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.

Some species were rare, the standard deviation was high.

2) 10 μm - 50 μm organism

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

Sampling	Phyla/Divisions	Species	Survival number/mL (Mean)	Minimum Dimension (μm)
Top	Chlorophyta	*Tetraselmis suecica*	2890.00 ± 80.00	11 ~ 12
	Bacillariophyta	*Chaetoceros* sp.	4.33 ± 1.15	20 ~ 40
		Ditylum Sp.	1.33 ± 0.58	25 ~ 48
		Ceratium sp.	2.00 ± 1.00	20 ~ 40
	Dinophyta	*Protoperidinium* sp.	2.33 ± 0.58	20 ~ 45
Middle	Chlorophyta	*Tetraselmis suecica*	2713.33 ± 25.17	11 ~ 12
	Bacillariophyta	*Ceratium* sp.	0.67 ± 0.58	20 ~ 40
		Chaetoceros sp.	2.67 ± 0.58	20 ~ 40
	Dinophyta	*Protoperidinium* sp.	2.67 ± 0.58	20 ~ 45
Bottom	Chlorophyta	*Tetraselmis suecica*	2750.00 ± 91.65	11 ~ 12
	Bacillariophyta	*Chaetoceros* sp.	1.33 ± 0.58	20 ~ 40
		Ceratium sp.	0.67 ± 0.58	20 ~ 40
	Dinophyta	*Protoperidinium* sp.	1.67 ± 0.58	20 ~ 45
Total	5 species 3 phyla/divisions		2791.00 ± 95.95	-
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.
Some species were rare, the standard deviation was high.

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)]	655667 ± 20251	Influent condition: Acceptable ($\geq 10^5$ ind./m^3)	
		M [b)]	749500 ± 18635		
		B [c)]	833667 ± 4252		
Control (untreated) seawater	Day 0 (C0)	T	493167 ± 22307	-	-
		M	498167 ± 21502		
		B	535167 ± 39116		
	Day 5 (C5)	T	277917 ± 22686	Effluent condition: Acceptable ($\geq 10^2$ ind./m^3)	
		M	239417 ± 2673		
		B	242833 ± 13042		
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		
	Day 5 (T5)	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-T5)/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 [a)]	2900.00 ± 78.50	Influent condition: Acceptable ($\geq 10^3$ ind./mL)	-
		M [b)]	2719.33 ± 25.58		
		B [c)]	2753.67 ± 92.74		
Control (untreated) seawater	Day 0 (C0)	T	2937.00 ± 89.82	-	-
		M	2705.33 ± 62.78		
		B	2788.33 ± 57.83		
	Day 5 (C5)	T	186.33 ± 9.02	Effluent condition: Acceptable ($\geq 10^2$ ind./ ml)	
		M	213.33 ± 12.50		
		B	216.67 ± 9.71		
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		
Treated ballast seawater	Day 5 (T5)	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-T5)/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 [a)]	10866.7 ± 1446.8	Influent condition: Acceptable ($\geq 10^4$ CFU/mL)	-
		M[b)]	28200.0 ± 1442.2		
		B[c)]	28200.0 ± 1928.7		
Control (untreated) seawater	Day 0 (C0)	T	47400.0 ± 4340.5	-	-
		M	25800.0 ± 1600.0		
		B	27933.3 ± 2052.6		
	Day 5 (C5)	T	26200.0 ± 2227.1	-	
		M	22800.0 ± 529.2		
		B	23833.3 ± 602.8		
Treated ballast seawater	Day 0 (T0)	T	20.0 ± 17.3	-	100 [d)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
	Day 5 (T5)	T	120800.0 ± 21659.2	-	-229.3[e)]
		M	97066.7 ± 6110.1		
		B	115200.0 ± 4866.2		

[a)] T: Top, [b)] M: Middle [c)] B: Bottom, [d)] Calculation = (C0-T0)/C0*100, [e)] Calculation = (C0-T5)/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 [a)]	0.0 ± 0.0	-	
		M [b)]	0.0 ± 0.0		
		B [c)]	2.7 ± 1.2		
Control (untreated) seawater	Day 0 (C0)	T	6.7 ± 1.2	-	-
		M	4.0 ± 2.0		
		B	6.0 ± 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 (<250 CFU/ 100ml)	100.0 [d)]
		M	0.0 ± 0.0		
		B	0.0 ± 0.0		
	Day 5 (T5)	T	0.3 ± 0.6	Effluent condition: Acceptable (<250 CFU/ 100ml)	96.0 [e)]
		M	0.0 ± 0.0		
		B	0.3 ± 0.6		

[a)] T: Top, [b)] M: Middle [c)] B: Bottom, [d)] Calculation = (C0-T0)/C0*100, [e)] Calculation = (C0-T5)/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 [a)]	26.0 ± 8.5	-	
		M [b)]	210.7 ± 28.9		
		B [c)]	194.7 ± 8.3		
Control (untreated) seawater	Day 0 (C0)	T	128.0 ± 13.9	-	-
		M	141.3 ± 11.5		
		B	150.7 ± 6.1		
	Day 5 (C5)	T	0.3 ± 0.6	-	
		M	0.7 ± 1.2		
		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		
	Day 5 (T5)	T	3.7 ± 5.5	Effluent condition: Acceptable (<100 CFU/ 100ml)	96.0 [e)]
		M	8.0 ± 4.0		
		B	5.0 ± 2.0		

[a)] T: Top, [b)] M: Middle [c)] B: Bottom, [d)] Calculation = (C0-T0)/C0*100, [e)] Calculation = (C0-T5)/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 [a)]	0.0 ± 0.0	-	-
		M [b)]	0.0 ± 0.0		
		B [c)]	0.0 ± 0.0		
Control (untreated) seawater	Day 0 (C0)	T	0.0 ± 0.0	-	-
		M	0.0 ± 0.0		
		B	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		
	Day 5 (T5)	T	0.0 ± 0.0	Effluent condition: Acceptable (<1 CFU/ 100ml)	N.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-0929	32.24±0.00	3.52±0.46	1.30	10.4±0.2
	OwM-0929	32.26±0.01	3.82±0.80	1.43	10.9±0.3
	OwB-0929	32.24±0.01	3.92±0.13	1.13	11.7±0.3

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: ≥ 10^5 individuals/m^3
  - The organism between 10 μm and 50 μm: ≥ 10^3 individuals / mL
  - Heterotrophic bacteria: ≥ 10^4 CFU/ mL

Sample		≥ 50 μm (individuals/m^3)	10 μm - 50 μm (individuals/mL)	Heterotrophic bacteria (CFU/mL)
Field	OwT	165289	2448	-
Lab	OwT	655667 ± 20251	2900.00 ± 78.50	10866.7 ± 1446.8
	OwM	749500 ± 18635	2719.33 ± 25.58	28200.0 ± 1442.2
	OwB	833667 ± 4252	2753.67 ± 92.74	28200.0 ± 1928.7

- 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	9 species 4 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	09:50~10:05	14:30
	OwM	09:48~10:06	14:30
	OwB	09:49~10:05	14:30
	C0T	10:17~10:39	16:30
	C0M	10:18~10:37	16:30
	C0B	10:16~10:37	16:30
	T0T	12:09~12:39	16:30
	T0M	10:56~12:41	16:30
	T0B	10:36~12:08	16:30
Day 5	C5T	12:30~13:24	16:52
	C5M	12:30~13:24	16:52
	C5B	12:30~13:24	16:52
	T5T	13:12~13:22	16:52
	T5M	12:51~13:21	16:52
	T5B	12:48~13:21	16:52
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	277917 ± 22686	186.33 ± 9.02
Middle	239417 ± 2673	213.33 ± 12.50
Bottom	242833 ± 13042	216.67 ± 9.71

3. Conclusion

3.1 Biological efficacy in treated ballast seawater by Purimar™ BWMS

- Treated ballast seawater by Purimar™ BWMS were capable of eliminating organism larger than 50 μm with an efficiency of 100%.
- Treated ballast seawater by Purimar™ BWMS were capable of eliminating organism between 10 and 50 μm with an efficiency of 100%.
- Treated ballast seawater by Purimar™ BWMS was capable of eliminating heterotrophic bacteria with an efficiency of 100 %. at day 0
- But the number of heterotrophic bacteria in treated ballast brackish water was increased at day 5 compared to control brackish water at day 0. Therefore, elimination efficacy of heterotrophic bacteria was reduced at day 5.
- Treated ballast seawater by Purimar™ BWMS were capable of eliminating *Escherichia coli* with an efficiency of 100, 96 % at day 0, 5
- Treated ballast seawater by Purimar™ BWMS were capable of eliminating *Enterococcus faecalis* with an efficiency of 100, 96.7 % at day 0, 5.
- *Vibrio cholera* O1, O139 were not detected.
- Therefore, treated ballast seawater by Purimar™ BWMS was capable of removing organism(> 50 μm, 10 ~ 50 μm) and a part of bacteria..

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-0929	R1	4.89	4.05	10.2
		R2	4.41	3.20	10.6
		R3	5.17	3.32	10.4
	OwM-0929	R1	5.51	4.22	10.6
		R2	4.86	3.05	11.2
		R3	5.38	4.18	10.8
	OwB-0929	R1	4.70	4.05	11.4
		R2	5.39	3.92	11.6
		R3	5.08	3.80	12.0

