Multi-laboratory validation of a new marine biodegradation screening test for chemical persistence assessment

Amelie Ott [a], Timothy J. Martin [a,1], Kishor Acharya [a], Delina Y. Lyon [b], Nik Robinson [c], Bob Rowles [d], Jason R. Snape [a,e,f], Ian Still [c,2], Graham F. Whale [g,3], Vurtice C. Albright III [h], Petra Bäverbäck [i], Nicola Best [j], Ruth Commander [k], Curtis Eickhoff [l], Sarah Finn [m], Björn Hidding [n], Heiko Maischak [o], Katherine A. Sowders [p], Masanori Taruki [q], Helen E. Walton [d], Aina Charlotte Wennberg [r], Russell J. Davenport [a]*

[a] Newcastle University, School of Engineering, Newcastle upon Tyne, NE1 7RU, United Kingdom

[b] Shell Oil Company, 150 N. Dairy Ashford Rd, Houston, TX 77079, United States

[c] European Oilfield Specialty Chemicals Association (EOSCA), Aberdeen, AB11 6YQ, United Kingdom

[d] Centre for Environment, Fisheries and Aquaculture Science (Cefas), Pakefield Road, Lowestoft, NR33 0HT, United Kingdom

[e] AstraZeneca Global Environment, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TF, United Kingdom

[f] University of Warwick, School of Life Sciences, Gibbet Hill Campus, Coventry CV4 7AL, United Kingdom

[g] Shell International Ltd, Risk Science Team, 4 York road, London, SE1 7NA, United Kingdom

[h] The Dow Chemical Company, Midland, Toxicology & Environmental Research & Consulting, MI 48674, United States

[i] Schlumberger, Sandslikroken 140, 5254 Sandsli, Norway

[j] Covance CRS Research Limited, Shardlow Business Park, London Road, Derby, DE72 2GD, United Kingdom

[k] Scymaris Ltd., Brixham Laboratory, Brixham, TQ5 8BA, United Kingdom

[l] Nautilus Environmental Company Inc., Burnaby, BC, V5A 4N7, Canada

[m] National Oilwell Varco (NOV), Flotta, Stromness, Orkney, KW16 3NP, United Kingdom

[n] BASF SE, Carl-Bosch-Straße 38, 67056 Ludwigshafen am Rhein, Germany

[o] Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany

[p] Baker Hughes – Environmental Services Group, 369 Marshall Ave., Webster Groves, Missouri 63119, United States

[q] Chemicals Evaluation and Research Institute, Japan, Kurume (CERI Kurume), 3-2-7 Miyanojin, Kurume-shi, Fukuoka 839-0801, Japan

[r] Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, 0349 Oslo, Norway

Present Addresses

[1] Department for Environment, Food and Rural Affairs, Lancaster House, Newcastle upon Tyne, NE4 7JB, United Kingdom

[2] Nalco Champion, WSW Building, Peterseat Drive, Aberdeen, AB12 3HT, United Kingdom

[3] Whale Environmental Consultancy Ltd., 55 Earlsway Curzon Park, Chester, CH4 8AZ, United Kingdom

SUPPORTING INFORMATION

Pages: 38

Figures: 21

Tables: 16

Biodegradation data supporting this publication is openly available under http://doi.org/10.25405/data.ncl.9938198.

Methods M1: Test protocol modifications in the $imBST_{MR}$ from the pre-validated marine erBST.

The new test ($imBST_{MR}$) was based on a previous intra-laboratory validated marine environmentally relevant BST (erBST) [1], but differed in following aspects to incorporate recommendations from stakeholders and other studies:[1–3]

- Terminology: While the microbiome in the erBST and $imBST_{MR}$ aims to better represent the samples environment, other BST conditions still do not represent the environment well e.g. high test chemical concentrations and high incubation temperatures. Consequently, the terminology "environmentally relevant" was replaced with improved/new for the $imBST_{MR}$.
- Biodegradation measurement: To overcome potential biodegradation underestimations in OECD 301B tests [1,4–6], the $imBST_{MR}$ monitored biodegradation with MRs in a modified OECD 301F test.
- TFF: In the $imBST_{MR}$, the TFF protocol was optimized to incorporate an additional filtrate pump to reduce membrane wall pressures. No backflushing was performed to preserve membrane integrity.
- Test chemicals: Due to equipment and licensing limitations at CROs, test chemicals were not radiolabeled (^{14}C) in the ring test. Higher test chemical concentrations were employed in the new and revised MR test in comparison to the pre-validation study.[1] In MR tests, chemical stock solutions were prepared with seawater instead of OECD mineral medium to circumvent seawater dilution in the test vessel (of bacterial cell concentrations and salinity).[1] However, it should be noted that the high salt

concentrations in seawater can modify the solubility and related properties of some organic chemicals.[7]

- Test medium: Phosphate nutrient additions (OECD mineral medium solution a) in the MR tests followed the OECD 301F protocol [8] and were 10 × higher than in the pre-validation study which followed the OECD 306 recipe.[1,9] The OECD guidelines do not explain this difference, but the OECD 306 method probably requires less phosphate due to the natural buffering capacity of seawater [10] and lower test chemical concentrations employed. To account for increased test chemical levels, more phosphate was added in the MR tests. However, it should be noted that this alteration was expected to have little or no effect as phosphate is added to excess in all OECD BSTs and no adverse effects have been observed with increased phosphate levels in BSTs.[10,11]

Figure S1. Locations of laboratories participating in the ring test.

