Test Report

Project name	Purimar™
Study number	TW-Land(6)
Test number	LET-10-007
Title	Efficacy test of Purimar™ Ballast Water Management System (land scale)
Prepared by	Byong-Jin, Kim

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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		Aug, 16, 2010	Aug, 16, 2010	Aug, 16, 2010
Sampling	1st	Aug, 19, 2010	Aug, 19, 2010	Aug, 19, 2010
	2nd	Aug, 24, 2010	Aug, 24, 2010	Aug, 24, 2010
Test substance	1st	Aug, 19, 2010	Aug, 19, 2010	Aug, 19, 2010
	2nd	Aug, 24, 2010	Aug, 24, 2010	Aug, 24, 2010
Test procedures (Bacteria test)	1st	Aug, 19, 2010	Aug, 19, 2010	Aug, 19, 2010
	2nd	Aug, 24, 2010	Aug, 24, 2010	Aug, 24, 2010
Observation and counting (≥50㎛, 10㎛-50㎛)	1st	Aug, 19, 2010	Aug, 19, 2010	Aug, 19, 2010
	2nd	Aug, 24, 2010	Aug, 24, 2010	Aug, 24, 2010
Observation and counting (Bacteria test)	1st	Aug, 20, 2010	Aug, 20, 2010	Aug, 20, 2010
	2nd	Aug, 21, 2010	Aug, 21, 2010	Aug, 21, 2010
	3rd	Aug, 25, 2010	Aug, 25, 2010	Aug, 25, 2010
	4th	Aug, 27, 2010	Aug, 27, 2010	Aug, 27, 2010
Raw data		Aug, 27, 2010	Aug, 27, 2010	Aug, 27, 2010
Test report		Sep, 17, 2010	Sep, 17, 2010	Sep, 17, 2010

Eunyang, Kang (Sign) — Sep. 17. 2010

Eun-Young, Kang / Quality Assurance — 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	Ha-na. Song (sign)	Sep. 17. 2010
	Eun-Jung. Jung (sign)	Sep. 17. 2010
	Sun-Ju Choi (sign)	Sep. 17. 2010
	Jae-woo, Ga (sign)	Sep. 17. 2010
QA/QC	Eun-young, Kang (sign)	Sep. 17, 2010
Efficacy Testing Manager	Byong-Jin Ka (sign)	17th, Sep, 2010

1. Results

1.1 Sampling point (Freshwater)

GPS coordinate	35°05.120N 128°56.812E

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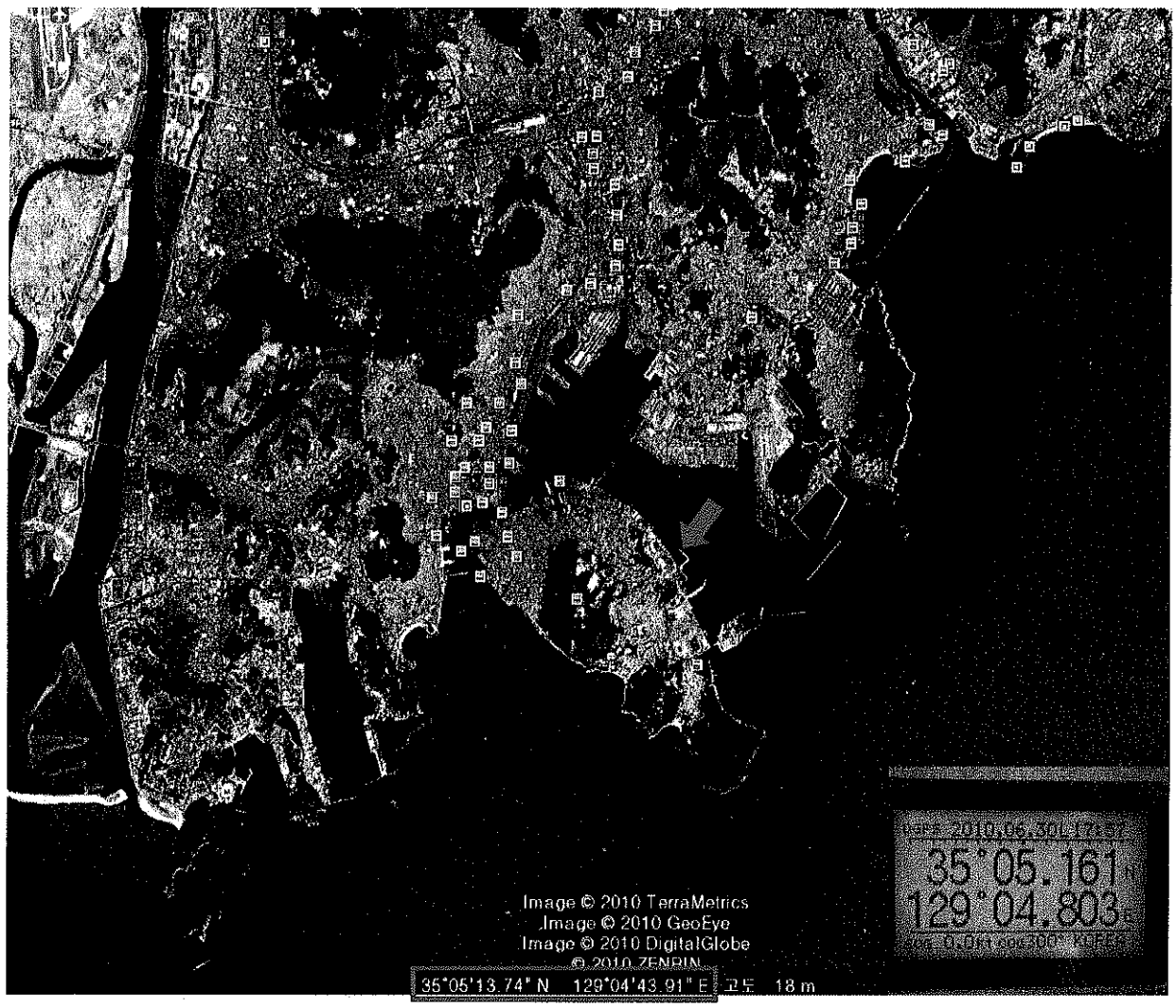
1.2 Sampling point(Seawater)

GPS coordinate	35°05.161N 129°04.803E



1.3 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-0819	16.60±0.28	11.00±0.85	5.60	66.3±0.3
	OwM-0819	17.25±0.07	10.85±1.06	6.40	67.7±1.6
	OwB-0819	16.40±0.42	9.70±0.00	6.70	65.8±1.4
Acceptability (Salinity : 3 ~ 32 PSU)		-	Acceptable (> 5 mg/L)	Acceptable (> 5 mg/L)	Acceptable (> 50 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-0819	7.78±0.01	22.37±0.00	18.63±0.00	8.07±0.01	23.2±0.1	1.010±0.000
	OwM-0819	7.82±0.00	22.37±0.01	18.63±0.00	7.98±0.00	22.0±0.1	1.020±0.000
	OwB-0819	7.65±0.01	22.36±0.00	18.61±0.00	7.92±0.00	24.6±0.0	0.991±0.000
	C0T-0819	7.63±0.01	23.11±0.01	19.57±0.00	7.90±0.01	27.1±0.1	2.713±0.006
	C0M-0819	7.51±0.00	23.16±0.01	19.54±0.00	7.80±0.01	21.3±0.1	2.477±0.006
	C0B-0819	7.68±0.00	23.14±0.01	19.61±0.00	7.70±0.01	22.7±0.1	2.430±0.000
	T0T-0819	7.40±0.01	23.11±0.01	18.65±0.01	7.53±0.01	29.2±0.1	0.284±0.000
	T0M-0819	7.77±0.00	23.70±0.01	18.67±0.00	7.71±0.01	22.7±0.1	0.223±0.000
	T0B-0819	7.66±0.00	23.43±0.00	18.85±0.00	7.78±0.01	22.5±0.1	0.230±0.000
Day 5	C5T-0824	6.92±0.01	26.65±0.01	19.83±0.01	6.24±0.01	2.3±0.1	0.206±0.000
	C5M-0824	7.03±0.00	26.49±0.01	19.70±0.01	6.38±0.00	1.5±0.1	0.301±0.000
	C5B-0824	6.92±0.00	26.67±0.01	19.70±0.00	6.29±0.01	2.0±0.1	0.223±0.000
	T5T-0824	7.21±0.00	25.99±0.00	18.83±0.00	6.54±0.00	2.7±0.1	0.025±0.000
	T5M-0824	7.05±0.01	26.10±0.01	18.83±0.01	6.44±0.00	0.1±0.0	0.022±0.000
	T5B-0824	7.03±0.00	26.06±0.01	18.86±0.00	6.56±0.01	0.4±0.0	0.017±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-0819	7.82±0.00	27.79±0.03	19.53±0.01	9.00±0.00	24.9±0.7
	OwM-0819	7.83±0.00	24.12±0.01	19.59±0.00	8.95±0.00	24.4±0.3
	OwB-0819	7.87±0.00	23.43±0.00	19.65±0.01	8.91±0.00	23.7±0.2
	C0T-0819	7.91±0.00	22.89±0.01	20.68±0.01	8.90±0.01	35.4±0.4
	C0M-0819	7.92±0.00	22.87±0.00	20.71±0.01	8.79±0.01	28.3±0.6
	C0B-0819	7.92±0.00	22.93±0.02	20.65±0.02	8.72±0.01	27.9±0.3
	T0T-0819	7.92±0.00	17.69±0.01	19.30±0.01	8.26±0.01	24.8±0.1
	T0M-0819	7.86±0.01	15.93±0.01	19.26±0.00	7.94±0.01	24.0±0.0
	T0B-0819	7.92±0.00	17.51±0.01	19.87±0.01	8.13±0.01	23.7±0.0
Day 5	C5T-0824	7.11±0.00	11.8±0.0	19.46±0.03	7.75±0.02	4.31±0.02
	C5M-0824	7.21±0.01	9.2±0.1	19.44±0.01	8.22±0.00	3.36±0.06
	C5B-0824	7.30±0.01	9.8±0.0	19.58±0.01	7.96±0.01	3.94±0.04
	T5T-0824	7.49±0.00	12.4±0.0	18.71±0.00	8.69±0.02	4.26±0.06
	T5M-0824	7.60±0.01	10.8±0.1	18.65±0.03	8.84±0.04	0.98±0.11
	T5B-0824	7.61±0.00	10.2±0.0	18.74±0.02	8.42±0.00	1.30±0.08