a) R: Replicate
b) TOC: Total 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
Day0	OwT-0929	R1	8.11	21.58	32.24	7.57	-	3.620
		R2	8.11	21.58	32.24	7.57	-	3.620
		R3	8.11	21.58	32.24	7.57	-	3.620
	OwM-0929	R1	8.11	21.58	32.25	7.57	-	3.420
		R2	8.11	21.58	32.26	7.58	-	3.420
		R3	8.11	21.57	32.26	7.58	-	3.420
	OwB-0929	R1	8.11	21.46	32.23	7.57	-	3.450
		R2	8.10	21.40	32.25	7.58	-	3.450
		R3	8.10	21.41	32.24	7.58	-	3.450
	C0T-0929	R1	8.04	21.99	32.15	7.53	-	7.850
		R2	8.04	22.01	32.15	7.53	-	7.850
		R3	8.04	22.01	32.17	7.53	-	7.850
	C0M-0929	R1	8.03	22.02	32.09	7.53	-	7.700
		R2	8.03	22.02	32.11	7.52	-	7.700
		R3	8.03	22.03	32.12	7.52	-	7.700
	C0B-0929	R1	8.03	22.48	32.06	7.48	-	6.900
		R2	8.03	22.51	32.06	7.48	-	6.900
		R3	8.03	22.52	32.06	7.49	-	6.900
	T0T-0929	R1	8.06	22.20	32.28	7.30	-	0.000
		R2	8.06	22.20	32.28	7.29	-	0.000
		R3	8.05	22.20	32.28	7.30	-	0.000
	T0M-0929	R1	7.99	22.11	32.19	7.24	-	0.000
		R2	7.98	22.16	32.18	7.22	-	0.000
		R3	7.98	22.18	32.19	7.23	-	0.000
	T0B-0929	R1	8.02	22.33	32.16	7.19	-	0.000
		R2	8.00	22.35	32.15	7.19	-	0.000
		R3	7.98	22.37	32.16	7.19	-	0.000
Day2	C2M-1001	R1	7.73	21.95	32.36	4.47	-	5.00
		R2	7.75	21.95	32.36	4.45	-	5.00
		R3	7.75	21.95	32.36	4.42	-	5.00
	T2M-1001	R1	8.02	21.88	32.47	7.28	-	0.00
		R2	8.03	21.89	32.47	7.28	-	0.00
		R3	8.02	21.89	32.46	7.27	-	0.00
Day5	C5T-1004	R1	7.44	22.64	32.15	6.89	-	2.080
		R2	7.43	22.65	32.15	6.88	-	2.080
		R3	7.46	22.68	32.14	6.86	-	2.080
	C5M-1004	R1	7.24	22.52	32.06	6.89	-	2.090
		R2	7.25	22.52	32.10	6.87	-	2.110
		R3	7.29	22.51	32.10	6.86	-	2.100
	C5B-1004	R1	7.33	22.58	32.14	6.89	-	2.080
		R2	7.33	22.60	32.14	6.88	-	2.080
		R3	7.33	22.60	32.15	6.87	-	2.080
	T5T-1004	R1	7.77	22.35	32.23	6.98	-	0.000
		R2	7.77	22.35	32.23	6.98	-	0.000
		R3	7.77	22.35	32.23	6.98	-	0.000
	T5M-1004	R1	7.69	22.24	32.14	7.04	-	0.000
		R2	7.69	22.25	32.17	7.04	-	0.000
		R3	7.70	22.24	32.20	7.04	-	0.000
	T5T-1004	R1	7.71	22.25	32.20	7.03	-	0.000
		R2	7.71	22.24	32.21	7.02	-	0.000
		R3	7.71	22.24	32.22	7.02	-	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-0929	R1	8.10	19.6	32.8	9.03	3.59
		R2	8.10	19.4	32.9	9.04	3.56
		R3	8.10	19.4	32.9	9.04	3.60
	OwM-0929	R1	8.18	14.5	32.4	9.51	4.15
		R2	8.18	14.5	32.6	9.62	4.13
		R3	8.18	14.4	32.6	9.64	4.16
	OwB-0929	R1	8.18	13.3	33.4	9.82	3.46
		R2	8.18	13.4	33.2	9.78	3.77
		R3	8.18	13.5	33.2	9.78	3.80
	C0T-0929	R1	8.20	13.8	33.3	9.35	4.54
		R2	8.20	13.8	33.4	9.38	4.31
		R3	8.20	13.8	33.4	9.39	4.51
	C0M-0929	R1	8.21	13.7	33.3	9.24	4.01
		R2	8.21	13.8	33.3	9.26	4.05
		R3	8.21	13.8	33.3	9.24	4.03
	C0B-0929	R1	8.23	11.7	31.8	9.42	4.58
		R2	8.23	11.7	31.8	9.36	4.48
		R3	8.23	11.7	31.8	9.36	4.48
	T0T-0929	R1	8.18	14.2	32.3	9.56	4.84
		R2	8.18	14.1	32.4	9.59	4.97
		R3	8.18	14.0	32.5	9.57	4.88
	T0M-0929	R1	8.24	10.4	32.2	9.68	3.43
		R2	8.24	10.3	32.2	9.67	3.51
		R3	8.24	10.4	32.2	9.64	3.50
	T0B-0929	R1	8.25	11.0	32.4	9.58	3.71
		R2	8.24	11.0	32.3	9.58	3.64
		R3	8.24	11.1	32.3	9.57	3.65
Day2	C2M-1001	R1	7.81	22.3	32.9	7.76	1.96
		R2	7.82	22.3	32.9	7.75	1.94
		R3	7.82	22.3	32.9	7.75	1.88
	T2M-1001	R1	8.08	22.4	33.5	8.35	1.44
		R2	8.08	22.4	33.5	8.34	1.46
		R3	8.08	22.4	33.5	8.34	1.48
Day 5	C5T-1004	R1	7.76	10.4	32.3	9.59	2.71
		R2	7.76	10.5	32.2	9.53	2.21
		R3	7.75	10.5	32.2	9.53	2.26
	C5M-1004	R1	7.75	11.6	32.2	9.17	2.14
		R2	7.75	11.6	32.2	9.17	2.09
		R3	7.75	11.6	32.3	9.16	2.10
	C5B-1004	R1	7.75	11.7	32.1	9.33	2.23
		R2	7.75	11.7	32.1	9.33	2.17
		R3	7.75	11.7	32.1	9.32	2.27
	T5T-1004	R1	7.91	10.7	32.2	9.53	1.94
		R2	7.91	10.7	32.1	9.50	2.13
		R3	7.91	10.7	32.2	9.50	2.24
	T5M-1004	R1	7.93	11.5	32.3	9.58	0.58
		R2	7.93	11.5	32.3	9.61	0.99
		R3	7.93	11.5	32.3	9.59	1.12
	T5T-1004	R1	7.94	10.2	32.4	9.63	1.51
		R2	7.94	10.2	32.4	9.63	1.54
		R3	7.94	10.3	32.4	9.61	1.62

a) R: Replicate

Appendix 4. Change of TRO concentration of test substances

Date	Sample ID	R [a)]	TRO con. (ppm)[b)]
Day 0	C0	R1	N.D
		R2	N.D
		R3	N.D
	T0	R1	0.71
		R2	0.74
		R3	0.72
Day 2	C2	R1	N.D
		R2	N.D
		R3	N.D
	T2	R1	0.19
		R2	0.18
		R3	0.18
Day 5	C5	R1	N.D
		R2	N.D
		R3	N.D
	T5	R1	N.D
		R2	N.D
		R3	N.D

a) R: Replicate

Appendix 5. Survival number of ≥ 50㎛ organism in original water(Influent water)

Date	Sample ID	Classification	Survival number /m^3			Mean	SD [b)]
			R1[a)]	R2	R3		
Day 0	OwT-0929	*Artemia salina*	169000	165000	164000	166000	2646
		Copepod nauplii	500	500	1000	667	289
		Paracalanus sp.	500	500	500	500	0
		Canthocalanus sp.	500	0	0	167	289
		Brachionus plicatilis	450000	490000	470000	470000	20000
		Coscinodiscus sp.	10000	10000	5000	8333	2887
		Protoperidinium sp.	5000	10000	15000	10000	5000
	OwM-0929	*Artemia salina*	163500	166500	165000	165000	1500
		Oithona sp.	500	1000	500	667	289
		Acartia sp.	500	500	500	500	0
		Brachionus plicatilis	565000	560000	590000	571667	16073
		Coscinodiscus sp.	5000	5000	10000	6667	2887
		Protoperidinium sp.	5000	5000	5000	5000	0
	OwB-0929	*Artemia salina*	152500	157000	161000	156833	4252
		Copepod nauplii	1000	500	1500	1000	500
		Paracalanus sp.	500	500	500	500	0
		Oithona sp.	500	0	500	333	289
		Brachionus plicatilis	670000	680000	665000	671667	7638
		Protoperidinium sp.	5000	0	5000	3333	2887

a) R: Replicate
b) S.D: Standard deviation
Some species were rare, the standard deviation was high.

Appendix 6. Survival number of ≥ 50μm organism in control (untreated) seawater

Date	Sample ID	Classification	Survival number/m^3			Mean	SD [b)]
			R1[a)]	R2	R3		
Day 0	C0T-0929	*Artemia salina*	82500	76000	80000	79500	3279
		Copepod nauplii	500	0	500	333	289
		Brachionus plicatilis	385000	420000	425000	410000	21794
		Protoperidinium sp.	0	5000	5000	3333	2887
	C0M-0929	*Artemia salina*	53500	49500	51500	51500	2000
		Brachionus plicatilis	450000	425000	460000	445000	18028
		Coscinodiscus sp.	0	0	5000	1667	2887
	C0B-0929	*Artemia salina*	25000	28000	27500	26833	1607
		Brachionus plicatilis	555000	480000	490000	508333	40723
Day 5	C5T-1004	*Artemia salina*	1250	1500	1000	1250	250
		Brachionus plicatilis	255000	300000	275000	276667	22546
	C5M-1004	*Artemia salina*	2750	2500	3000	2750	250
		Brachionus plicatilis	235000	240000	235000	236667	2887
	C5B-1004	*Artemia salina*	3000	3000	2500	2833	289
		Brachionus plicatilis	225000	245000	250000	240000	13229

a) R: Replicate
b) S.D: Standard deviation
Some species were rare, the standard deviation was high.

Appendix 7. Survival number of ≥ 50㎛ organism in treated ballast seawater by treatment of Purimar™ BWMS

Date	Sample ID	Classification	Survival number /m³			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	T0T - 0929	-	0	0	0	0.0	0.0
	T0M- 0929	-	0	0	0	0.0	0.0
	T0B- 0929	-	0	0	0	0.0	0.0
Day 5	T5T - 1004	-	0	0	0	0.0	0.0
	T5M- 1004	-	0	0	0	0.0	0.0
	T5B- 1004	-	0	0	0	0.0	0.0

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

Appendix 8. 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-0929	*Tetraselmis suecica*	2890	2810	2970	2890.00	80.00
		Chaetoceros sp.	3	5	5	4.33	1.15
		Ditylum Sp.	1	2	1	1.33	0.58
		Ceratium sp.	2	3	1	2.00	1.00
		Protoperidinium sp.	3	2	2	2.33	0.58
	OwM-0929	*Tetraselmis suecica*	2740	2690	2710	2713.33	25.17
		Ceratium sp.	1	0	1	0.67	0.58
		Chaetoceros sp.	3	2	3	2.67	0.58
		Protoperidinium sp.	2	3	3	2.67	0.58
	OwB-0929	*Tetraselmis suecica*	2850	2730	2670	2750.00	91.65
		Chaetoceros sp.	2	1	1	1.33	0.58
		Ceratium sp.	1	0	1	0.67	0.58
		Protoperidinium sp.	2	2	1	1.67	0.58

a) R: Replicate
b) S.D: Standard deviation
Some species were rare, the standard deviation was high.