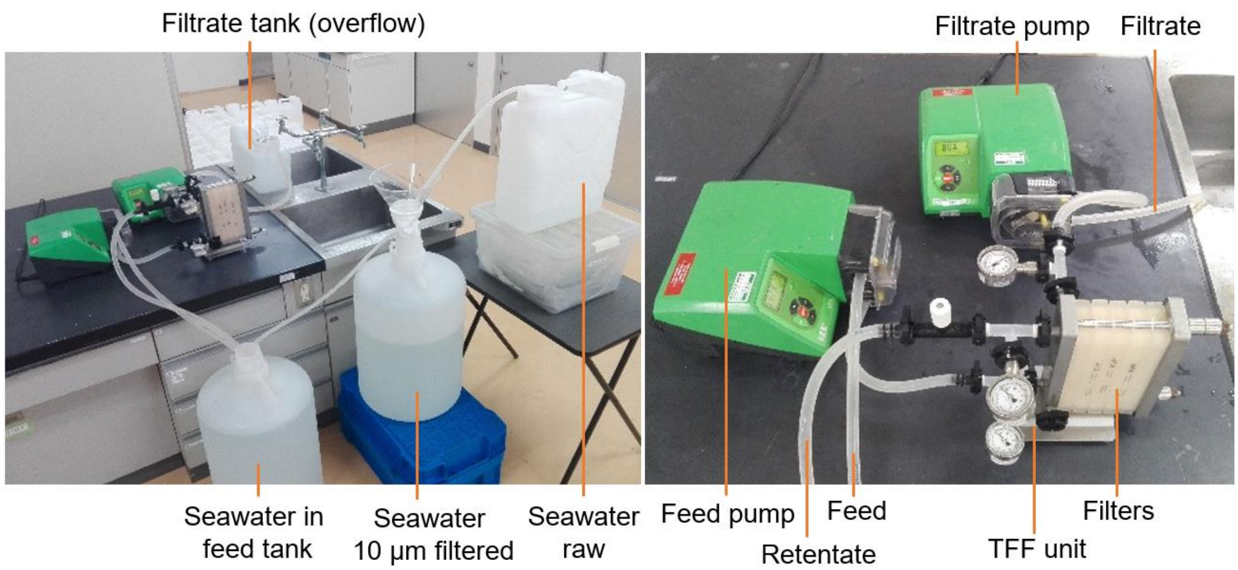


Figure S2. Example tangential flow filtration setup to increase bacterial cell numbers in seawater.

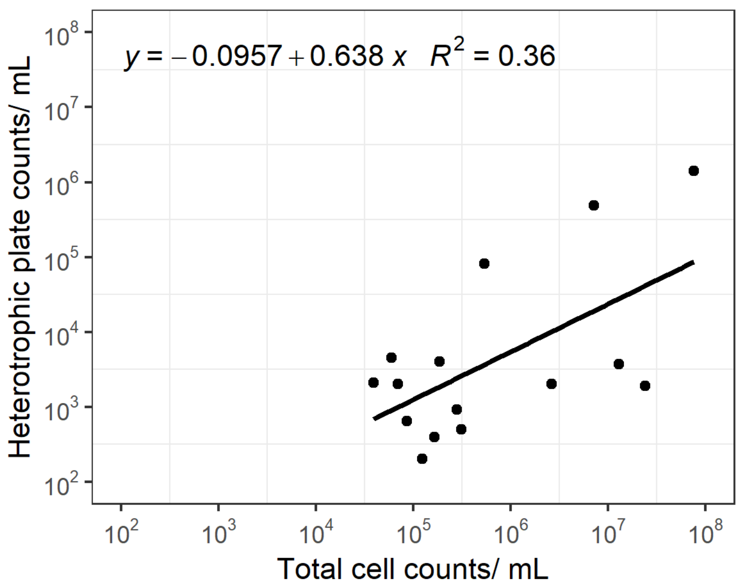


Figure S3. Correlation and linear regression between heterotrophic plate counts (measured using different culture methods) and total cell concentrations (measured by flow cytometry) in seawater samples (S1, S2, S3) where both measurement methods were conducted.

Note: For the following imBST$_{MR}$ and mBST$_{MR}$ biodegradation plots, every 20th data point was plotted for CRO A, C, D, F, H, K, L, M (automatic recordings every 4- 7 hours) and every 3rd data point for CRO I (manual daily recordings on weekdays). For the OECD306$_{CB}$ biodegradation plots, individual measurements of the sacrificial BOD bottles are plotted together with a line representing the arithmetic mean.