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

1.4 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*	132500 ± 5268	130 ~ 180
		Crustacean nauplii	1500 ± 500	120 ~ 160
		Oithona sp.	1833 ± 289	110 ~ 150
		Sinocalanus sp.	333 ± 289	150 ~ 180
	Rotifera	*Brachionus* ssp.	1018333 ± 46458	85 ~ 150
	Dinophyta	*Protoperidinium* sp.	8333 ± 2887	51 ~ 53
Middle	Arthropoda	*Artemia salina*	170500 ± 4822	130 ~ 180
		Crustacean nauplii	667 ± 289	120 ~ 160
		Pseudodiaptomus sp.	500 ± 0	100 ~ 140
		Oithona sp.	1667 ± 577	110 ~ 150
	Rotifera	*Brachionus* ssp.	1105000± 37749	85 ~ 150
	Dinophyta	*Protoperidinium* sp.	13333 ± 2887	51 ~ 53
Bottom	Arthropoda	*Artemia salina*	166000 ± 6062	130 ~ 180
		Crustacean nauplii	1500 ± 500	120 ~ 160
		Pseudodiaptomus sp.	500 ± 500	120 ~ 160
	Rotifera	*Brachionus* ssp.	1098333 ± 57951	85 ~ 150
Total	7 species 3 phyla/divisions		1240278 ± 68254	-
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*	1123 ± 55	11 ~ 12
	Bacillariophyta	*Thalassiosira* sp.	3 ± 1	20 ~ 40
		Chaetoceros sp.	6 ± 2	15 ~ 45
		Ditilum sp.	1 ± 1	30 ~ 45
	Dinophyta	*Protoperidinium* sp.	6 ± 1	20 ~ 45
Middle	Chlorophyta	*Tetraselmis suecica*	1190 ± 60	11 ~ 12
	Bacillariophyta	*Thalassiosira* sp.	3 ± 1	20 ~ 40
		Chaetoceros sp.	5 ± 1	15 ~ 45
	Dinophyta	*Protoperidinium* sp.	9 ± 2	20 ~ 45
Bottom	Chlorophyta	*Tetraselmis suecica*	1063 ± 38	11 ~ 12
	Bacillariophyta	*Thalassiosira* sp.	2 ± 1	20 ~ 40
		Ditilum sp.	1 ± 1	30 ~ 45
	Dinophyta	*Protoperidinium* sp.	6 ± 1	20 ~ 45
Total	5 species 3 phyla/divisions		1140 ± 67	-
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

1.5 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)]	1162833 ± 41038	Influent condition: Acceptable ($\geq 10^5$ ind./m^3)	
		M [b)]	1291667 ± 43921		
		B [c)]	1266333 ± 63447		
Control (untreated) brackish water	Day 0 (C0)	T	886000 ± 38636	-	-
		M	1083833 ± 46522		
		B	903333 ± 31608		
	Day 5 (C5)	T	24917 ± 1010	Effluent condition: Acceptable ($\geq 10^2$ ind./m^3)	
		M	23667 ± 764		
		B	21000 ± 1090		
Treated ballast brackish water	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)]	1140 ± 55	Influent condition: Acceptable ($\geq 10^3$ ind./mL)	-
		M [b)]	1207 ± 59		
		B [c)]	1073 ± 38		
Control (untreated) brackish water	Day 0 (C0)	T	1181 ± 39	-	-
		M	1163 ± 92		
		B	1074 ± 56		
	Day 5 (C5)	T	126 ± 8	Effluent condition: Acceptable ($\geq 10^2$ ind./ ml)	
		M	105 ± 7		
		B	101 ± 10		
Treated ballast brackish water	Day 0 (T0)	T	0 ± 0	Effluent condition : Acceptable (<10 ind./ ml)	100.0 [d)]
		M	0 ± 0		
		B	0 ± 0		
Treated ballast brackish water	Day 5 (T5)	T	0 ± 0	Effluent condition : Acceptable (<10 ind./ ml)	100.0 [e)]
		M	0 ± 0		
		B	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.

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)]	15000 ± 1311	Influent condition: Acceptable ($\geq 10^4$ CFU/mL)	-
		M [b)]	18667 ± 2157		
		B [c)]	12333± 1137		
Control (untreated) brackish water	Day 0 (C0)	T	42600 ± 4331	-	-
		M	24400 ± 1637		
		B	18667 ± 808		
	Day 5 (C5)	T	38400 ± 1000	-	
		M	62600 ± 10912		
		B	54533 ± 643		
Treated ballast brackish water	Day 0 (T0)	T	67 ± 35	-	99.8 [d)]
		M	50 ± 35		
		B	57 ± 15		
	Day 5 (T5)	T	101600 ± 3418	-	-138.8 [e)]
		M	63533 ± 2610		
		B	53867 ± 3331		

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)]	350 ± 115	-	
		M [b)]	280 ± 56		
		B [c)]	317 ± 25		
Control (untreated) brackish water	Day 0 (C0)	T	1173 ± 110	-	-
		M	993 ± 15		
		B	950 ± 26		
	Day 5 (C5)	T	4 ± 2	-	
		M	2 ± 1		
		B	1 ± 1		
Treated ballast brackish water	Day 0 (T0)	T	0 ± 0	Effluent condition: Acceptable (<250 CFU/ 100ml)	100.0 [d)]
		M	0 ± 0		
		B	0 ± 0		
	Day 5 (T5)	T	0 ± 0	Effluent condition: Acceptable (<250 CFU/ 100ml)	100.0 [e)]
		M	0 ± 1		
		B	0 ± 1		

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)]	290 ± 53	-	
		M[b)]	267 ± 32		
		B[c)]	283 ± 15		
Control (untreated) brackish water	Day 0 (C0)	T	TNTC (> 2000)[d)]	-	-
		M	TNTC (> 2000)[d)]		
		B	TNTC (> 2000)[d)]		
	Day 5 (C5)	T	22 ± 2	-	
		M	1 ± 1		
		B	17 ± 10		
Treated ballast brackish water	Day 0 (T0)	T	0 ± 0	Effluent condition: Acceptable (<100 CFU/ 100ml)	100.0 [e)]
		M	0 ± 0		
		B	0 ± 0		
	Day 5 (T5)	T	20 ± 3	Effluent condition: Acceptable (<100 CFU/ 100ml)	99.4 [f)]
		M	16 ± 2		
		B	1 ± 1		

a) T: Top, b) M: Middle c) B: Bottom,
d) Colonies were too numerous to count (TNTC), used upper limit count with smallest filtration volume,
e) Calculation = (C0-T0)/C0*100, f) 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	-	-
		M [b)]	0 ± 0		
		B [c)]	0 ± 0		
Control (untreated) brackish water	Day 0 (C0)	T	0 ± 0	-	-
		M	0 ± 0		
		B	0 ± 0		
	Day 5 (C5)	T	0 ± 0	-	
		M	0 ± 0		
		B	0 ± 0		
Treated ballast brackish water	Day 0 (T0)	T	0 ± 0	Effluent condition: Acceptable (<1 CFU/ 100ml)	N.D [d)]
		M	0 ± 0		
		B	0 ± 0		
	Day 5 (T5)	T	0 ± 0	Effluent condition: Acceptable (<1 CFU/ 100ml)	N.D
		M	0 ± 0		
		B	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: 3 ~ 22 PSU (Salinity should be separated by at least 10 PSU)
  - DOC: > 5 mg/L
  - POC: > 5 mg/L
  - TSS: > 50 mg/L

Date	Sample ID & lot No.	Salinity [a)] (‰)	DOC (mg/L)	POC (mg/L)	TSS (mg/L)
Day 0	OwT-0819	18.63±0.00	11.00±0.85	5.60	66.3±0.3
	OwM-0819	18.63±0.00	10.85±1.06	6.40	67.7±1.6
	OwB-0819	18.61±0.00	9.70±0.00	6.70	65.8±1.4

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	714000	1780	-
Lab	OwT	1162833 ± 41038	1140 ± 55	15000 ± 1311
	OwM	1291667 ± 43921	1207 ± 59	18667 ± 2157
	OwB	1266333 ± 63447	1073 ± 38	12333± 1137

- 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	7 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	11:26~11:37	17:00
	OwM	11:26~11:37	
	OwB	11:25~11:36	
	C0T	12:45~12:53	16:40
	C0M	12:45~12:52	
	C0B	12:44~12:52	
	T0T	15:21~15:37	19:00
	T0M	15:16~15:37	
	T0B	14:48~15:37	
Day 5	C5T	15:56~16:27	20:30
	C5M	15:56~16:27	
	C5B	15:55~16:26	
	T5T	16:30~16:49	20:30
	T5M	16:20~16:48	
	T5B	16:09~16:48	
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	24917 ± 1010	126 ± 8
Middle	23667 ± 764	105 ± 7
Bottom	21000 ± 1090	101 ± 10

3. Conclusion

3.1 Biological efficacy in treated ballast brackish water by Purimar™ BWMS

- Treated ballast brackish water by Purimar™ BWMS were capable of eliminating organism larger than 50 μm with an efficiency of 100%.
- Treated ballast brackish water by Purimar™ BWMS were capable of eliminating organism between 10 and 50 μm with an efficiency of 100%.
- Treated ballast brackish water by Purimar™ BWMS was capable of eliminating heterotrophic bacteria with an efficiency of 99.8 %. at day 0.
- But treated ballast brackish water by Purimar™ BWMS was incapable of eliminating heterotrophic bacteria at day 5.
- Treated ballast brackish water by Purimar™ BWMS were capable of eliminating *Escherichia coli* with an efficiency of 100% at day 0, 5.
- Treated ballast brackish water by Purimar™ BWMS were capable of eliminating *Enterococcus faecalis* with an efficiency of 100, 99.4% at day 0, 5.
- *Vibrio cholera* O1, O139 were not detected.
- Therefore, treated ballast brackish water by Purimar™ BWMS was capable of removing zooplankton, phytoplankton 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-0819	R1	16.40	10.40	66.5
		R2	16.80	11.60	66.5
		R3	-	-	66.0
	OwM-0819	R1	17.20	10.10	66.5
		R2	17.30	11.60	69.5
		R3	-	-	67.0
	OwB-0819	R1	16.10	9.70	65.0
		R2	16.70	9.70	67.5
		R3	-	-	65.0