Appendix 9. Survival number of 10μm - 50μm organism in control (untreated) seawater

Date	Sample ID	Classification	Survival number / ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	C0T - 0929	*Tetraselmis suecica*	3030	2850	2920	2933.33	90.74
		Chaetoceros sp.	1	2	2	1.67	0.58
		Protoperidinium sp.	2	3	1	2.00	1.00
	C0M- 0929	*Tetraselmis suecica*	2650	2680	2770	2700.00	62.45
		Ceratium sp.	1	1	2	1.33	0.58
		Chaetoceros sp.	2	1	2	1.67	0.58
		Protoperidinium sp.	3	2	2	2.33	0.58
	C0B- 0929	*Tetraselmis suecica*	2800	2720	2830	2783.33	56.86
		Ditylum Sp.	2	1	3	2.00	1.00
		Chaetoceros sp.	1	1	2	1.33	0.58
		Protoperidinium sp.	2	2	1	1.67	0.58
Day 5	C5T- 1004	*Tetraselmis suecica*	186	175	193	184.67	9.07
		Chaetoceros sp.	1	2	2	1.67	0.58
	C5M- 1004	*Tetraselmis suecica*	213	226	201	213.33	12.50
	C5B- 1004	*Tetraselmis suecica*	205	218	223	215.33	9.29
		Chaetoceros sp.	1	1	2	1.33	0.58

a) R: Replicate
b) S.D: Standard deviation
Some species were rare, the standard deviation was high.

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

Date	Sample ID	Classification	Survival number / ml			Mean	SD [b)]
			R1[a)]	R2	R3		
Day 0	T0T-0929	-	0	0	0	-	-
	T0M-0929	-	0	0	0	-	-
	T0B-0929	-	0	0	0	-	-
Day 5	T5T-1004	-	0	0	0	-	-
	T5M-1004	-	0	0	0	-	-
	T5B-1004	-	0	0	0	-	-

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

Appendix 11. Number of heterotrophic bacteria in original water, control (untreated) seawater, treated ballast seawater by treatment of Purimar™ BWMS

Date	Sample ID		CFU/mL			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (09/29)	Ow-0929	Top	9200	11800	11600	10866.7	1446.8
		Middle	27000	29800	27800	28200.0	1442.2
		Bottom	29000	26000	29600	28200.0	1928.7
	C0-0929	Top	42400	50200	49600	47400.0	4340.5
		Middle	24200	27400	25800	25800.0	1600.0
		Bottom	26200	30200	27400	27933.3	2052.6
	T0-0929	Top	10	40	10	20.0	17.3
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
Day5 (10/04)	C5-1004	Top	28600	25800	24200	26200.0	2227.1
		Middle	22600	23400	22400	22800.0	529.2
		Bottom	23200	23900	24400	23833.3	602.8
	T5-1004	Top	96000	130400	136000	120800.0	21659.2
		Middle	102400	98400	90400	97066.7	6110.1
		Bottom	112800	112000	120800	115200.0	4866.2

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

Appendix 12. Number of *Escherichia coli* in original water, control (untreated) seawater, treated ballast seawater by treatment of Purimar™ BWMS

Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (09/29)	Ow-0929	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	2	4	2	2.7	1.2
	C0-0929	Top	6	6	8	6.7	1.2
		Middle	6	2	4	4.0	2.0
		Bottom	4	10	4	6.0	3.5
	T0-0929	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 (10/04)	C5-1004	Top	0	0	0	0.0	0.0
		Middle	0	0	1	0.3	0.6
		Bottom	0	0	0	0.0	0.0
	T5-1004	Top	1	0	0	0.3	0.6
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	1	0.3	0.6

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

Appendix 13. Number of *Enterococcus faecalis* in original water, control (untreated) seawater, treated ballast seawater by treatment of Purimar™ BWMS

Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (09/29)	Ow-0929	Top	10	32	20	26.0	8.5
		Middle	196	244	192	210.7	28.9
		Bottom	188	192	204	194.7	8.3
	C0-0929	Top	144	120	120	128.0	13.9
		Middle	148	128	148	141.3	11.5
		Bottom	144	156	152	150.7	6.1
	T0-0929	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 (10/04)	C5-1004	Top	1	0	0	0.3	0.6
		Middle	0	2	0	0.7	1.2
		Bottom	0	0	0	0.0	0.0
	T5-1004	Top	0	7	4	3.7	5.5
		Middle	3	1	5	8.0	4.0
		Bottom	3	5	7	5.0	2.0

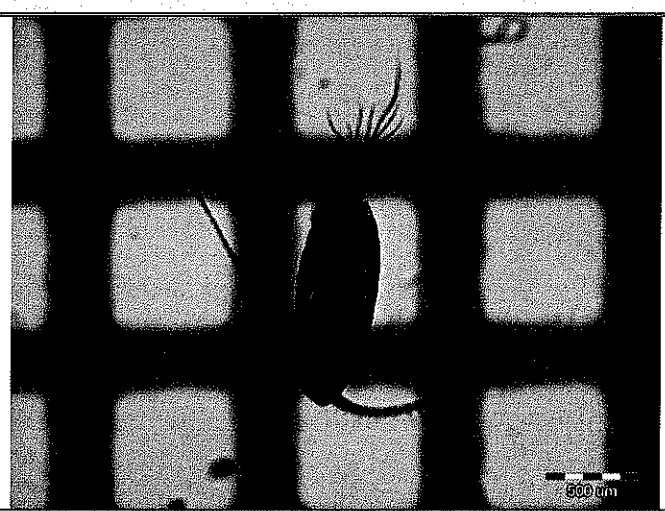
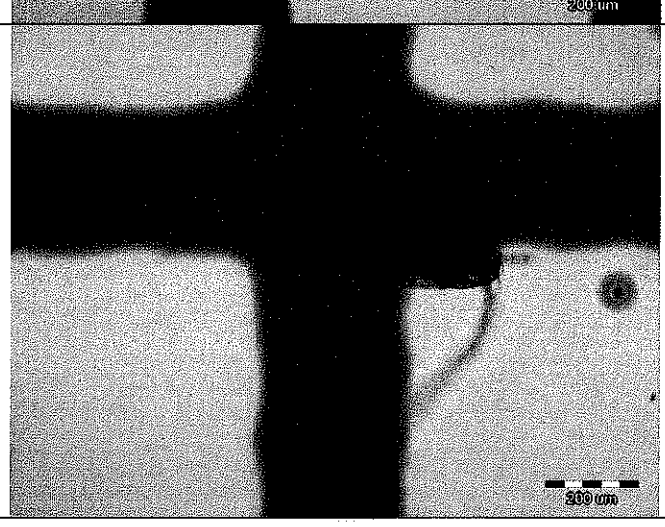
a) R: Replicate
b) S.D: Standard deviation

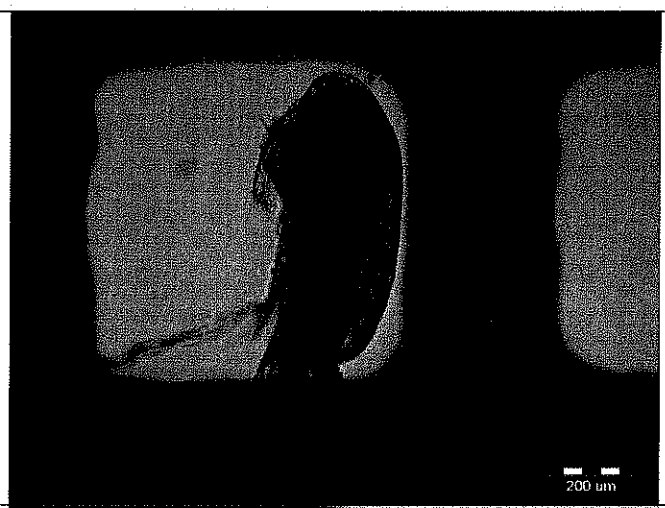
Appendix 14. Number of *Vibrio cholera* O1, O139 in original water, control (untreated) seawater, treated ballast seawater by treatment of Purimar™ BWMS

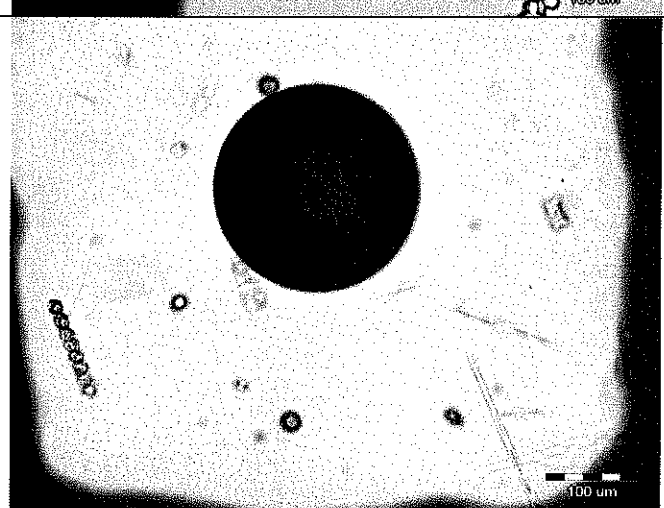
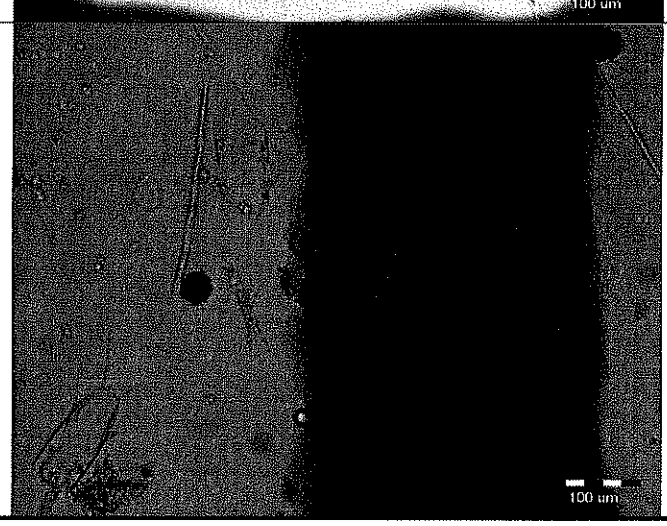
Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (09/29)	Ow-0929	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
	C0-0929	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
	T0-0929	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
Day5 (10/04)	C5-1004	Top	0	0	0	0.0	0.0
		Middle	0	0	0	0.0	0.0
		Bottom	0	0	0	0.0	0.0
	T5-1004	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 15. The microscope image of ≥ 50㎛ in original water (Day0)

Specific Name	Original water
Canthocalanus sp.*	
*Artemia salina***	
Acartia sp.**	