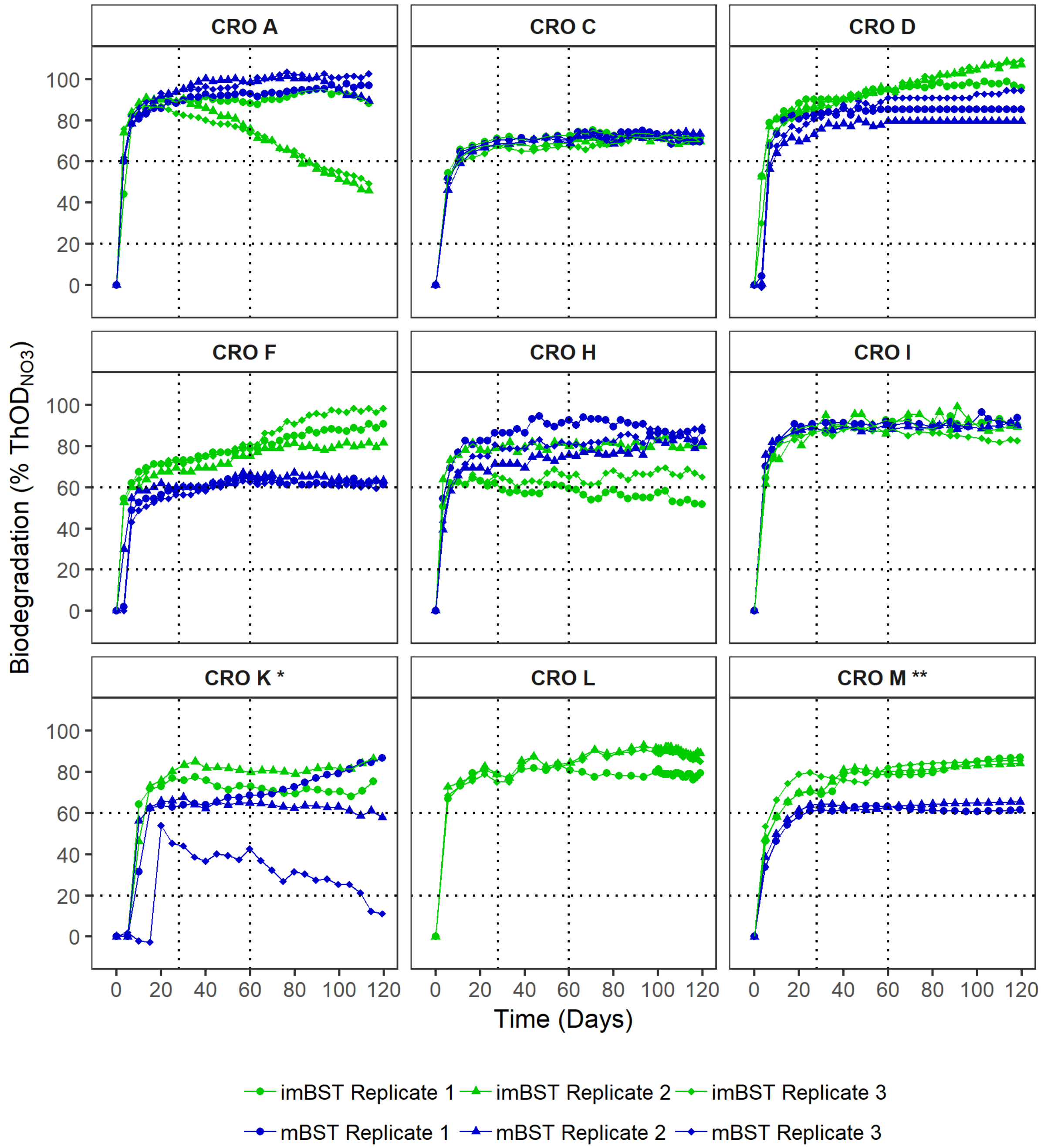
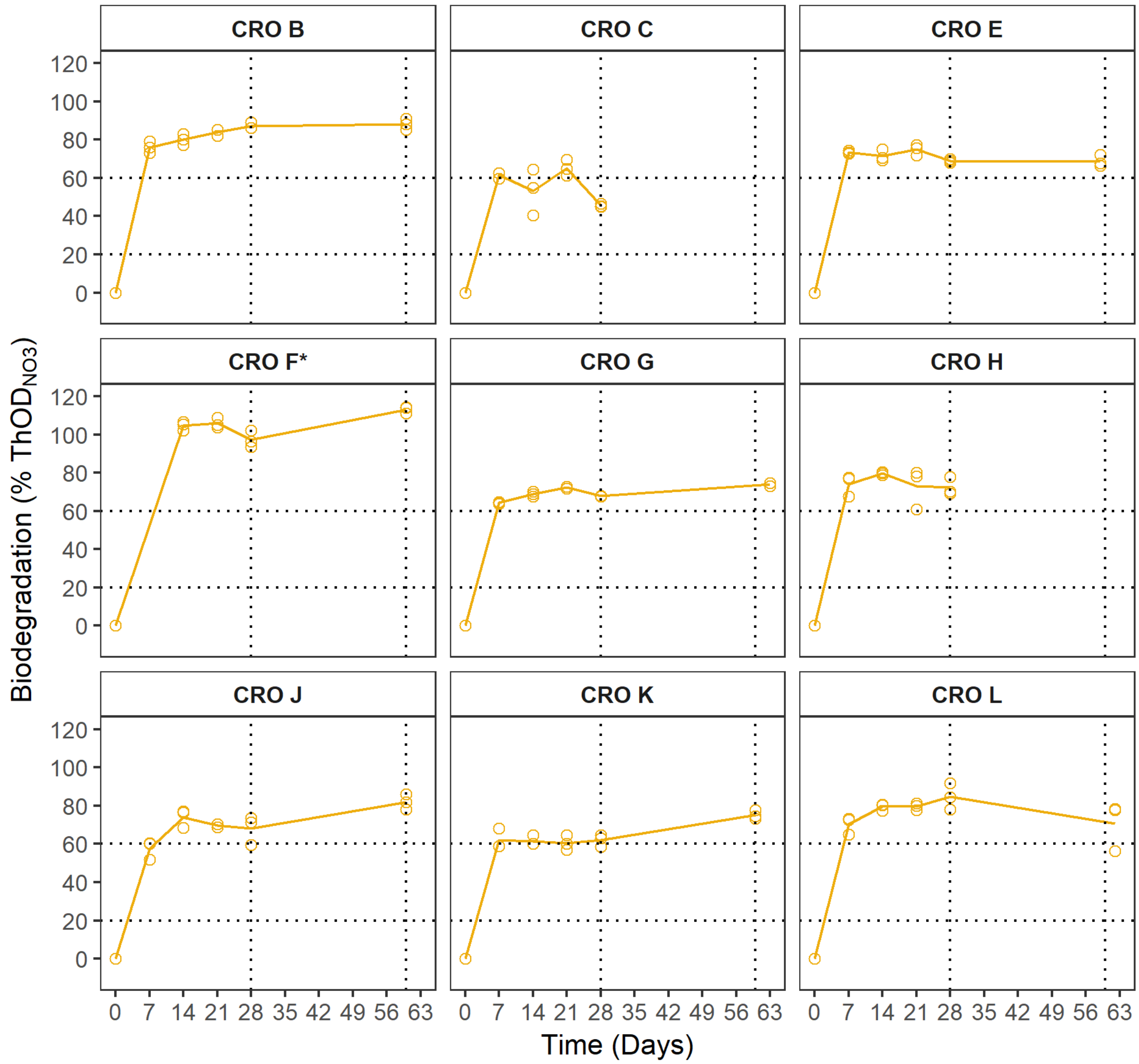


Figure S4. Biodegradation of sodium benzoate in the mBST$_{MR}$ and imBST$_{MR}$. * For removed outlier, see Figure S20. ** Biodegradation based on CO_2 production instead of O_2 consumption.