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
Day 0	OwT-0819	R1	7.77	22.37	18.63	8.07	23.1	1.010
		R2	7.78	22.37	18.63	8.08	23.3	1.010
		R3	7.78	22.37	18.63	8.07	23.1	1.010
	OwM-0819	R1	7.82	22.37	18.63	7.98	21.9	1.020
		R2	7.82	22.37	18.63	7.98	22.0	1.020
		R3	7.82	22.38	18.63	7.98	22.1	1.020
	OwB-0819	R1	7.65	22.36	18.61	7.92	24.6	0.991
		R2	7.65	22.36	18.61	7.92	24.6	0.991
		R3	7.66	22.36	18.61	7.92	24.6	0.991
	C0T-0819	R1	7.63	23.11	19.57	7.90	27.1	2.710
		R2	7.63	23.11	19.57	7.91	27.2	2.720
		R3	7.64	23.10	19.57	7.90	27.1	2.710
	C0M-0819	R1	7.51	23.16	19.54	7.80	21.4	2.480
		R2	7.51	23.16	19.54	7.80	21.3	2.480
		R3	7.51	23.17	19.54	7.81	21.3	2.470
	C0B-0819	R1	7.68	23.14	19.61	7.70	22.6	2.430
		R2	7.68	23.14	19.61	7.70	22.7	2.430
		R3	7.68	23.15	19.61	7.71	22.7	2.430
	T0T-0819	R1	7.39	23.10	18.64	7.53	29.1	0.284
		R2	7.40	23.11	18.65	7.54	29.2	0.284
		R3	7.40	23.11	18.65	7.53	29.3	0.284
	T0M-0819	R1	7.77	23.70	18.67	7.71	22.7	0.223
		R2	7.77	23.70	18.67	7.71	22.8	0.223
		R3	7.77	23.71	18.67	7.72	22.7	0.223
	T0B-0819	R1	7.66	23.43	18.85	7.78	22.6	0.230
		R2	7.66	23.43	18.85	7.78	22.5	0.230
		R3	7.66	23.43	18.85	7.77	22.5	0.230
Day 5	C5T-0824	R1	6.91	26.65	19.84	6.25	2.2	0.206
		R2	6.92	26.64	19.83	6.24	2.3	0.206
		R3	6.92	26.65	19.83	6.24	2.3	0.206
	C5M-0824	R1	7.03	26.49	19.70	6.38	1.6	0.301
		R2	7.03	26.50	19.70	6.38	1.5	0.301
		R3	7.03	26.49	19.69	6.38	1.5	0.301
	C5B-0824	R1	6.92	26.66	19.70	6.29	2.0	0.223
		R2	6.92	26.67	19.70	6.29	2.1	0.223
		R3	6.92	26.68	19.70	6.30	2.0	0.223
	T5T-0824	R1	7.21	25.99	18.83	6.54	2.7	0.025
		R2	7.21	25.99	18.83	6.54	2.7	0.025
		R3	7.21	25.99	18.83	6.54	2.6	0.025
	T5M-0824	R1	7.04	26.10	18.83	6.44	0.1	0.022
		R2	7.05	26.11	18.83	6.44	0.1	0.022
		R3	7.05	26.10	18.82	6.44	0.1	0.022
	T5B-0824	R1	7.03	26.05	18.86	6.55	0.4	0.017
		R2	7.03	26.06	18.86	6.56	0.4	0.017
		R3	7.03	26.07	18.86	6.56	0.4	0.017

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-0819	R1	7.82	27.81	19.54	9.00	25.6
		R2	7.82	27.79	19.53	9.00	24.3
		R3	7.82	27.76	19.53	9.00	24.7
	OwM-0819	R1	7.83	24.11	19.59	8.95	24.3
		R2	7.83	24.13	19.59	8.95	24.8
		R3	7.83	24.13	19.59	8.95	24.2
	OwB-0819	R1	7.87	23.43	19.65	8.91	23.8
		R2	7.87	23.43	19.64	8.91	23.5
		R3	7.87	23.43	19.65	8.91	23.7
	C0T-0819	R1	7.91	22.88	20.68	8.90	35.5
		R2	7.91	22.89	20.68	8.91	35.7
		R3	7.91	22.89	20.67	8.90	35.0
	C0M-0819	R1	7.92	22.87	20.71	8.80	28.8
		R2	7.92	22.87	20.71	8.79	27.6
		R3	7.92	22.87	20.70	8.79	28.6
	C0B-0819	R1	7.92	22.94	20.63	8.71	27.7
		R2	7.92	22.93	20.67	8.72	27.8
		R3	7.92	22.91	20.65	8.72	28.3
	T0T-0819	R1	7.92	17.69	19.31	8.27	24.8
		R2	7.92	17.69	19.30	8.27	24.8
		R3	7.92	17.70	19.30	8.25	24.7
	T0M-0819	R1	7.86	15.93	19.26	7.93	24.0
		R2	7.86	15.93	19.26	7.93	24.0
		R3	7.87	15.94	19.26	7.95	24.0
	T0B-0819	R1	7.92	17.51	19.86	8.13	23.7
		R2	7.92	17.51	19.88	8.13	23.7
		R3	7.92	17.52	19.88	8.12	23.7
Day 5	C5T-0824	R1	7.11	11.8	19.45	7.75	4.33
		R2	7.11	11.8	19.49	7.74	4.30
		R3	7.11	11.8	19.43	7.77	4.29
	C5M-0824	R1	7.21	9.1	19.43	8.22	3.34
		R2	7.21	9.2	19.44	8.22	3.42
		R3	7.22	9.2	19.44	8.22	3.31
	C5B-0824	R1	7.29	9.8	19.58	7.97	3.92
		R2	7.30	9.8	19.59	7.96	3.99
		R3	7.30	9.8	19.58	7.96	3.92
	T5T-0824	R1	7.49	12.4	18.71	8.70	4.21
		R2	7.49	12.4	18.71	8.69	4.23
		R3	7.49	12.4	18.71	8.67	4.33
	T5M-0824	R1	7.60	10.7	18.68	8.81	0.967
		R2	7.60	10.9	18.63	8.83	0.873
		R3	7.61	10.9	18.65	8.89	1.090
	T5B-0824	R1	7.61	10.2	18.76	8.42	1.29
		R2	7.61	10.2	18.73	8.42	1.38
		R3	7.61	10.2	18.72	8.42	1.23

a) R: Replicate

Appendix 4. 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-0819	*Artemia salina*	137500	127000	133000	132500	5268
		Crustacean nauplii	1500	1000	2000	1500	500
		Oithona sp.	1500	2000	2000	1833	289
		Sinocalanus sp.	500	0	500	333	289
		Brachionus ssp.	965000	1040000	1050000	1018333	46458
		Protoperidinium sp.	10000	10000	5000	8333	2887
	OwM-0819	*Artemia salina*	172500	165000	174000	170500	4822
		Crustacean nauplii	500	1000	500	667	289
		Pseudodiaptomus sp.	500	500	500	500	0
		Oithona sp.	1000	2000	2000	1667	577
		Brachionus ssp.	1140000	1065000	1110000	1105000	37749
		Protoperidinium ssp.	15000	10000	15000	13333	2887
	OwB-0819	*Artemia salina*	172500	165000	160500	166000	6062
		Crustacean nauplii	1000	1500	2000	1500	500
		Pseudodiaptomus sp.	1000	0	500	500	500
		Brachionus ssp.	1165000	1060000	1070000	1098333	57951

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

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

Date	Sample ID	Classification	Survival number /m^3			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	C0T - 0819	*Artemia salina*	19500	17500	17000	18000	1323
		Crustacean nauplii	500	1500	1000	1000	500
		Oithona sp.	500	0	500	333	289
		Brachionus ssp.	875000	900000	825000	866667	38188
	C0M- 0819	*Artemia salina*	8000	10500	9500	9333	1258
		Crustacean nauplii	1000	500	500	667	289
		Oithona sp.	500	500	500	500	0
		Brachionus ssp.	1090000	1020000	1110000	1073333	47258
	C0B- 0819	*Artemia salina*	16000	14000	16500	15500	1323
		Crustacean nauplii	500	500	1000	667	289
		Oithona sp.	500	0	1000	500	500
		Brachionus ssp.	850000	910000	900000	886667	32146
Day 5	C5T - 0824	*Brachionus* ssp.	25750	23750	24250	24583	1041
		Oithona sp.	250	250	500	333	144
	C5M- 0824	*Brachionus* ssp.	23500	23000	24500	23667	764
	C5B- 0824	*Brachionus* ssp.	22000	20500	20000	20833	1041
		Oithona sp.	250	0	250	167	144

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

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

Date	Sample ID	Classification	Survival number /m³			Mean	SD [b]
			R1[a]	R2	R3		
Day 0	T0T-0819	-	0	0	0	0	0
	T0M-0819	-	0	0	0	0	0
	T0B-0819	-	0	0	0	0	0
Day 5	T5T-0824	-	0	0	0	0	0
	T5M-0824	-	0	0	0	0	0
	T5B-0824	-	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-0819	*Tetraselmis suecica*	1120	1070	1180	1123	55
		Thalassiosira sp.	2	3	3	3	1
		Chaetoceros sp.	6	8	5	6	2
		Ditilum sp.	1	1	2	1	1
		Protoperidinium sp.	7	5	7	6	1
	OwM-0819	*Tetraselmis suecica*	1190	1130	1250	1190	60
		Thalassiosira sp.	3	4	2	3	1
		Chaetoceros sp.	5	4	6	5	1
		Protoperidinium sp.	9	10	7	9	2
	OwB-0819	*Tetraselmis suecica*	1020	1080	1090	1063	38
		Thalassiosira sp.	3	2	2	2	1
		Ditilum sp.	1	2	1	1	1
		Protoperidinium sp.	5	5	7	6	1

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

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

Date	Sample ID	Classification	Survival number / ml			Mean	SD [b)]
			R1[a)]	R2	R3		
Day 0	C0T - 0819	*Tetraselmis suecica*	1210	1130	1180	1173	40
		Thalassiosira sp.	2	3	2	2	1
		Chaetoceros sp.	1	2	1	1	1
		Protoperidinium sp.	4	4	5	4	1
	C0M- 0819	*Tetraselmis suecica*	1120	1260	1090	1157	91
		Thalassiosira sp.	2	1	1	1	1
		Chaetoceros sp.	1	2	2	2	1
		Protoperidinium sp.	3	4	2	3	1
	C0B- 0819	*Tetraselmis suecica*	1080	1010	1120	1070	56
		Chaetoceros sp.	2	2	3	2	1
		Protoperidinium sp.	1	3	2	2	1
Day 5	C5T- 0824	*Tetraselmis suecica*	124	128	114	122	7
		Thalassiosira sp.	2	3	2	2	1
		Protoperidinium sp.	1	2	1	1	1
	C5M- 0824	*Tetraselmis suecica*	105	96	107	103	6
		Thalassiosira sp.	1	0	1	1	1
		Protoperidinium sp.	2	1	1	1	1
	C5B- 0824	*Tetraselmis suecica*	89	108	98	98	10
		Thalassiosira sp.	0	1	1	1	1
		Protoperidinium sp.	2	1	2	2	1

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

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

Date	Sample ID	Classification	Survival number / ml			Mean	SD [b)]
			R1[a)]	R2	R3		
Day 0	T0T-0819	-	0	0	0	0	0
	T0M-0819	-	0	0	0	0	0
	T0B-0819	-	0	0	0	0	0
Day 5	T5T-0824	-	0	0	0	0	0
	T5M-0824	-	0	0	0	0	0
	T5B-0824	-	0	0	0	0	0