Paracalanus* sp.*	
Oithona* sp.**	
Copepod nauplii***	

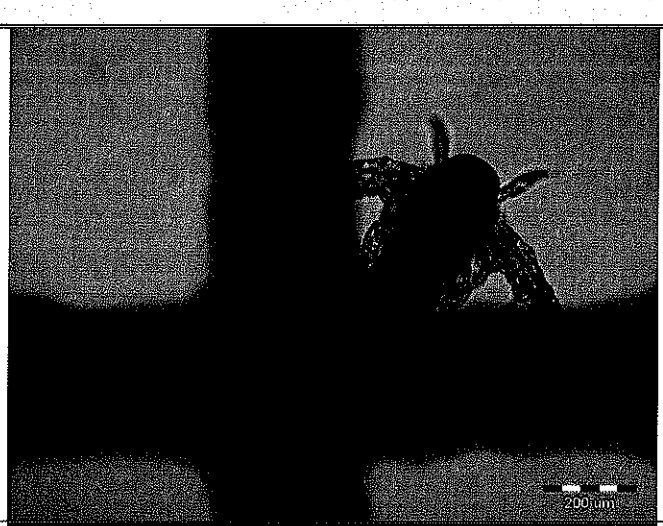
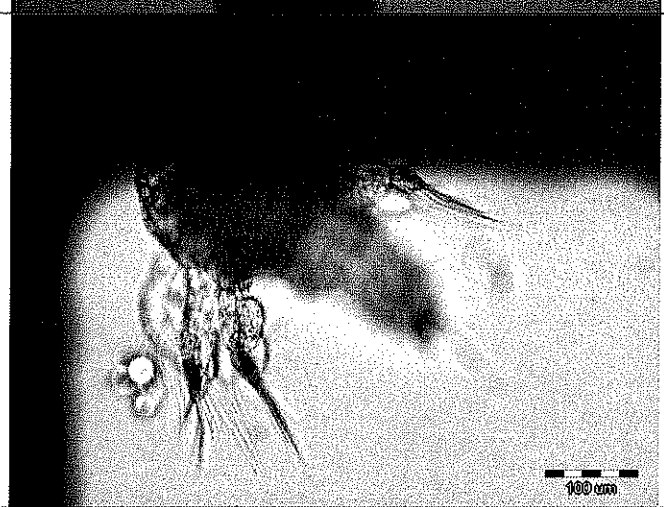
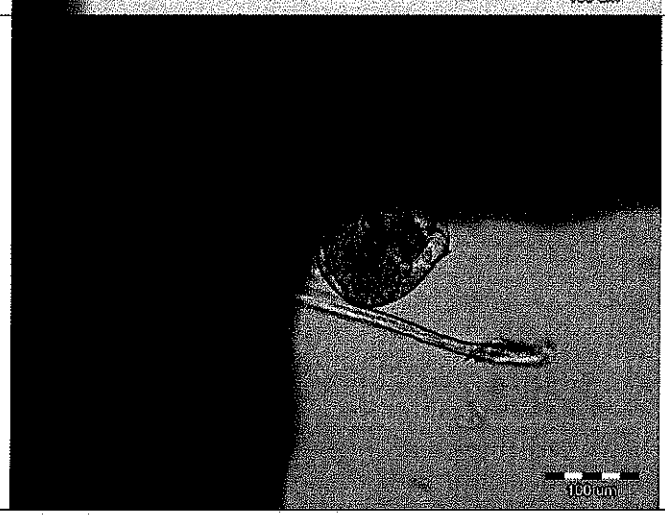
Brachionus plicatilis***	
Coscinodiscus* sp.**	
Protoperidinium* sp.**	

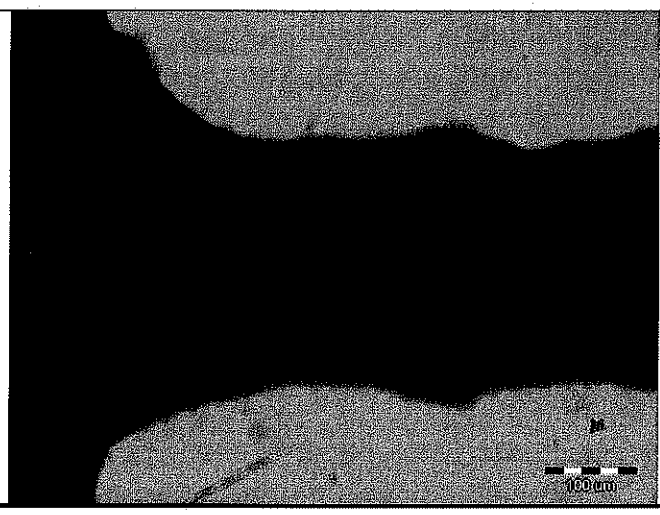
* Magnification : 40

* *Magnification : 100

** *Magnification : 200

Appendix 16. The microscope image of ≥ 50㎛ in control (untreated) seawater by treatment of Purimar™ BWMS (Day0)

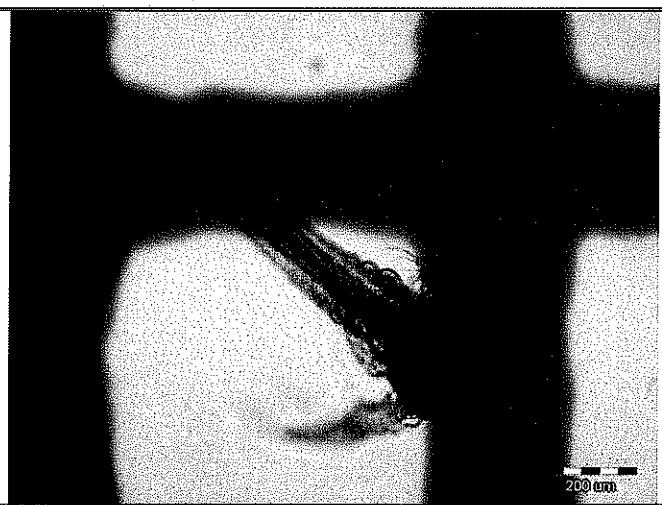
Specific Name	Control (untreated) seawater
*Artemia salina**	
Copepod nauplii**	
*Brachionus plicatilis***	

Protoperidinium* sp.*	

* Magnification : 100

** Magnification : 200

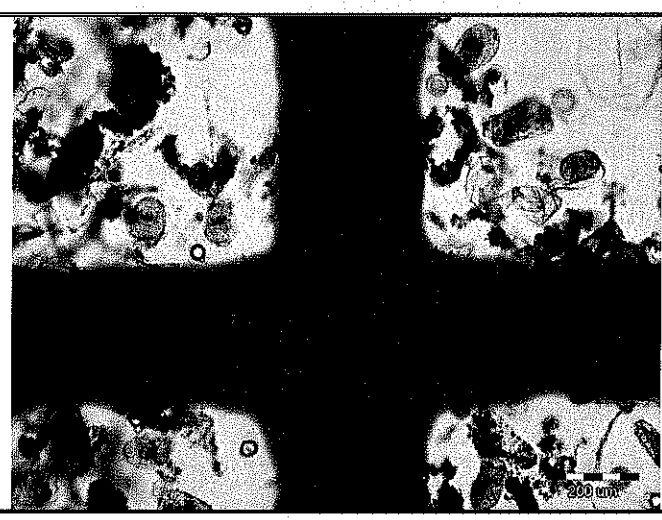
Appendix 17. The microscope image of ≥ 50㎛ in control (untreated) seawater by treatment of Purimar™ BWMS (Day5)

Specific Name	Control (untreated) seawater
*Artemia salina**	
*Brachionus plicatilis***	

*Magnification : 100

** Magnification : 200

Appendix 18. The microscope image of ≥ 50㎛ in treated ballast seawater by treatment of Purimar™ BWMS (Day0)

Specific Name	Treated ballast seawater
-	

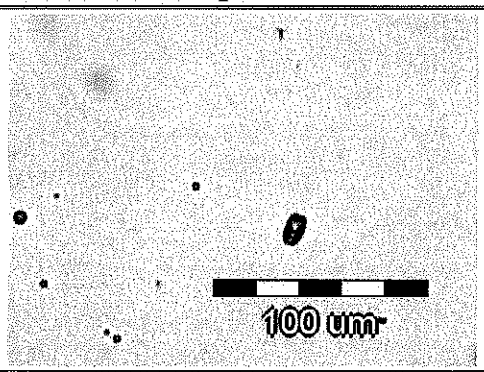
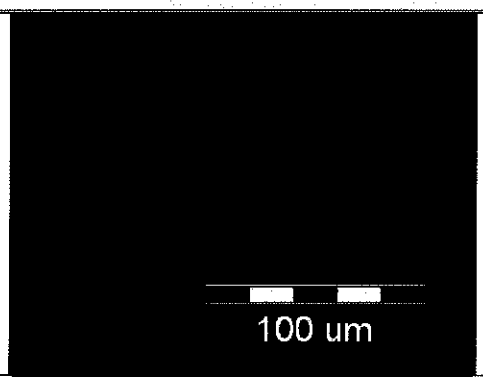
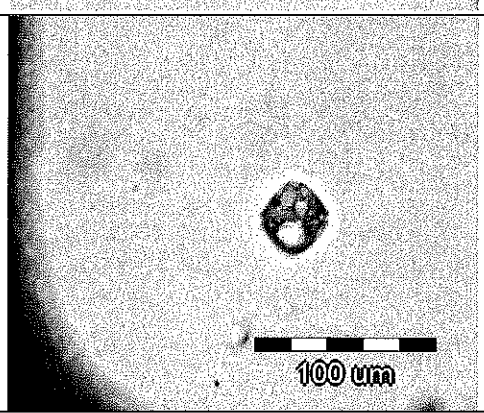
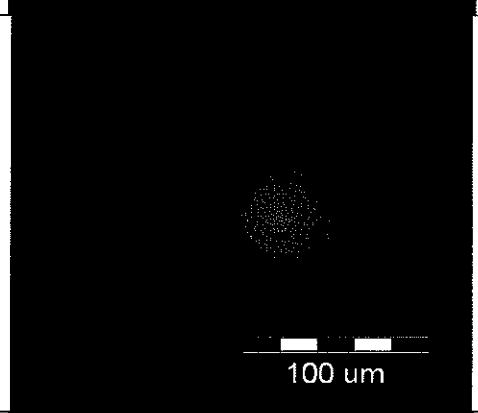
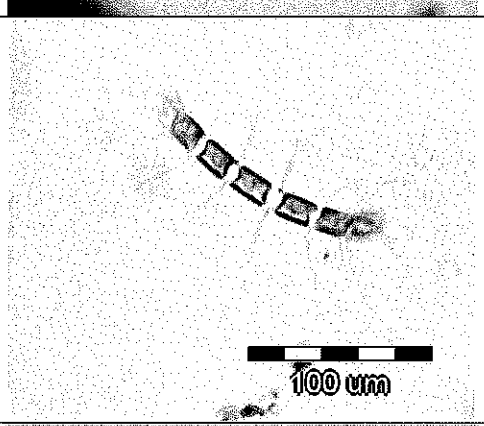
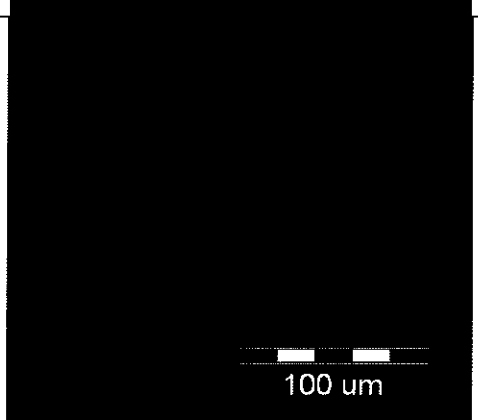
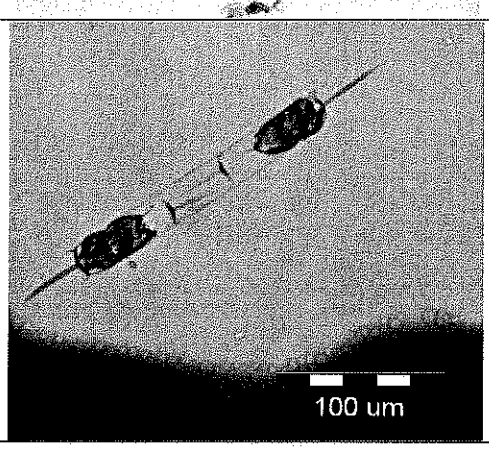
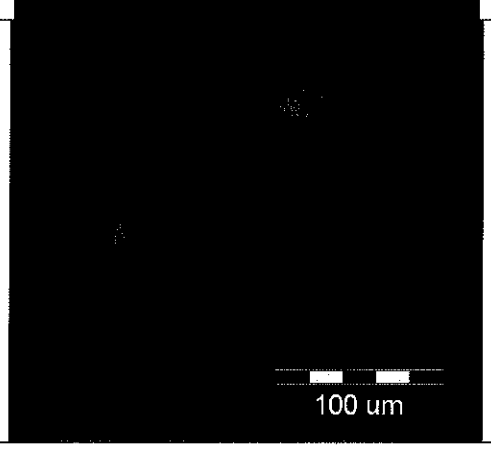
Magnification : 10