108

109 **Figure S5.** Biodegradation of sodium benzoate in the OECD306$_{CB}$. * For removed outlier, see Figure S19.

110

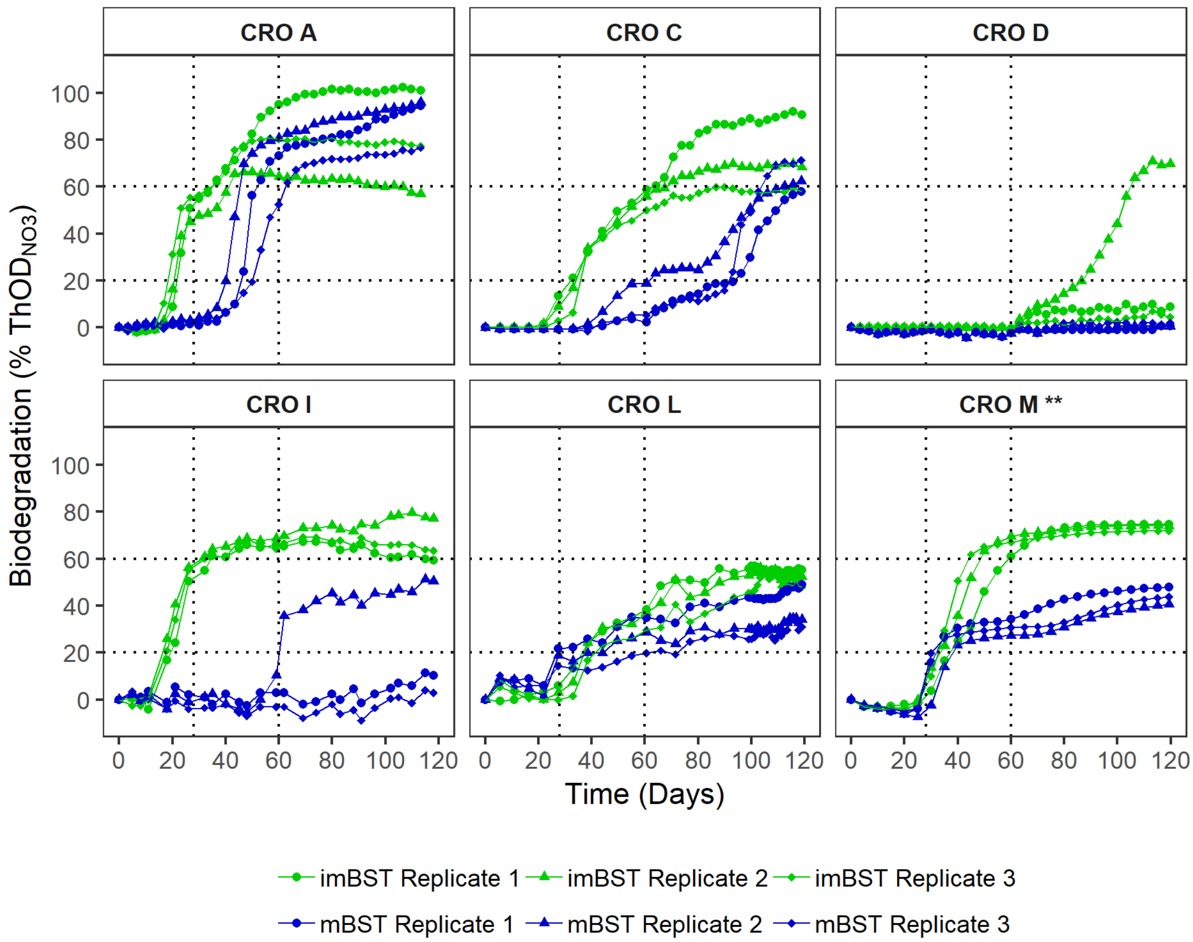


Figure S6. Biodegradation of triethanolamine in the $mBST_{MR}$ and $imBST_{MR}$. ** Biodegradation based on CO_2 production instead of O_2 consumption.