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

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

Date	Sample ID		CFU /mL			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (08/19)	Ow-0819	Top	15200	13600	16200	15000	1311
		Middle	19600	16200	20200	18667	2157
		Bottom	11400	13600	12000	12333	1137
	C0-0819	Top	25000	30900	31400	42600	4331
		Middle	23000	26200	24000	24400	1637
		Bottom	17800	18800	19400	18667	808
	T0-0819	Top	30	100	70	67	35
		Middle	10	70	70	50	35
		Bottom	70	60	40	57	15
Day5 (08/24)	C5-0824	Top	27300	25400	25500	38400	1000
		Middle	50000	41900	42400	62600	10912
		Bottom	35700	34900	35400	54533	643
	T5-0824	Top	72200	75000	74800	101600	6835
		Middle	41500	46800	45000	63533	5220
		Bottom	52800	57600	51200	53867	3331

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

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

Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (08/19)	Ow-0819	Top	310	480	260	350	115
		Middle	330	290	220	280	56
		Bottom	340	320	290	317	25
	C0-0819	Top	1100	1120	1300	1173	110
		Middle	980	990	1010	993	15
		Bottom	970	960	920	950	26
	T0-0819	Top	0	0	0	0	0
		Middle	0	0	0	0	0
		Bottom	0	0	0	0	0
Day5 (08/24)	C5-0824	Top	5	6	2	4	2
		Middle	1	3	3	2	1
		Bottom	1	0	1	1	1
	T5-0824	Top	0	0	0	0	0
		Middle	0	1	0	0	1
		Bottom	0	0	1	0	1

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

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

Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (08/19)	Ow-0819	Top	250	350	270	290	53
		Middle	290	230	280	267	32
		Bottom	300	280	270	283	15
	C0-0819	Top	> 2000 [c]	> 2000	> 2000	> 2000	-
		Middle	> 2000	> 2000	> 2000	> 2000	-
		Bottom	> 2000	> 2000	> 2000	> 2000	-
	T0-0819	Top	0	0	0	0	0
		Middle	0	0	0	0	0
		Bottom	0	0	0	0	0
Day5 (08/24)	C5-0824	Top	22	23	20	22	2
		Middle	1	0	1	1	1
		Bottom	22	6	24	17	10
	T5-0824	Top	18	24	18	20	3
		Middle	18	16	14	16	2
		Bottom	1	1	0	1	1

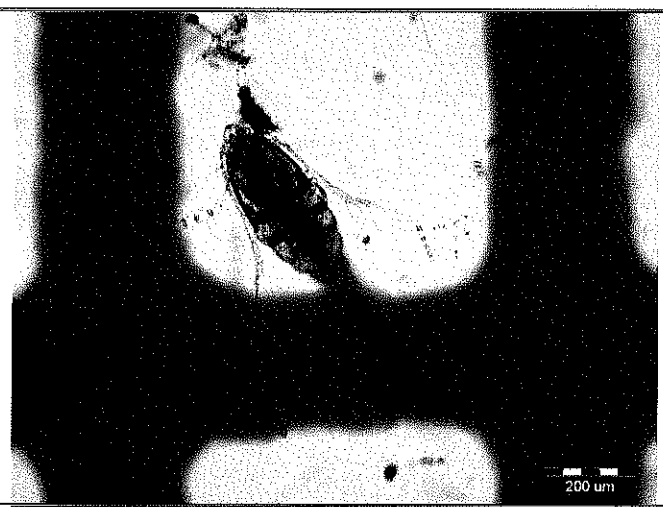
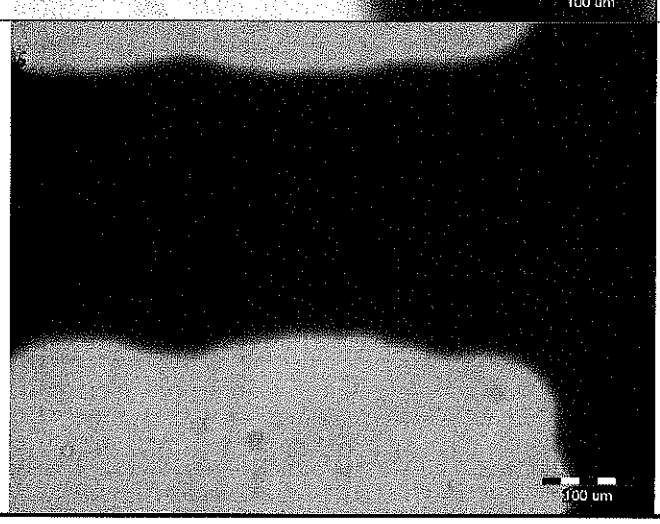
a) R: Replicate
b) S.D: Standard deviation
c) Colonies were too numerous to count (TNTC), used upper limit count with smallest filtration volume

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

Date	Sample ID		CFU /100ml			Mean	SD [b]
			R1[a]	R2	R3		
Day 0 (08/19)	Ow-0819	Top	0	0	0	0	0
		Middle	0	0	0	0	0
		Bottom	0	0	0	0	0
	C0-0819	Top	0	0	0	0	0
		Middle	0	0	0	0	0
		Bottom	0	0	0	0	0
	T0-0819	Top	0	0	0	0	0
		Middle	0	0	0	0	0
		Bottom	0	0	0	0	0
Day5 (08/24)	C5-0824	Top	0	0	0	0	0
		Middle	0	0	0	0	0
		Bottom	0	0	0	0	0
	T5-0824	Top	0	0	0	0	0
		Middle	0	0	0	0	0
		Bottom	0	0	0	0	0

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

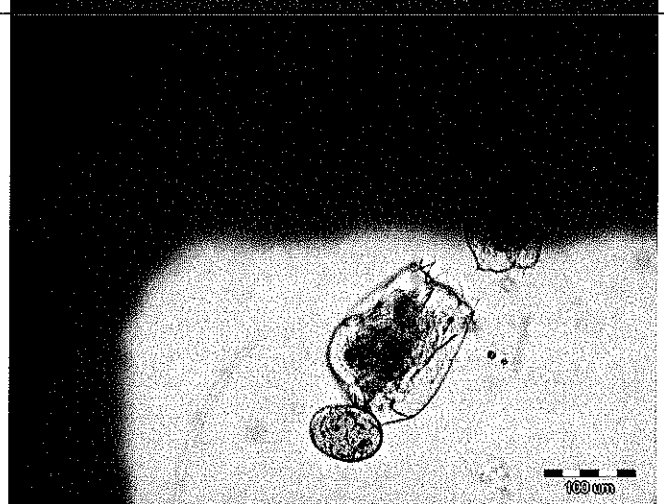
Appendix 14. The microscope image of ≥ 50㎛ in original water (Day0)

Specific Name	Original water
*Sinocalanus sp.**	
*Pseudodiaptomus sp.**	
*Protoperidinium sp.***	

* Magnification : 10

** Magnification : 20

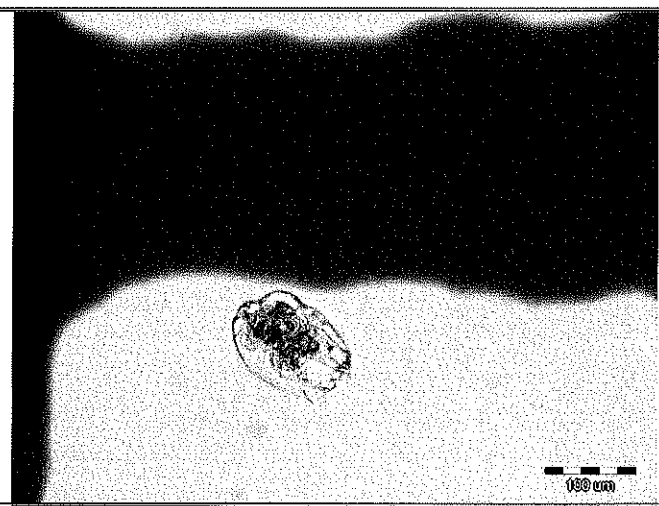
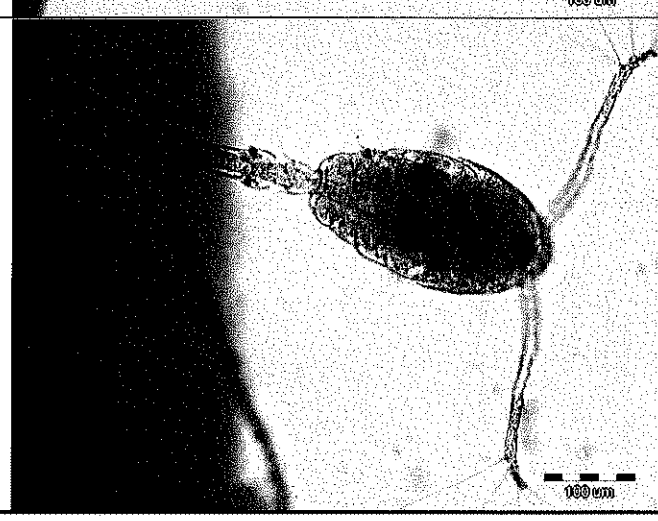
Appendix 15. The microscope image of ≥ 50㎛ in control (untreated) brackish water by treatment of Purimar™ BWMS (Day0)

Specific Name	Control (untreated) brackish water
*Artemia salina**	
Brachionus ssp.**	
*Oithona sp.***	

* Magnification : 10

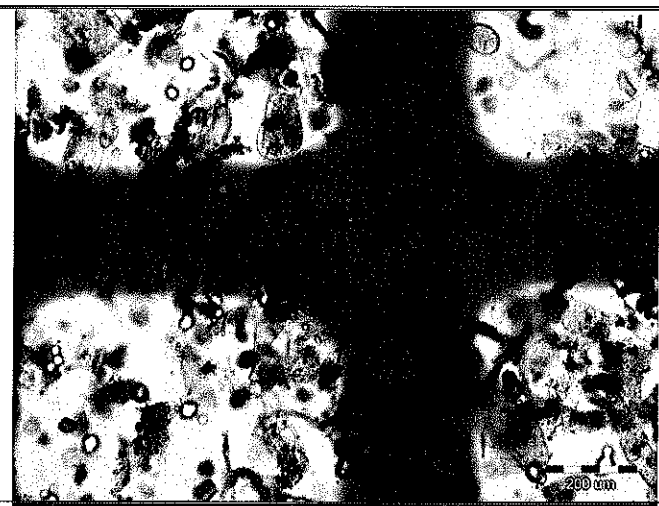
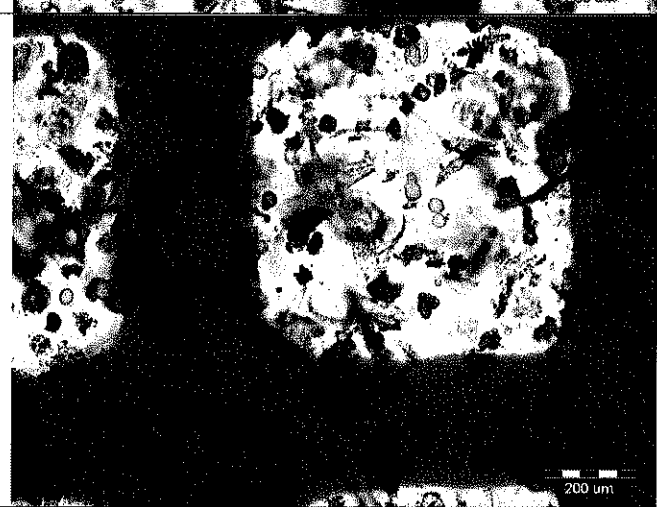
** Magnification : 20

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

Specific Name	Control (untreated) brackish water
***Brachionus* ssp.**	
Oithona sp.	