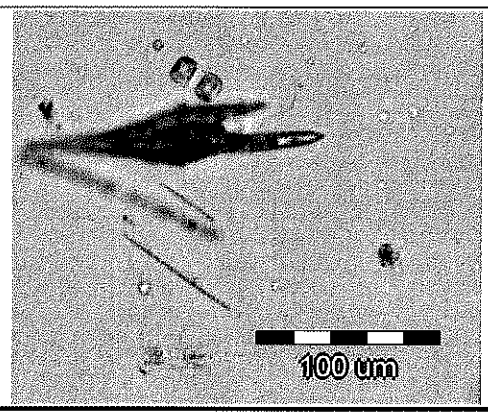
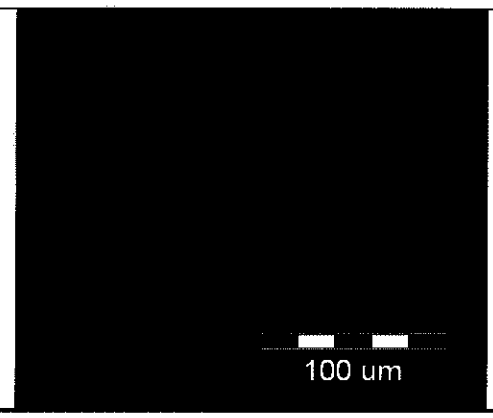
Appendix 19. The microscope image of ≥ 50㎛ in treated ballast seawater by treatment of Purimar™ BWMS (Day5)

Specific Name	Treated ballast seawater
-	

Magnification : 10

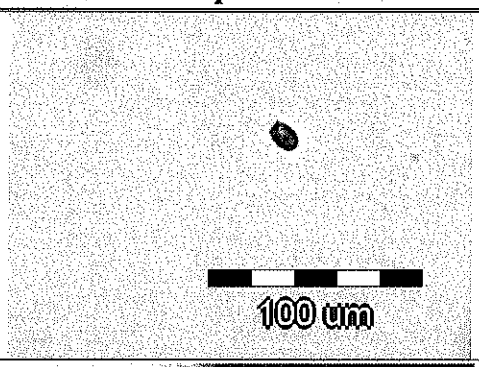
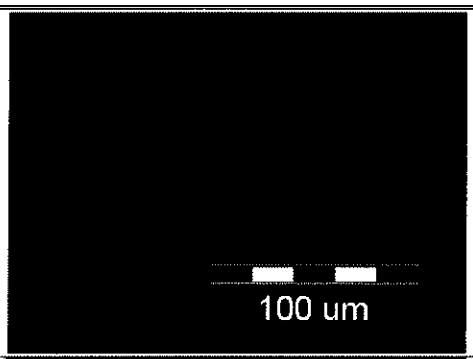
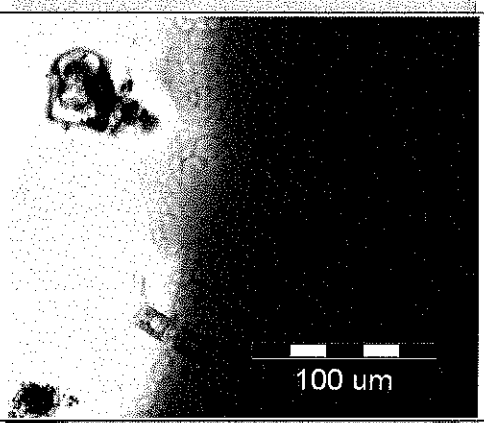
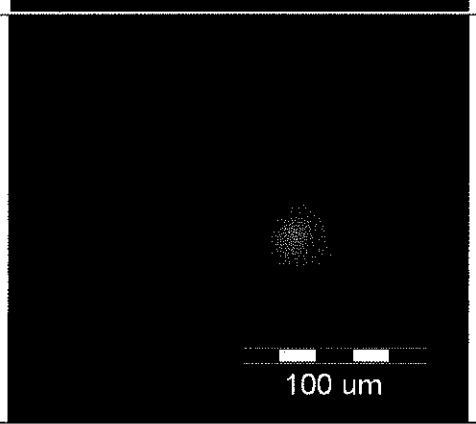
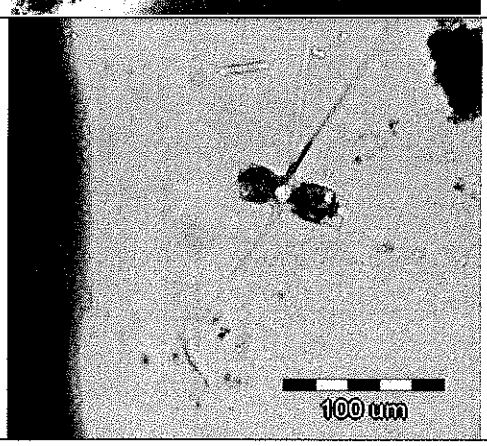
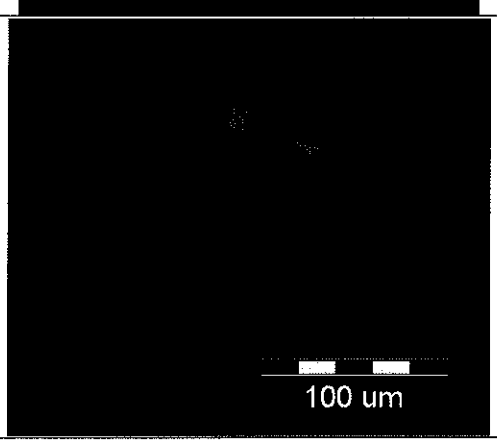
Appendix 20. The microscope image of 10㎛−50㎛ in original water (Day0)

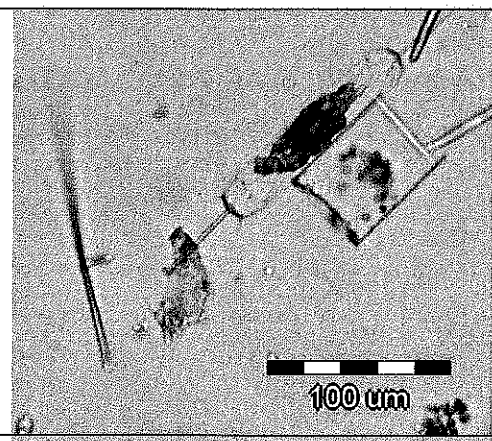
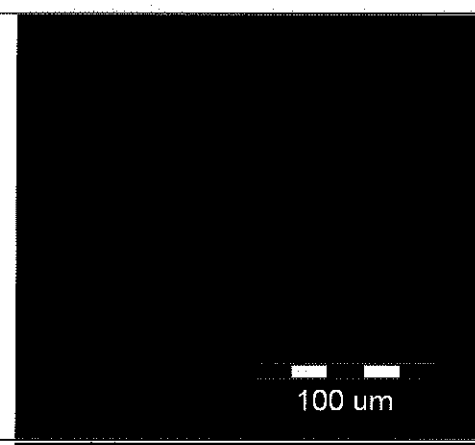
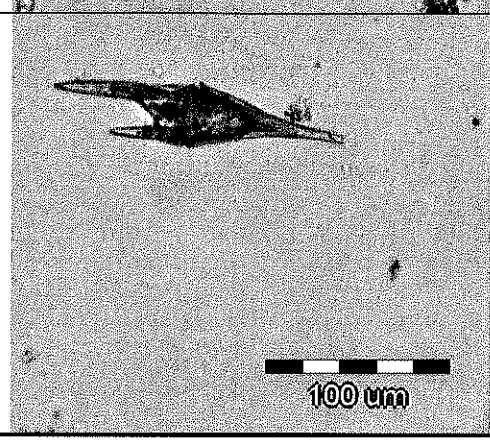
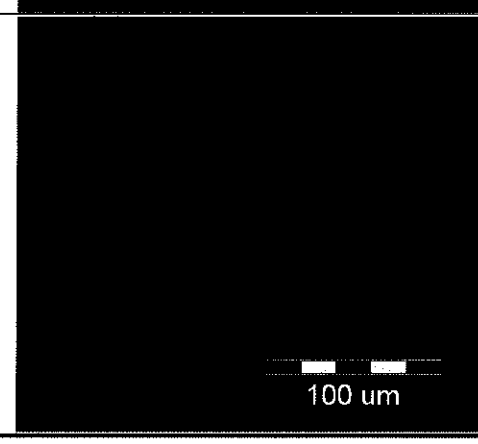
Specific Name	Control (untreated) seawater	
	Optical	Fluorescence
Tetraselmis suecica		
Protoperidinium sp.		
Chaetoceros sp.		
Ditylum Sp.		