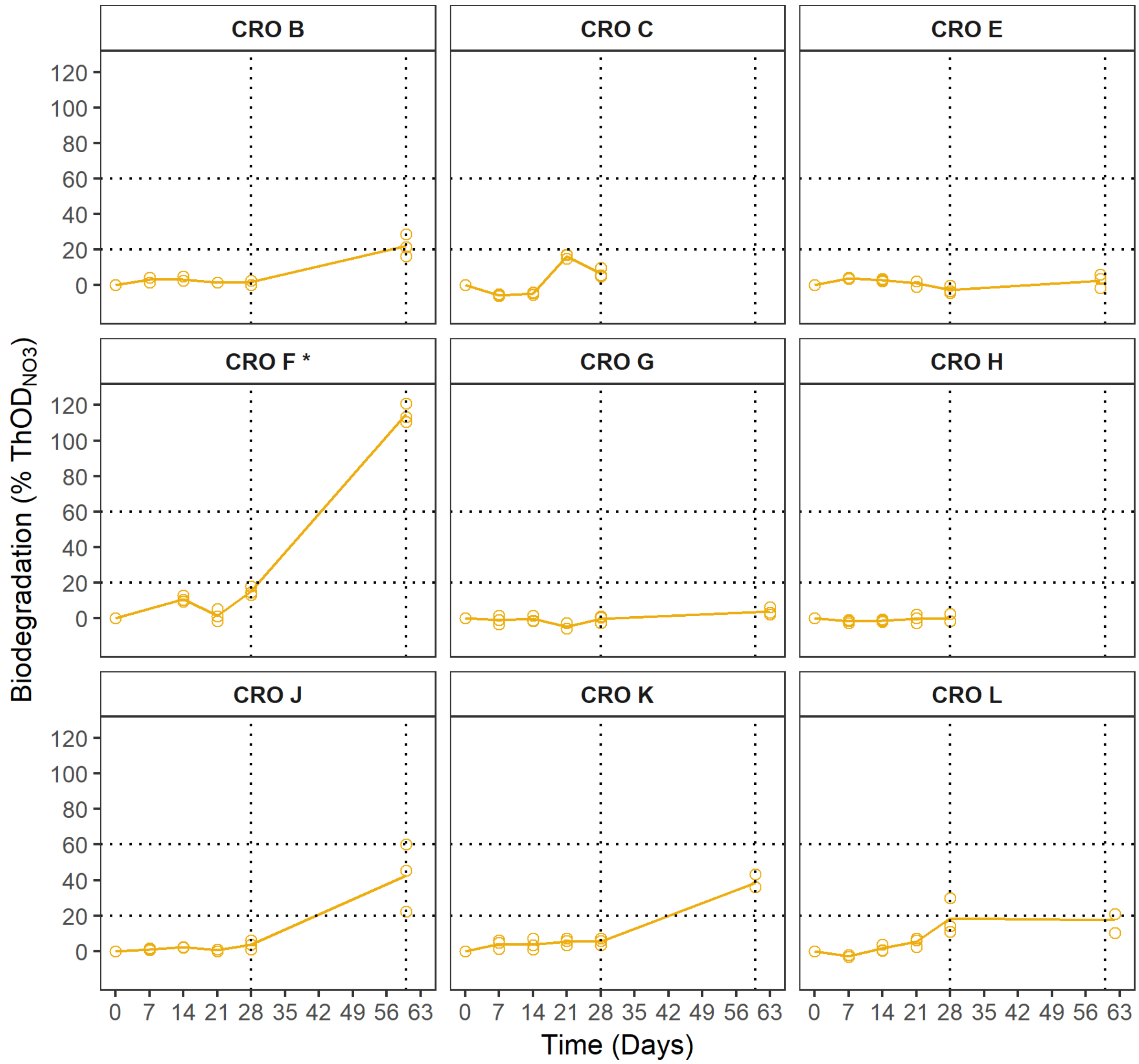
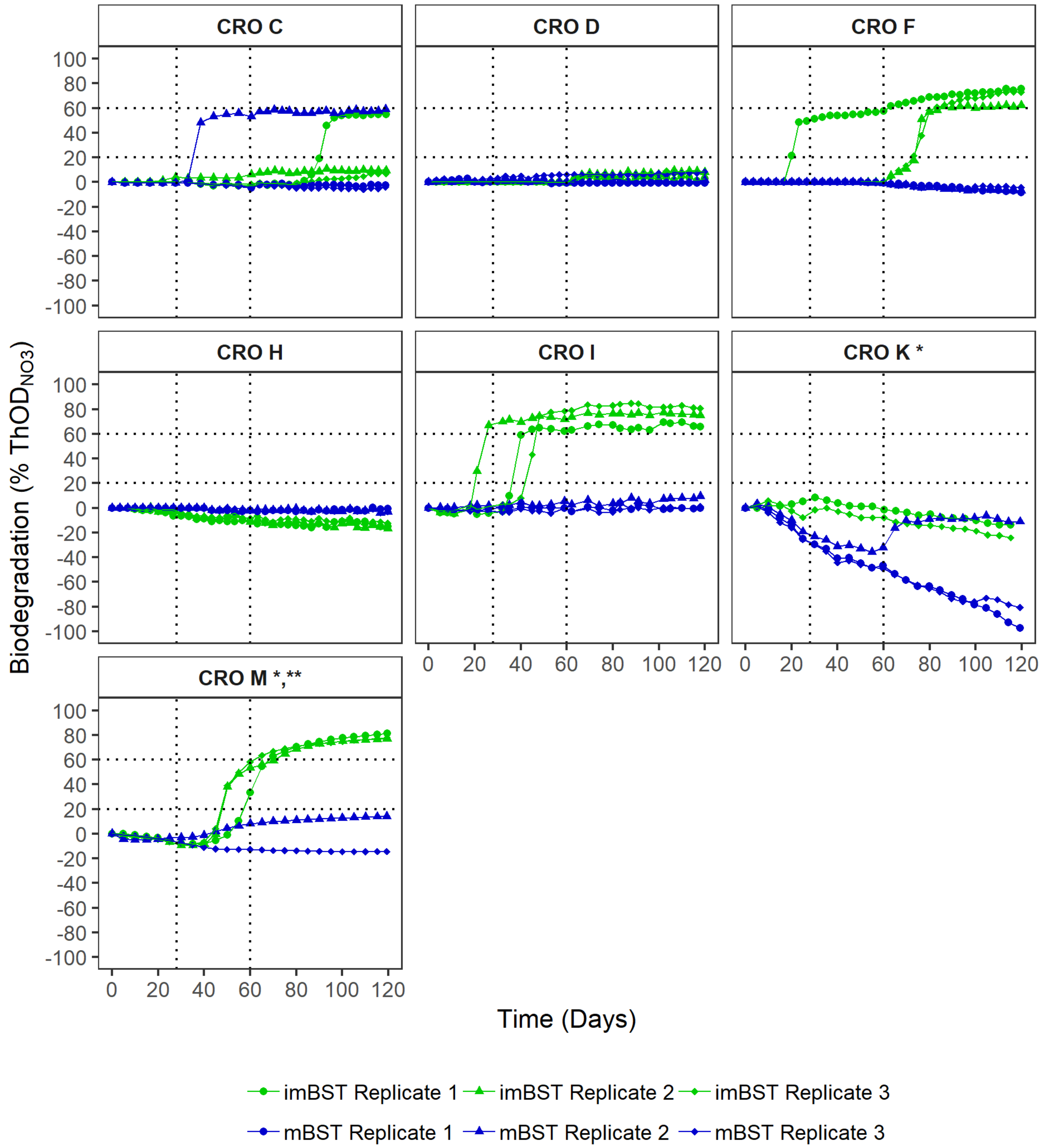