Magnification : 20

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

Specific Name	Treated ballast brackish water
-	
-	
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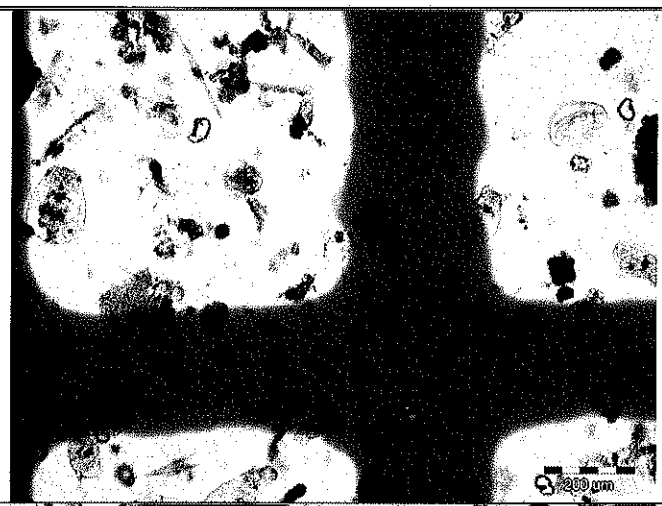
Magnification : 10

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

Specific Name	Treated ballast brackish water
-	
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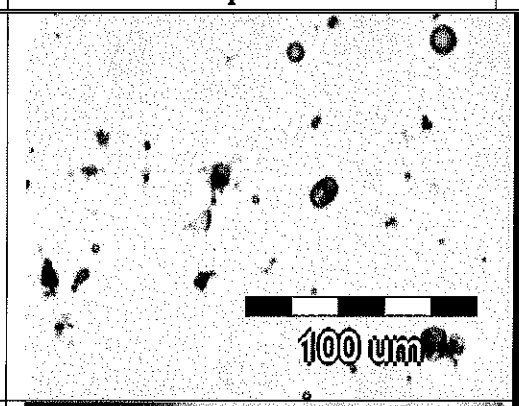
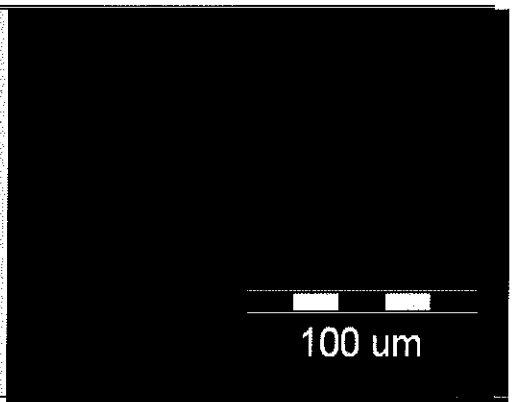
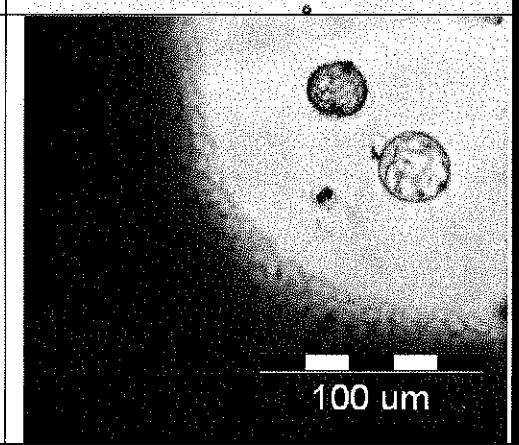
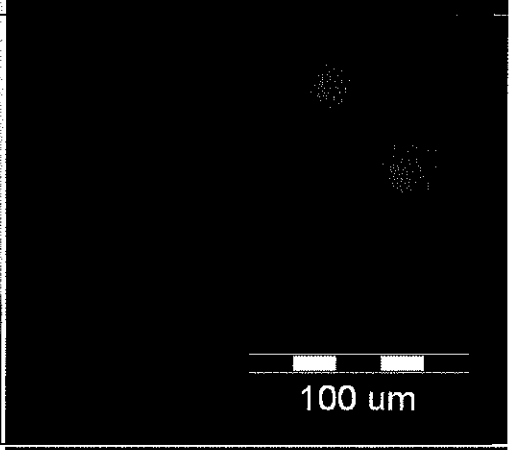
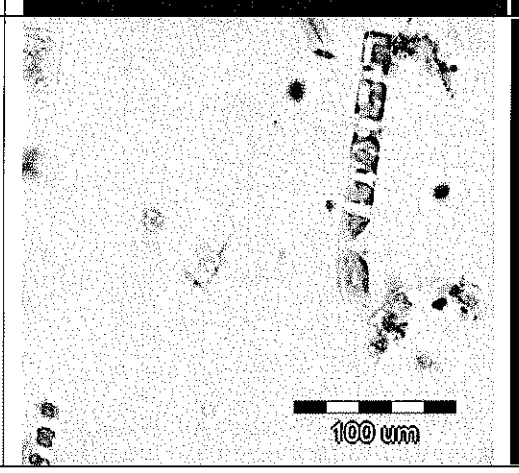
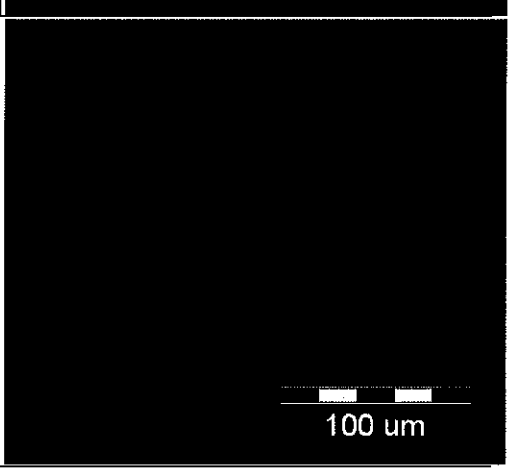
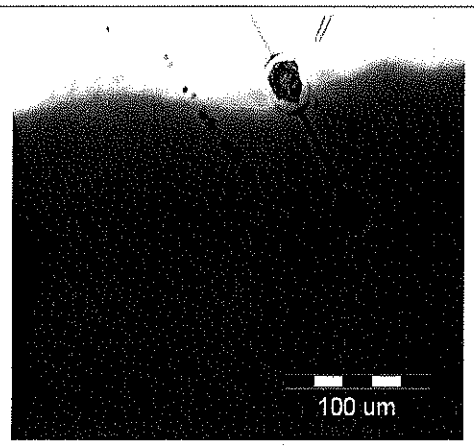
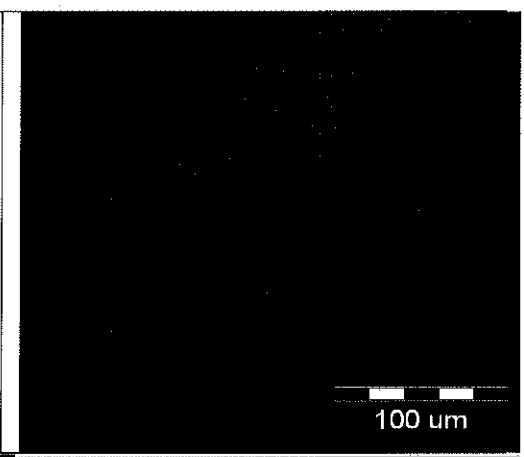
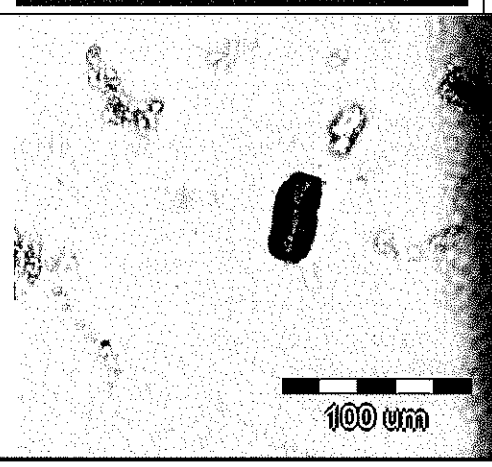
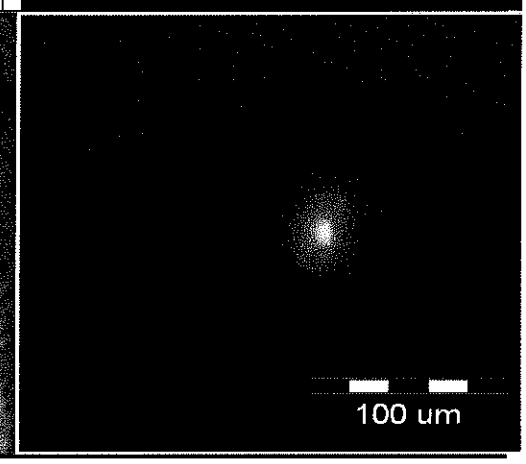
Magnification : 10