***Ceratium* sp.**		

Magnification : 200

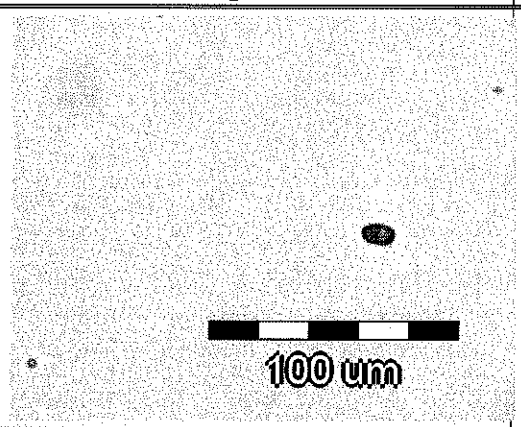
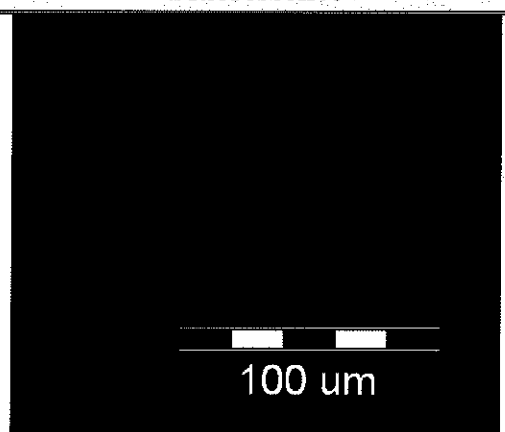
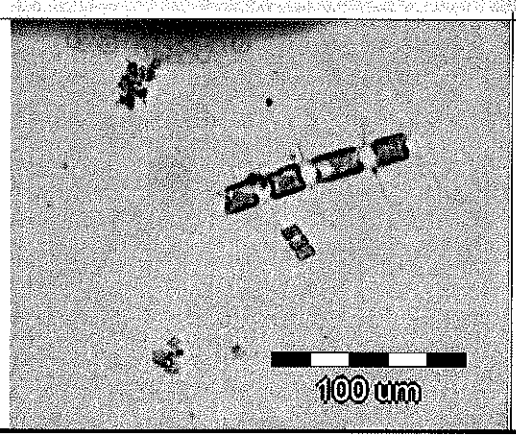
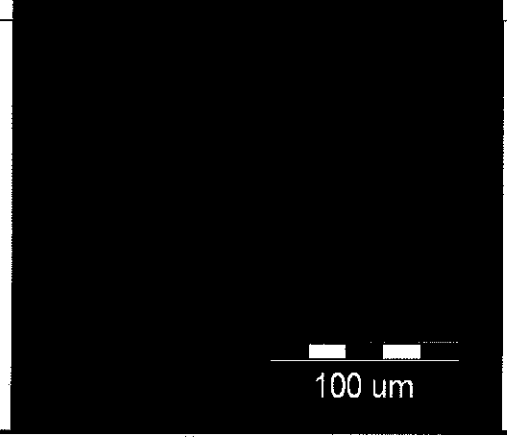
Appendix 21. The microscope image of 10㎛−50㎛ in control (untreated) seawater by treatment of Purimar™ BWMS (Day0)

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

***Ditylum* Sp.**		
***Ceratium* sp.**		

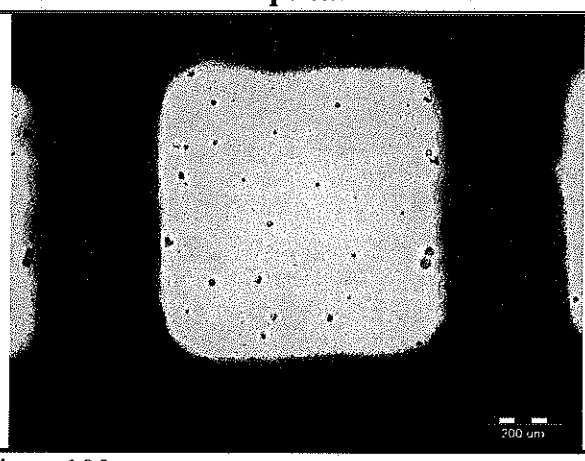
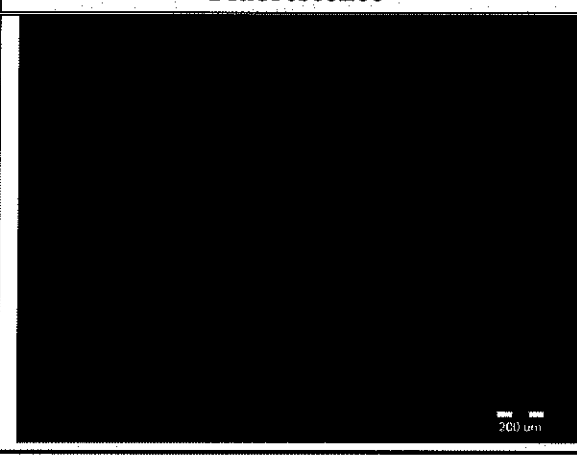
Magnification : 200

Appendix 22. 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		
Chaetoceros sp.		

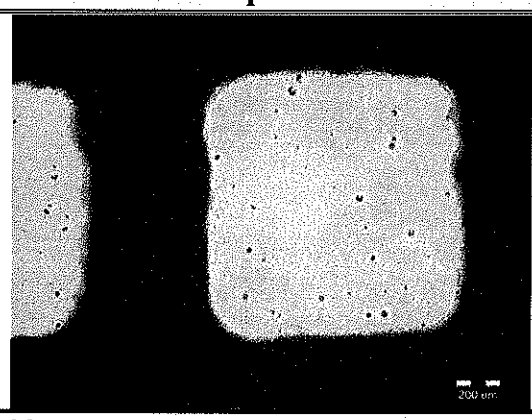
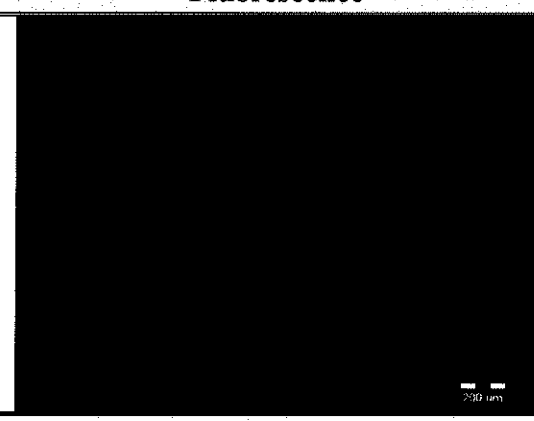
Magnification : 200

Appendix 23. The microscope image of 10㎛~50㎛ in treated ballast seawater by treatment of Purimar™ BWMS (Day0)

Specific Name	Treated ballast seawater	
	Optical	Fluorescence
-		

Magnification : 100

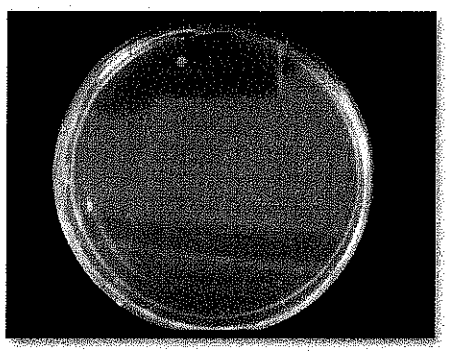
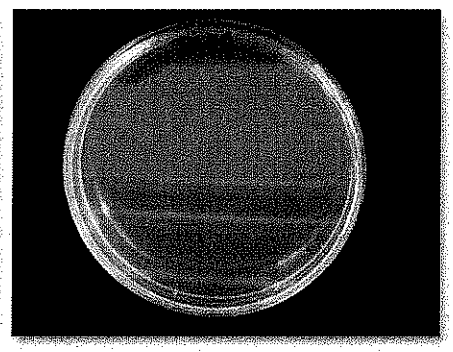
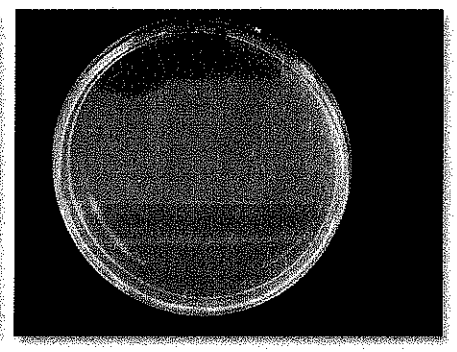
Appendix 24. The microscope image of 10 ㎛–50 ㎛ in treated ballast seawater by treatment of Purimar™ BWMS (Day5)

Specific Name	Treated ballast seawater	
	Optical	Fluorescence
-		

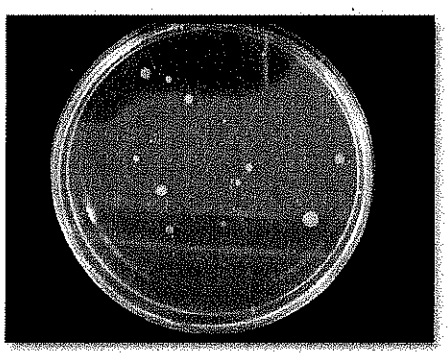
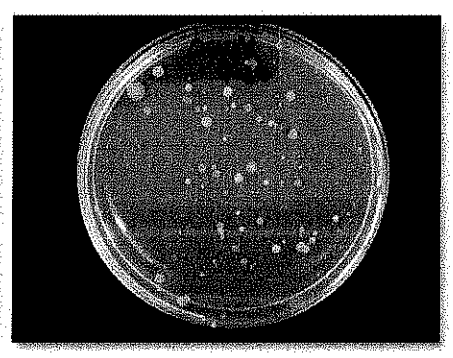
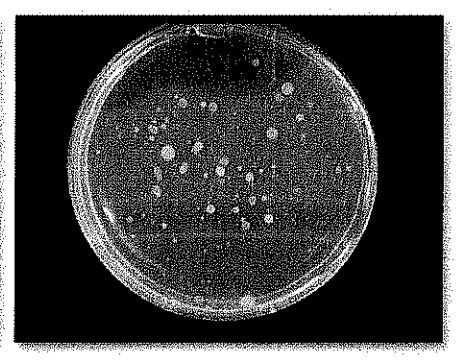
Magnification : 200

Appendix 25. The image of heterotrophic bacteria: Day 0

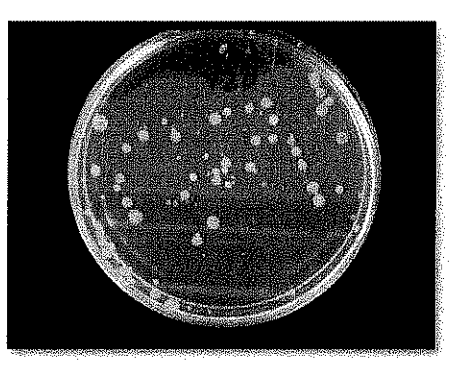
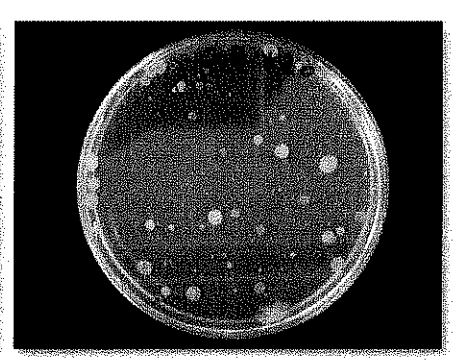
Treated ballast seawater