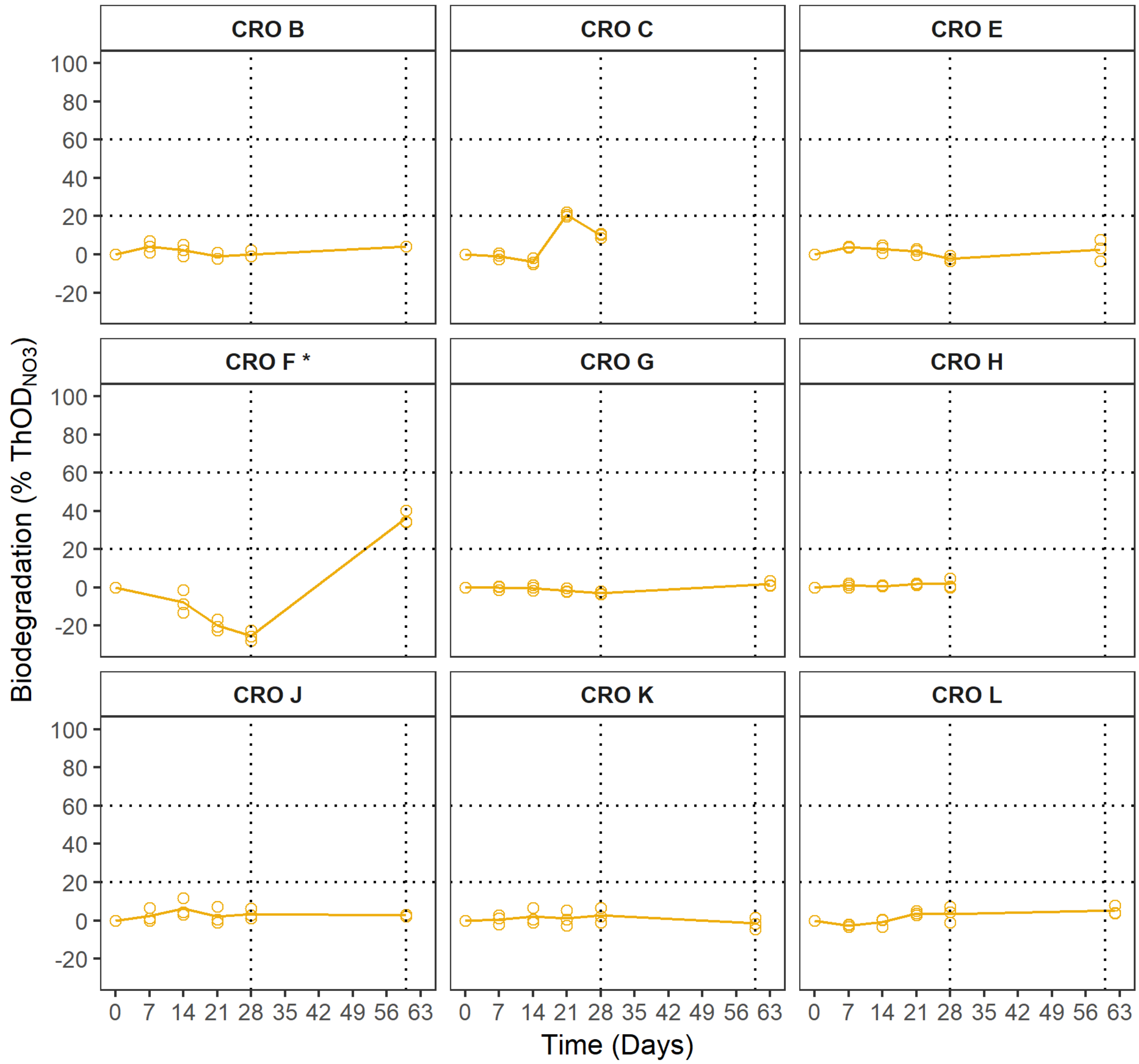