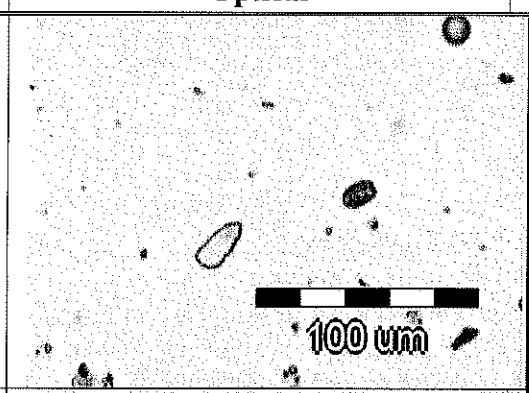
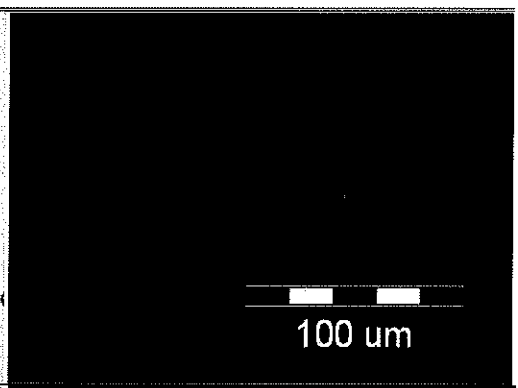
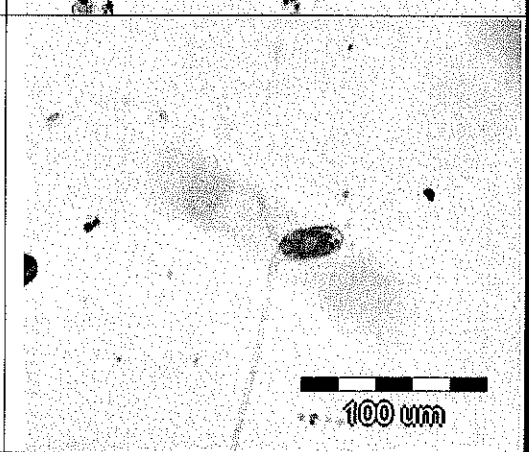
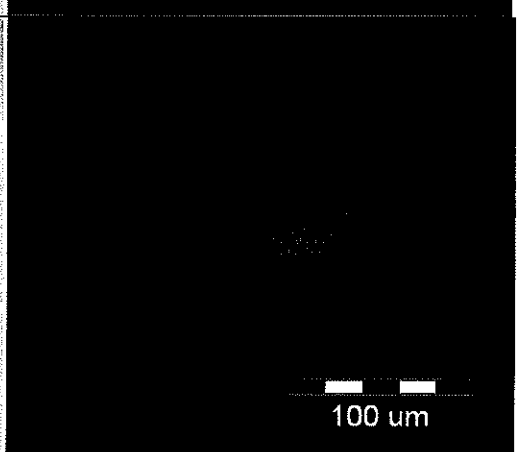
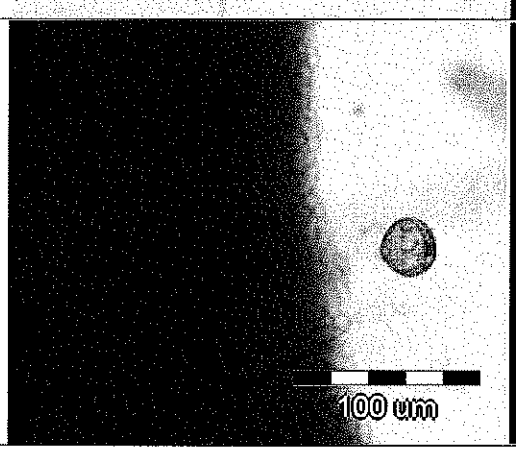
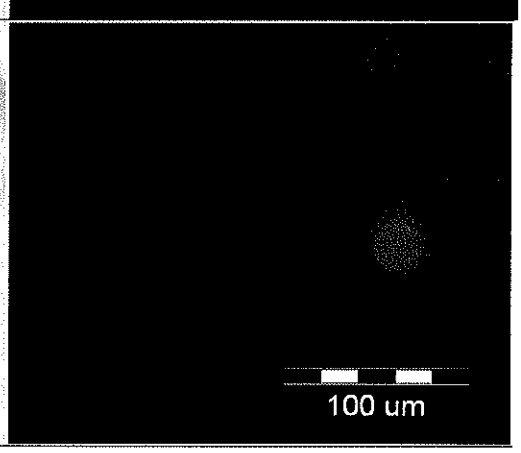
Appendix 19. The microscope image of 10㎛−50㎛ in original water (Day0)

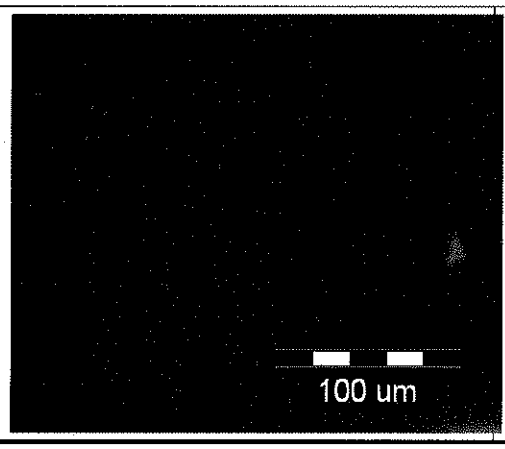
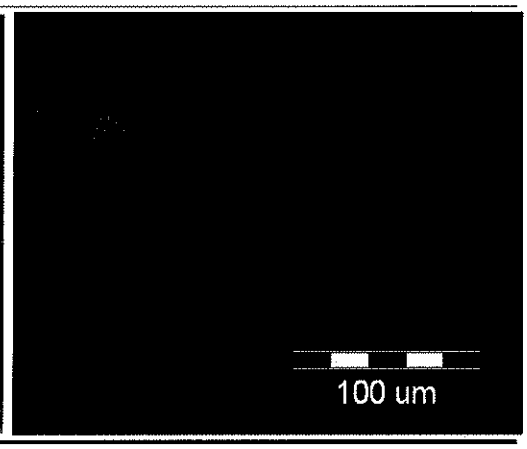
Specific Name	Control (untreated) brackish water	
	Optical	Fluorescence
Tetraselmis suecica		
Protoperidinium sp.		
Chaetoceros sp.		

***Ditylum* sp.**		
***Thalassiosira* sp.**		

Magnification : 200

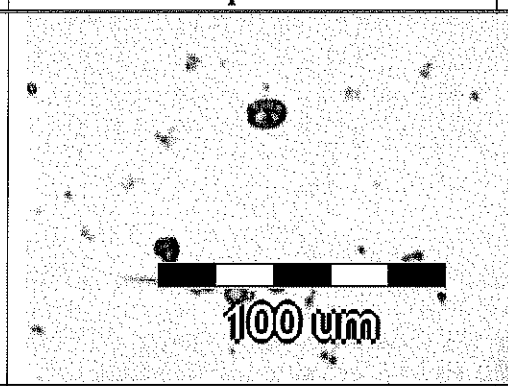
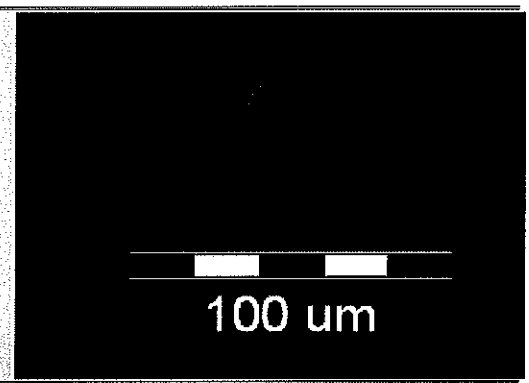
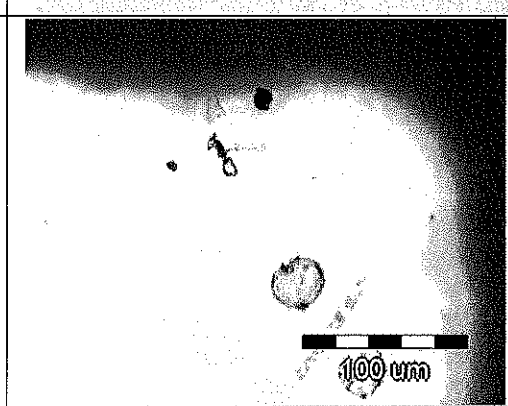
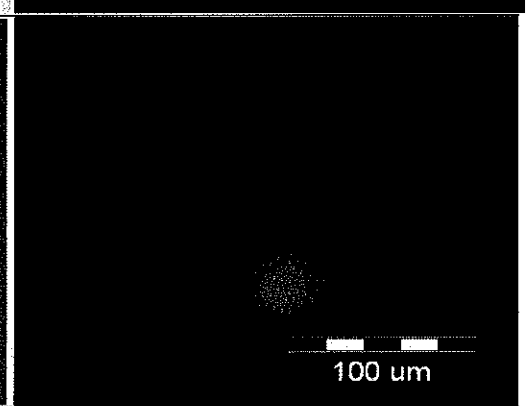
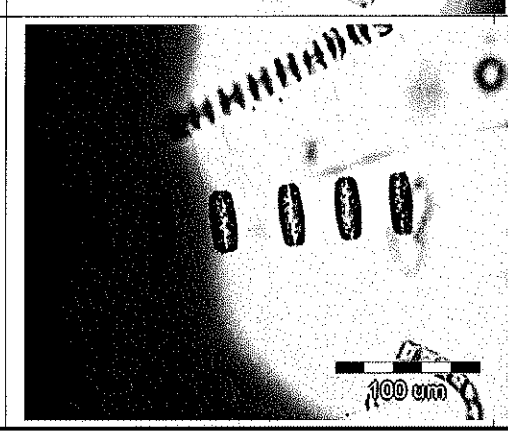
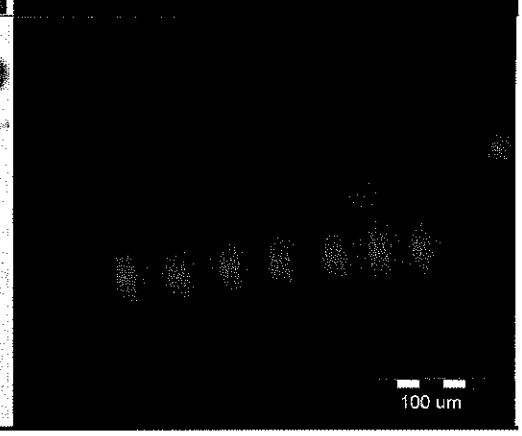
Appendix 20. The microscope image of 10㎛~50㎛ in control (untreated) brackish water by treatment of Purimar™ BWMS (Day0)

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

***Thalassiosira* sp.**		

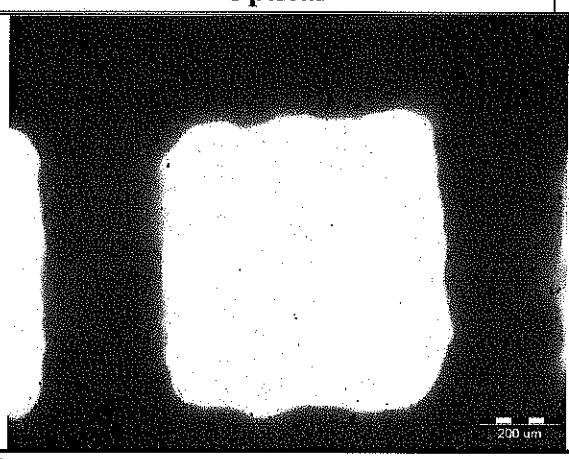
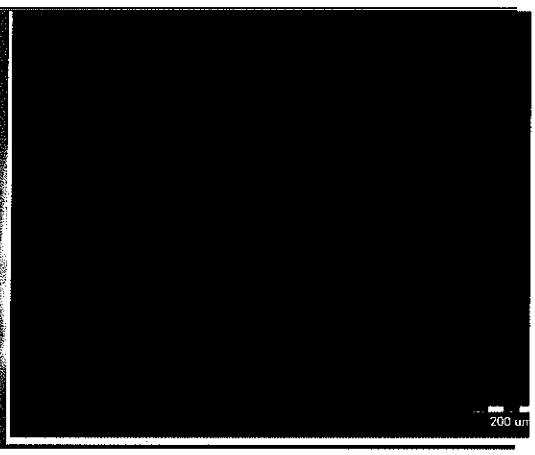
Magnification : 200

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

Specific Name	Control (untreated) brackish water	
	Optical	Fluorescence
Tetraselmis suecica		
Protoperidinium sp.		
Thalassisosira sp.		