Top	Middle	Bottom
		

Original water

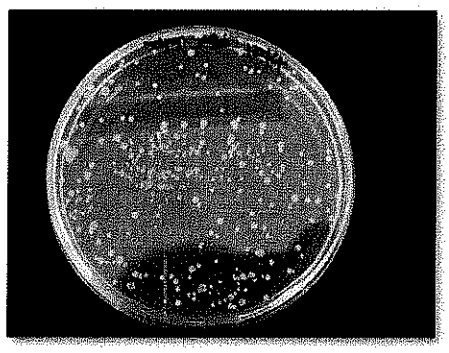
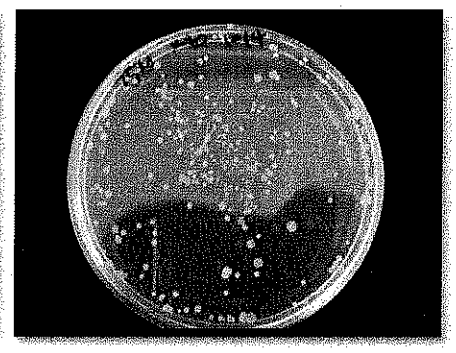
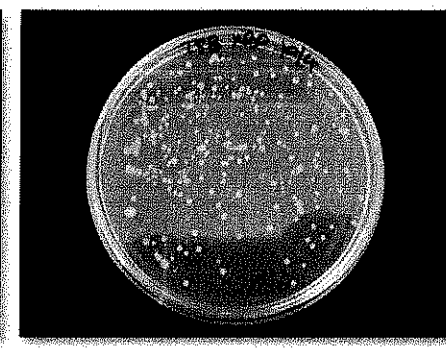
Top	Middle	Bottom
		

Control (untreated) seawater

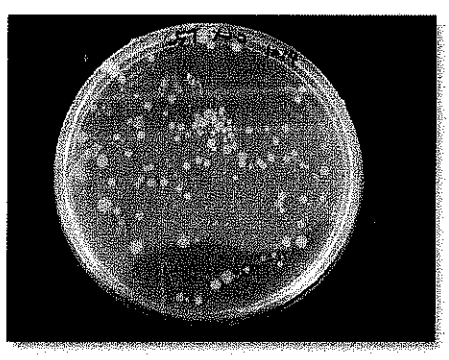
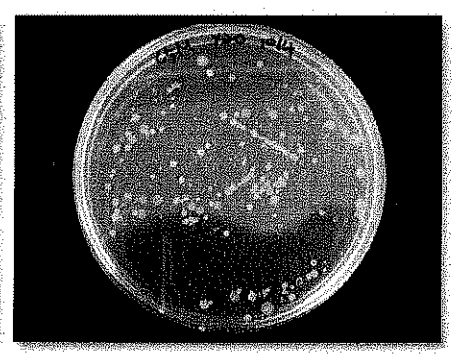
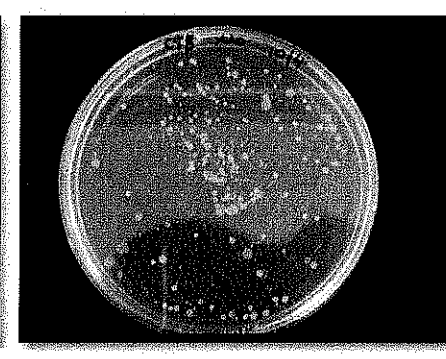
Top	Middle	Bottom
		

Appendix 26. The image of heterotrophic bacteria: Day 5

Treated ballast seawater

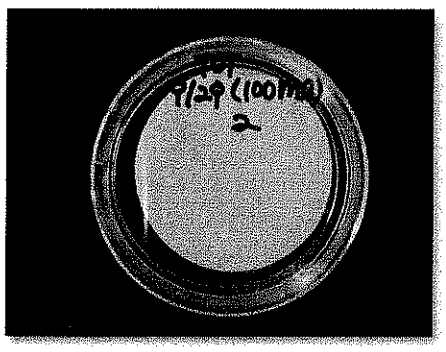
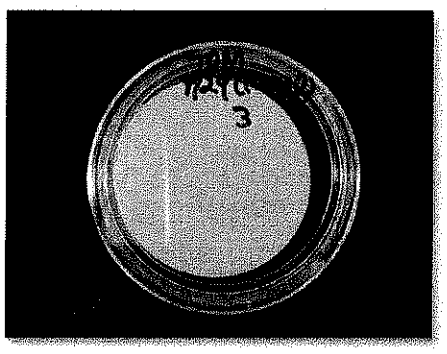
Top	Middle	Bottom
		

Control (untreated) seawater

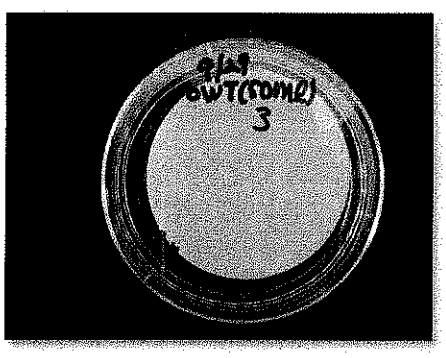
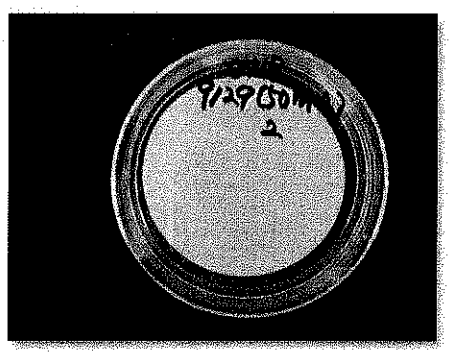
Top	Middle	Bottom
		

Appendix 27. The image of *Escherichia coli*: Day 0

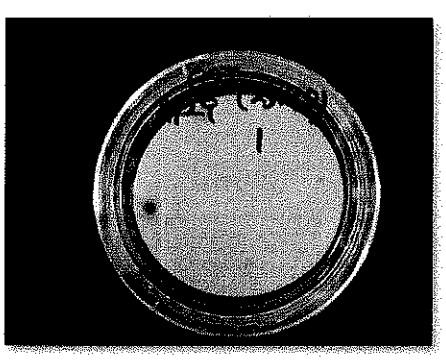
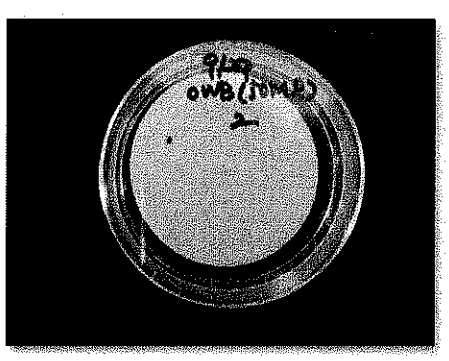
Treated ballast seawater

Top	Middle	Bottom
		

Original water

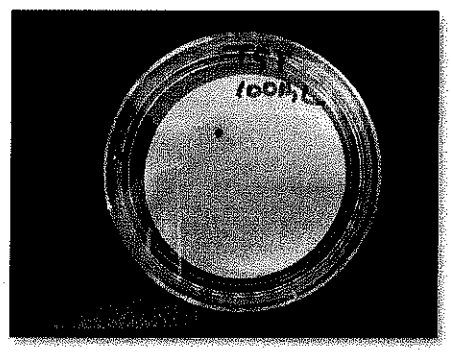
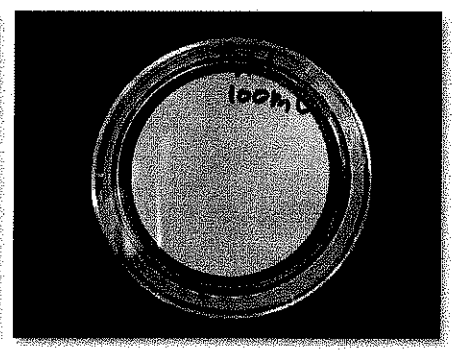
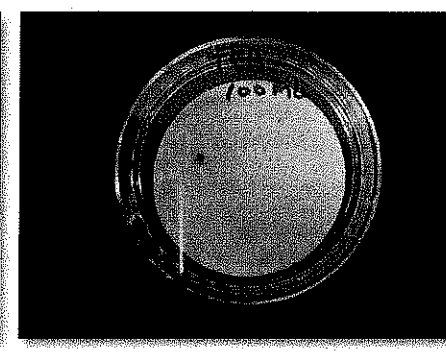
Top	Middle	Bottom
		

Control (untreated) seawater

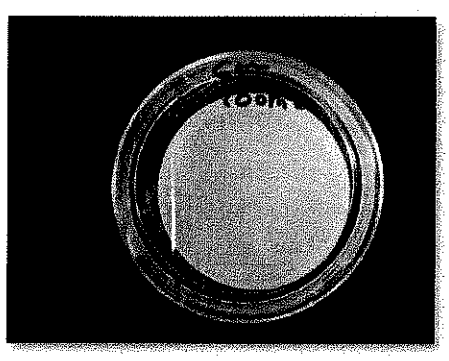
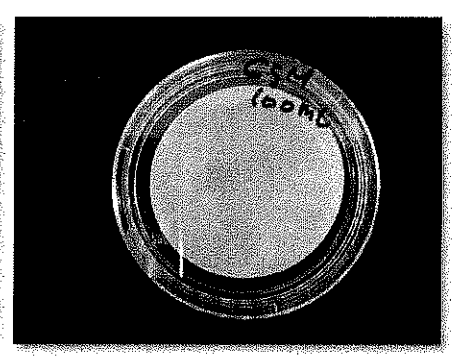
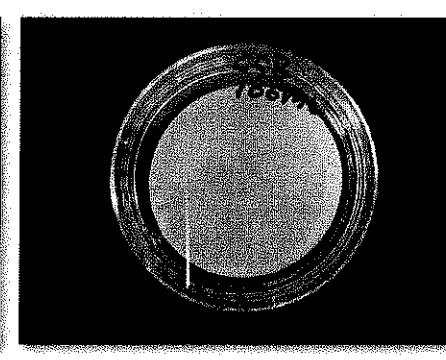
Top	Middle	Bottom
		