Figure S7. Biodegradation of triethanolamine in the OECD306$_{CB}$. * For removed outlier, see Figure S19.



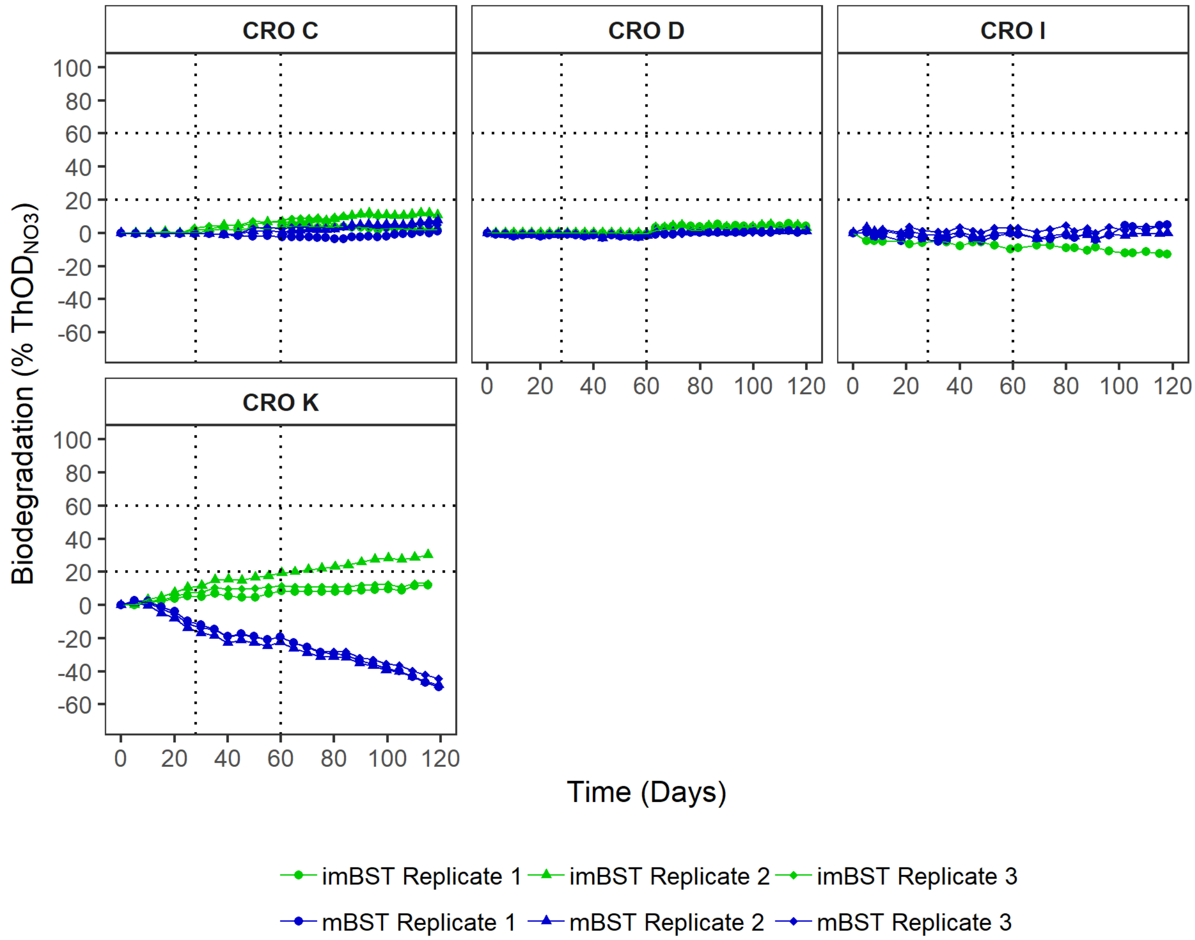
117

118 **Figure S8.** Biodegradation of 4-nitrophenol in the mBST$_{MR}$ and imBST$_{MR}$. * For removed outlier, see
119 Figure S20. ** Biodegradation based on CO_2 production instead of O_2 consumption.



120

121 **Figure S9.** Biodegradation of 4-nitrophenol in the OECD306$_{CB}$. * For removed outlier, see Figure S19.



122

123 **Figure S10.** Biodegradation of anionic polyacrylamide in the mBST$_{MR}$ and imBST$_{MR}$.