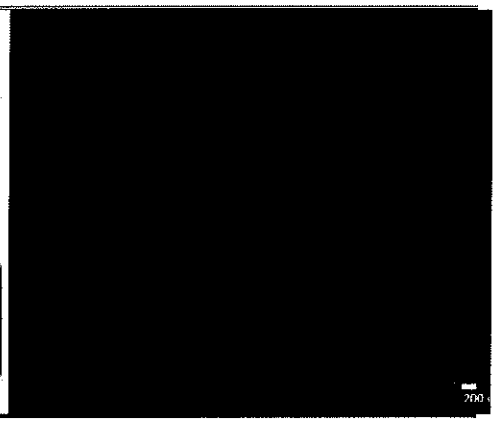
Magnification : 200

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

Specific Name	Treated ballast brackish water	
	Optical	Fluorescence
-		

Magnification : 100

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 brackish water

Top **Middle** **Bottom**

Original water

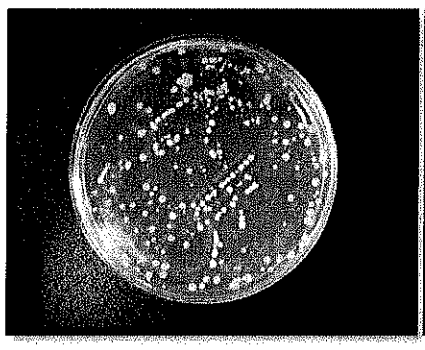
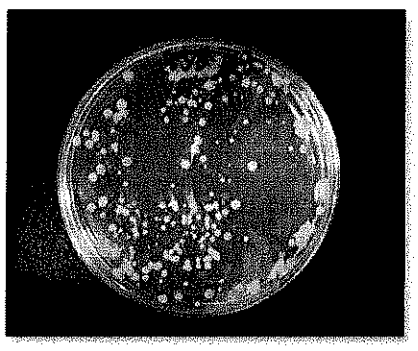
Top **Middle** **Bottom**

Control (untreated) brackish water

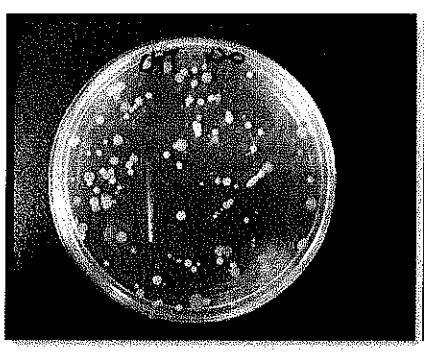
Top **Middle** **Bottom**

Appendix 25. The image of heterotrophic bacteria: Day 5

Treated ballast brackish water

Top	Middle	Bottom
		

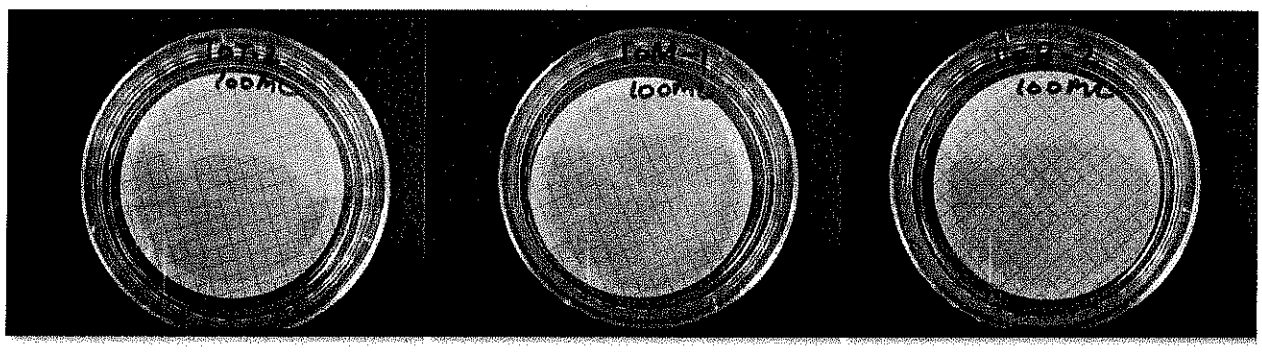
Control (untreated) brackish water

Top	Middle	Bottom
		

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

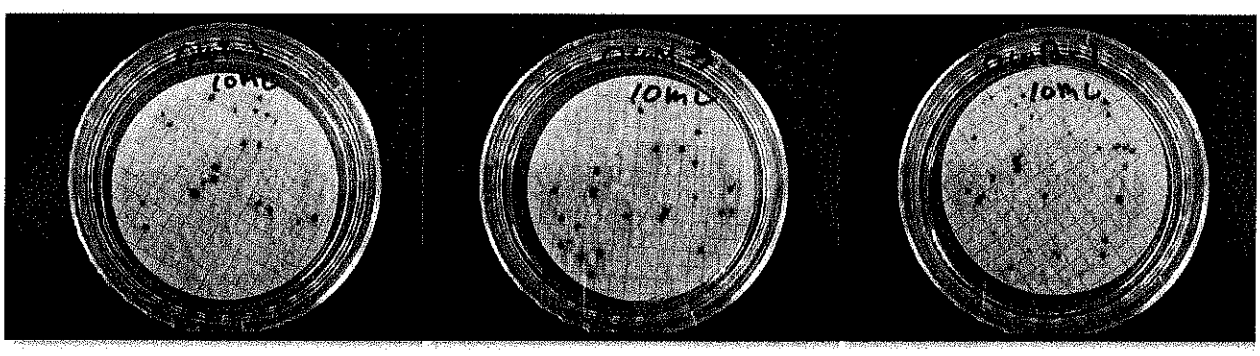
Treated ballast brackish water

Top **Middle** **Bottom**



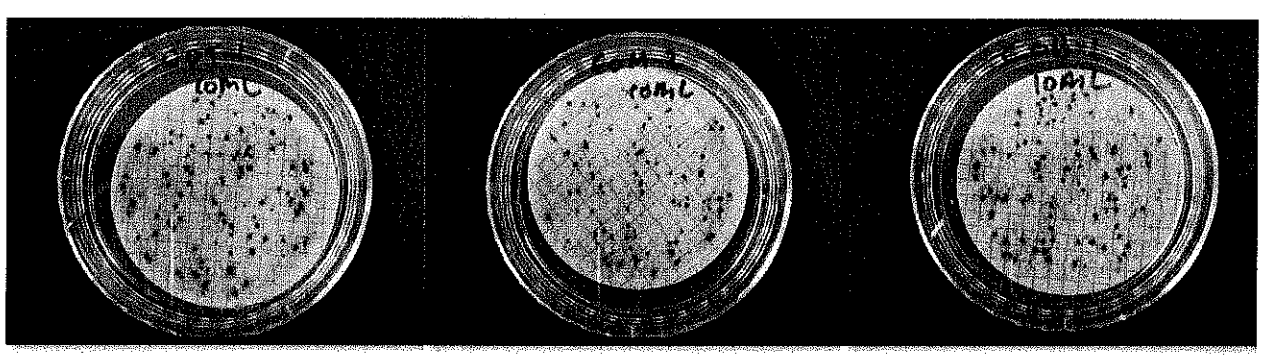
Original water

Top **Middle** **Bottom**



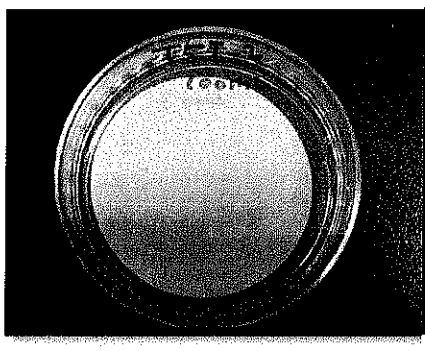
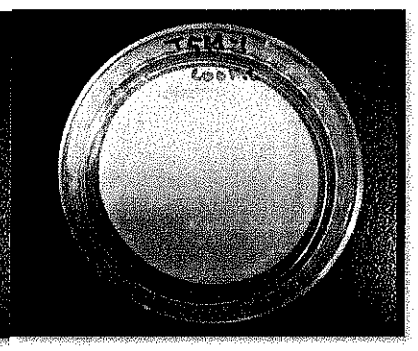
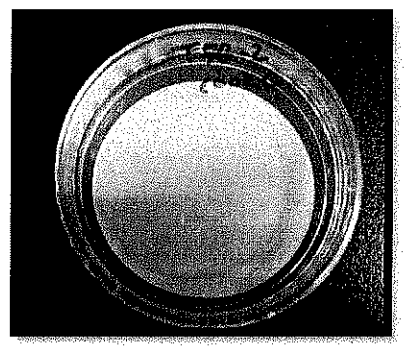
Control (untreated) brackish water

Top **Middle** **Bottom**

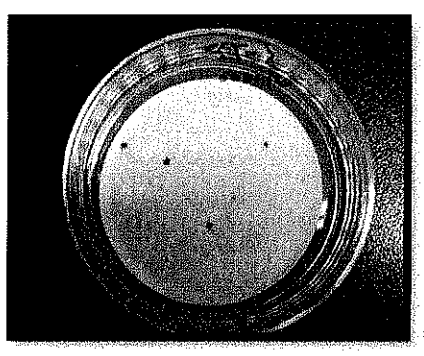
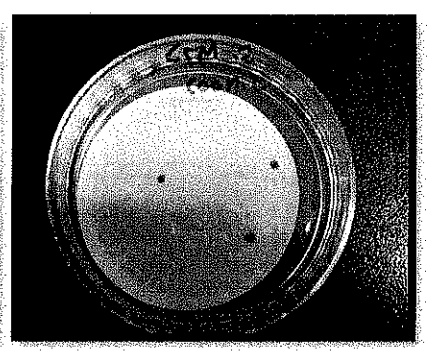
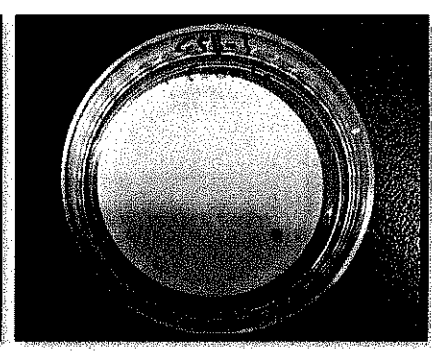