Appendix 28. The image of *Escherichia coli*: Day 5

Treated ballast seawater

Top	Middle	Bottom
		

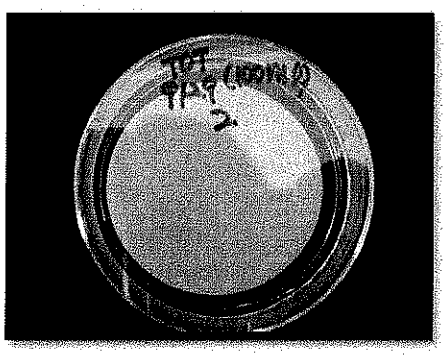
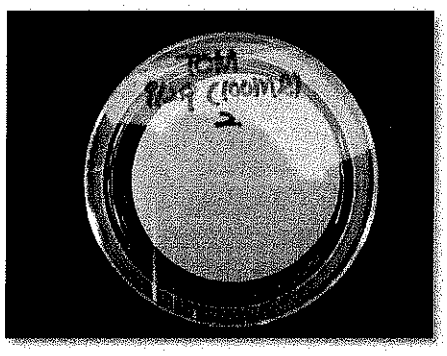
Control (untreated) seawater

Top	Middle	Bottom
		

Appendix 29. The image of *Enterococcus faecalis*: Day 0

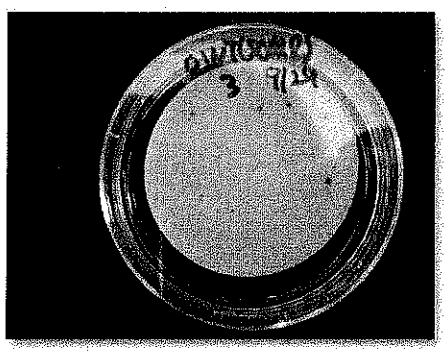
Treated ballast seawater

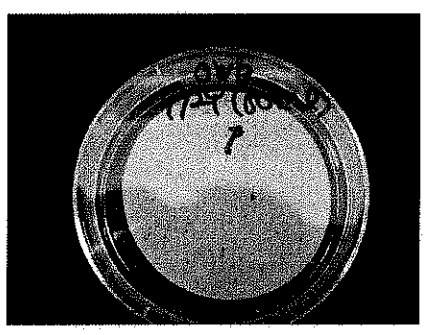
Top **Middle** **Bottom**

Original water

Top **Middle** **Bottom**

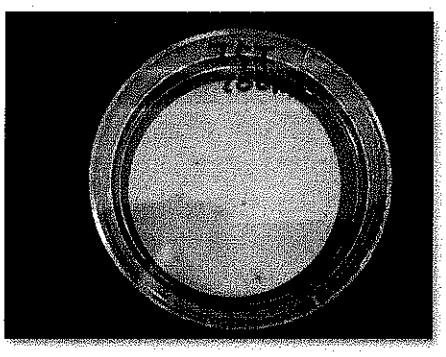
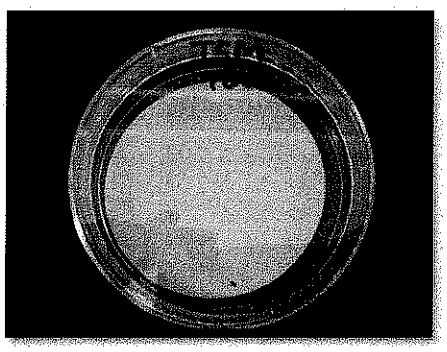
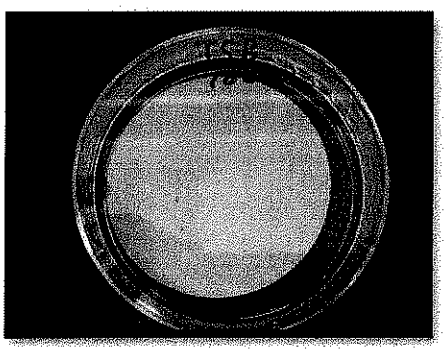
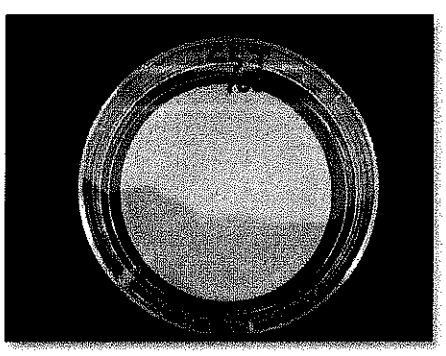
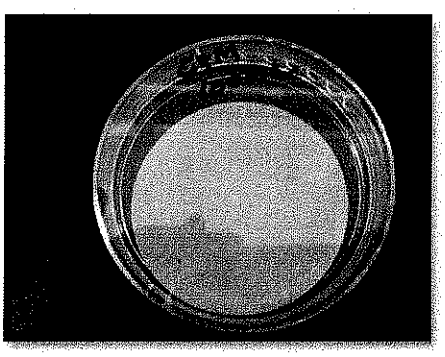
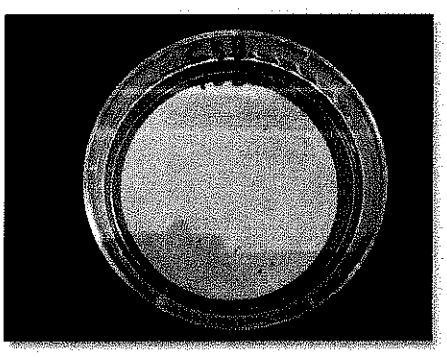




Control (untreated) seawater

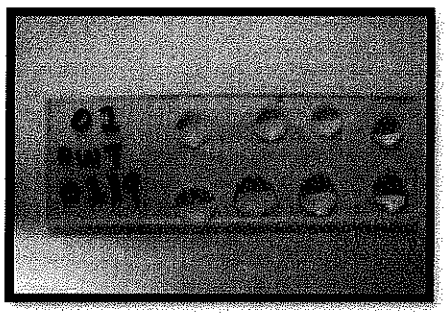
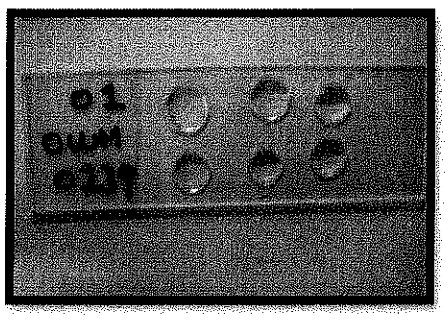
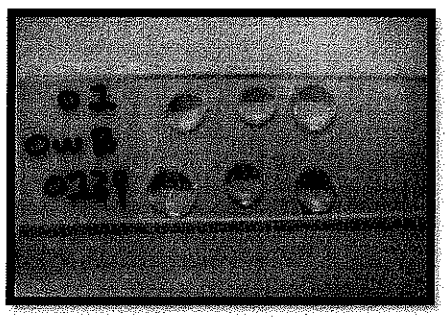
Top **Middle** **Bottom**

Appendix 30. The image of *Enterococcus faecalis*: Day 5

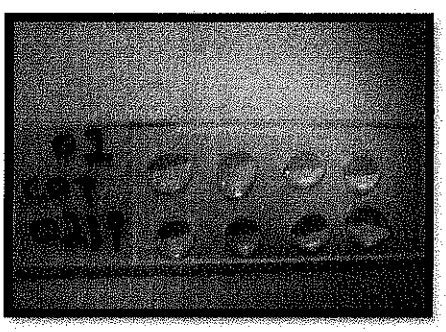
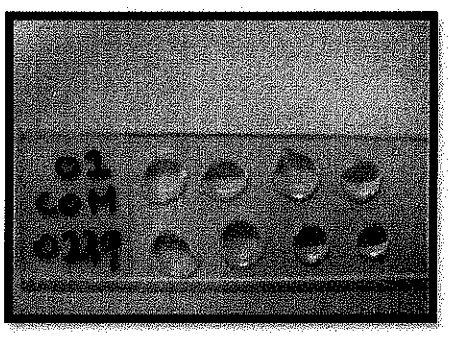
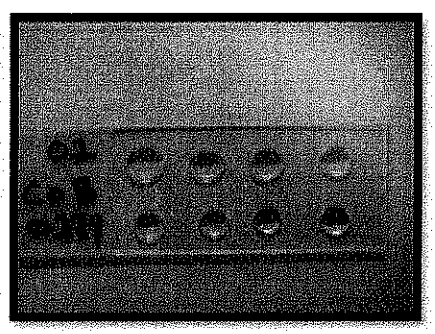
Treated ballast seawater		
Top	**Middle**	**Bottom**
		
Control (untreated) seawater		
Top	**Middle**	**Bottom**
		

Appendix 31. The image of *Vibrio cholera* O1, O139: Day 0

Original water

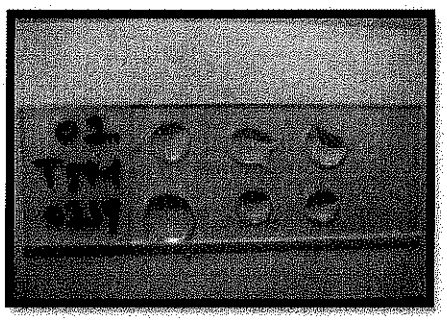
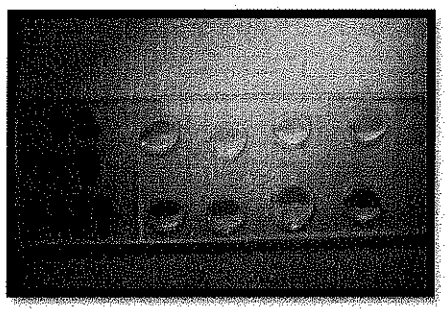
Top	Middle	Bottom
		

Control (untreated) seawater

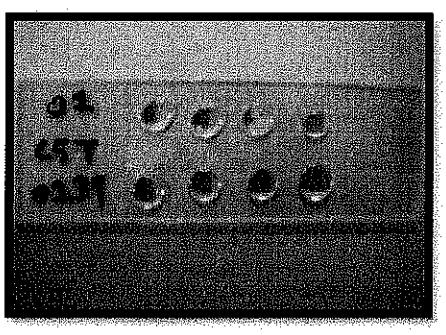
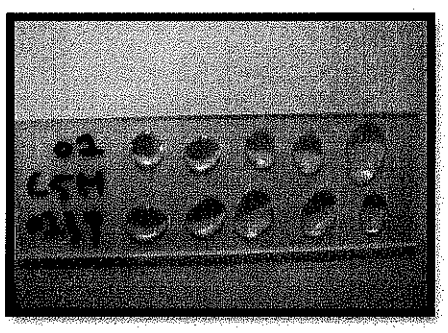
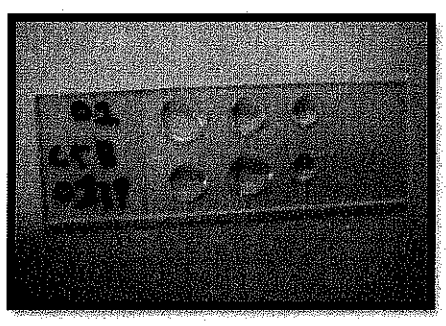
Top	Middle	Bottom
		

Appendix 32. The image of *Vibrio cholera* O1, O139: Day 5

Treated ballast seawater

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