Appendix 27. The image of *Escherichia coli*: Day 5

Treated ballast brackish water

Top	Middle	Bottom
		

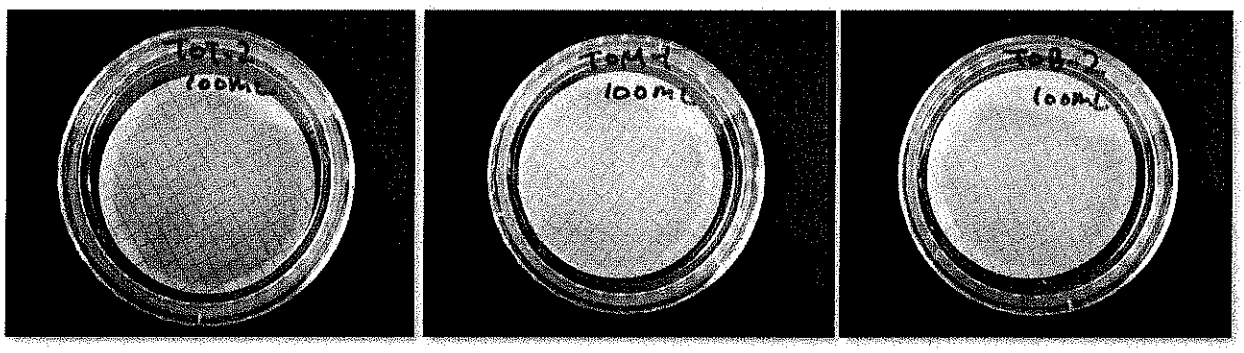
Control (untreated) brackish water

Top	Middle	Bottom
		

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

Treated ballast brackish water

Top **Middle** **Bottom**

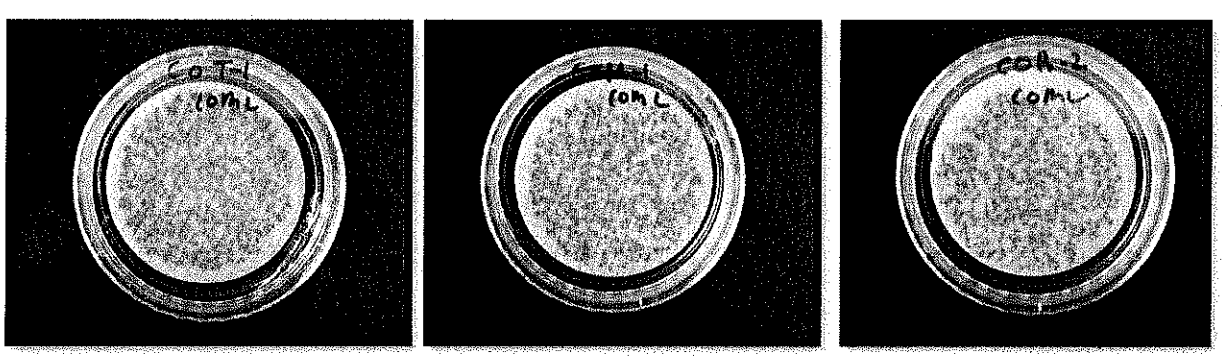


Original water

Top **Middle** **Bottom**

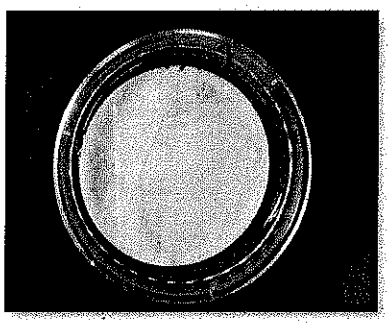
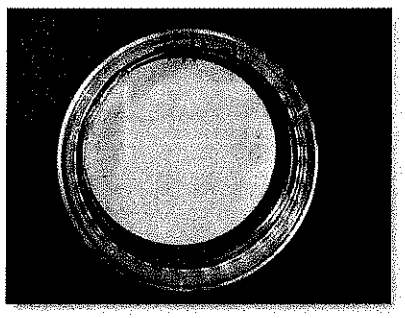
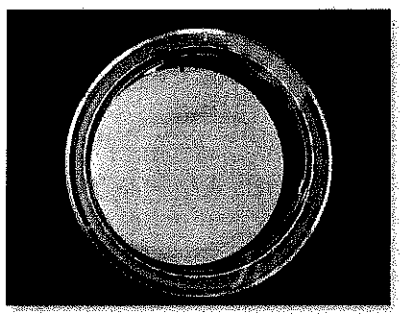
Control (untreated) brackish water

Top **Middle** **Bottom**

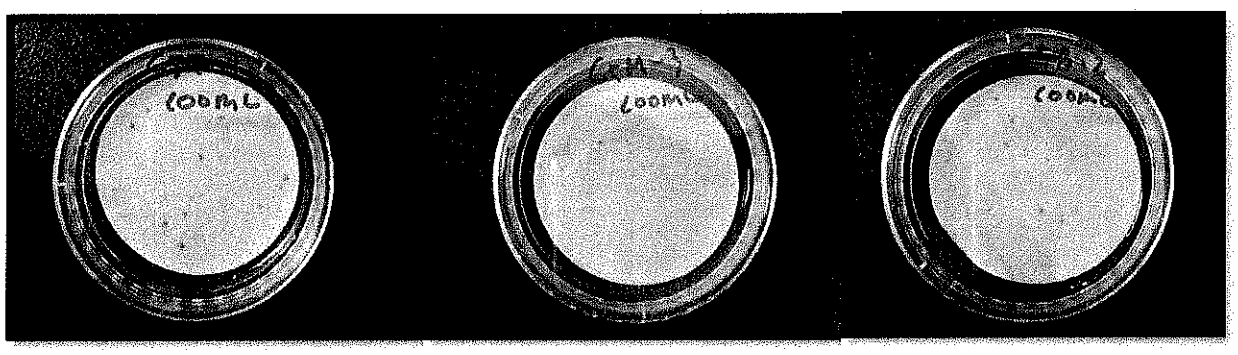


Appendix 29. The image of *Enterococcus faecalis*: Day 5

Treated ballast brackish water

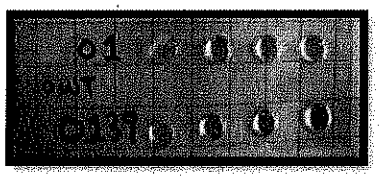
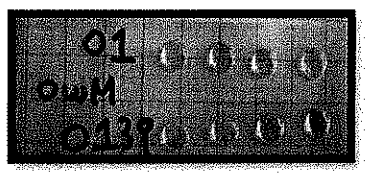
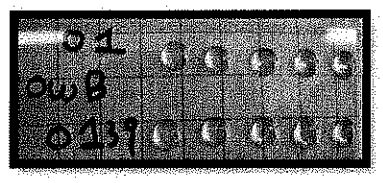
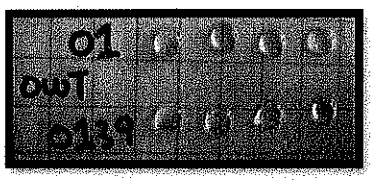
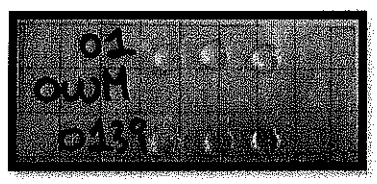
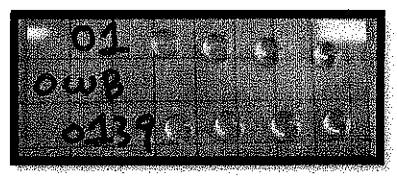
Top	Middle	Bottom
		

Control (untreated) brackish water

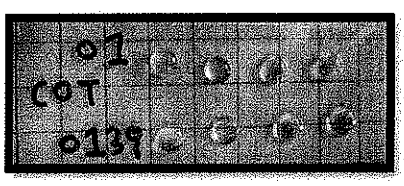
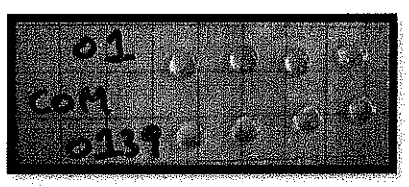
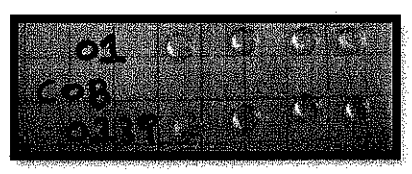
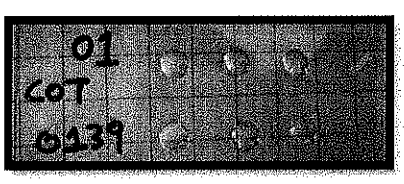
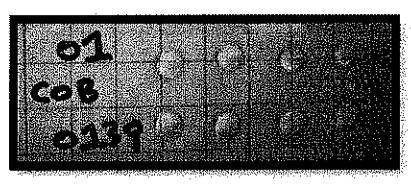
Top	Middle	Bottom
		

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

Treated ballast brackish water

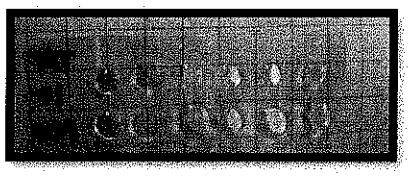
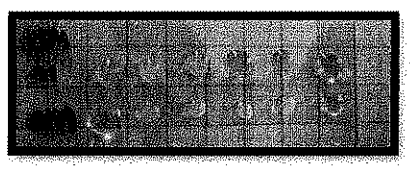
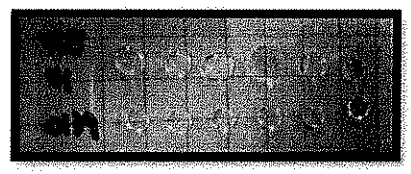
Top	Middle	Bottom
		
		

Control (untreated) brackish water

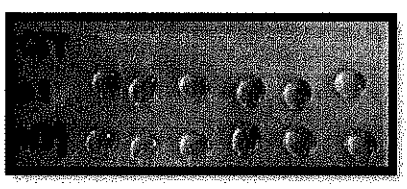
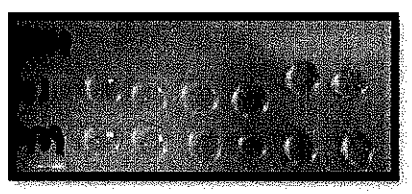
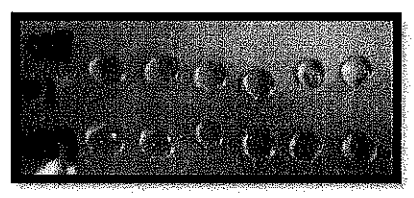
Top	Middle	Bottom
		
		

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

Treated ballast brackish water

Top	Middle	Bottom
		

Control (untreated) brackish water

Top	Middle	Bottom
		

5. Attachment

5.1 Filtered natural brackish water(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