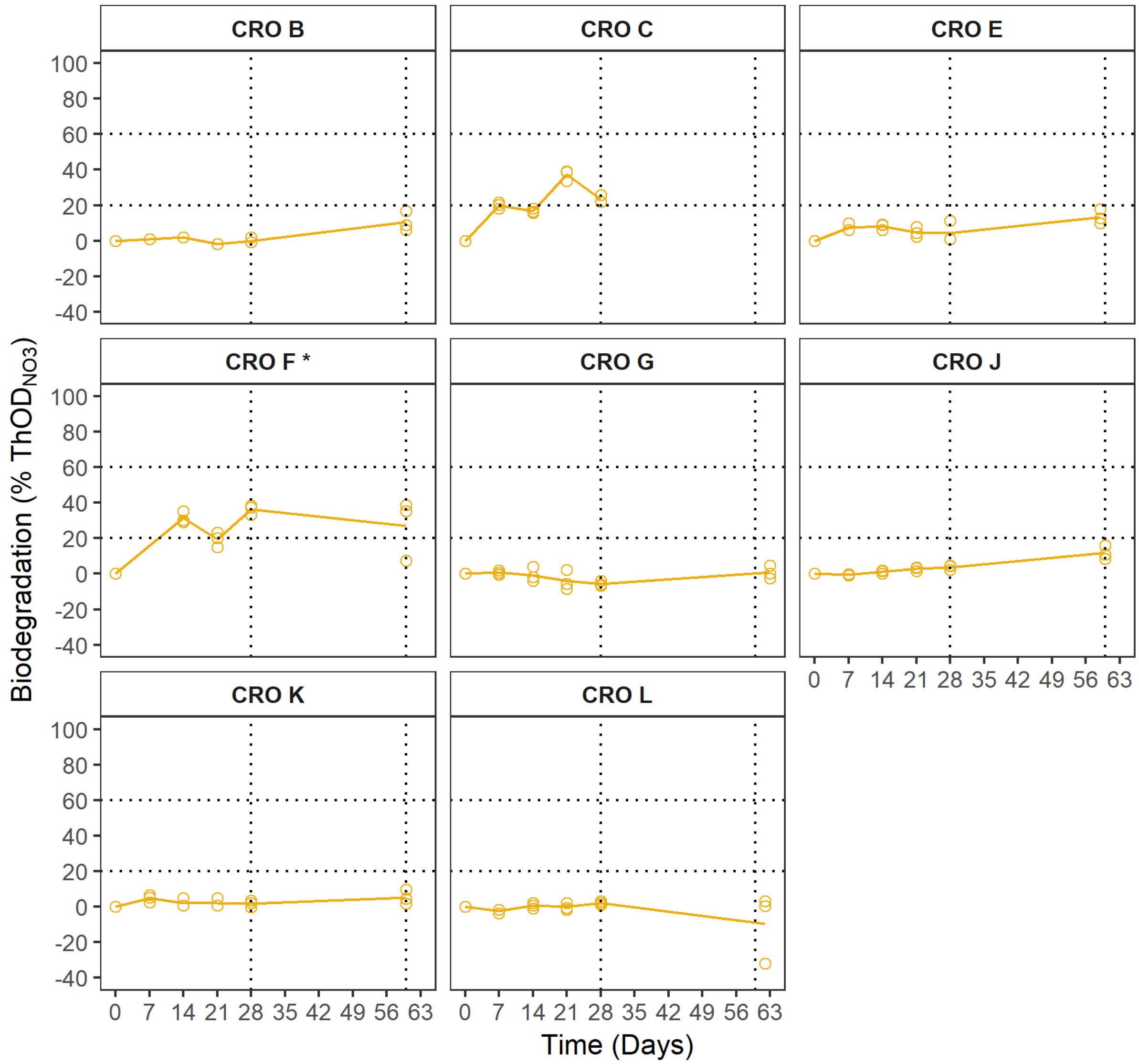


Figure S11. Biodegradation of anionic polyacrylamide in the OECD306$_{CB}$. * For removed outlier, see Figure S19.

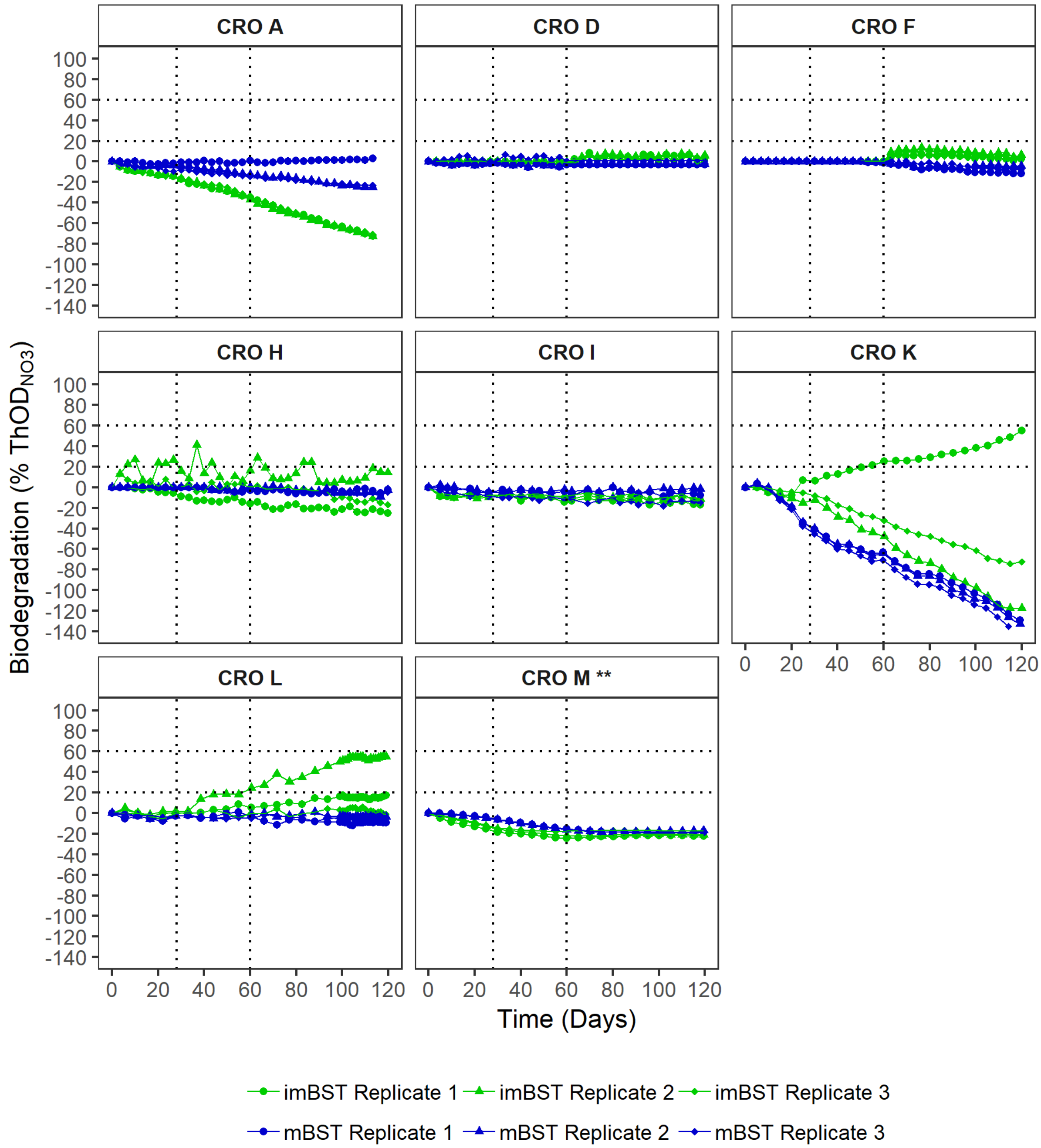


Figure S12. Biodegradation of pentachlorophenol in $mBST_{MR}$ and $imBST_{MR}$. ** Biodegradation based on CO_2 production instead of O_2 consumption.

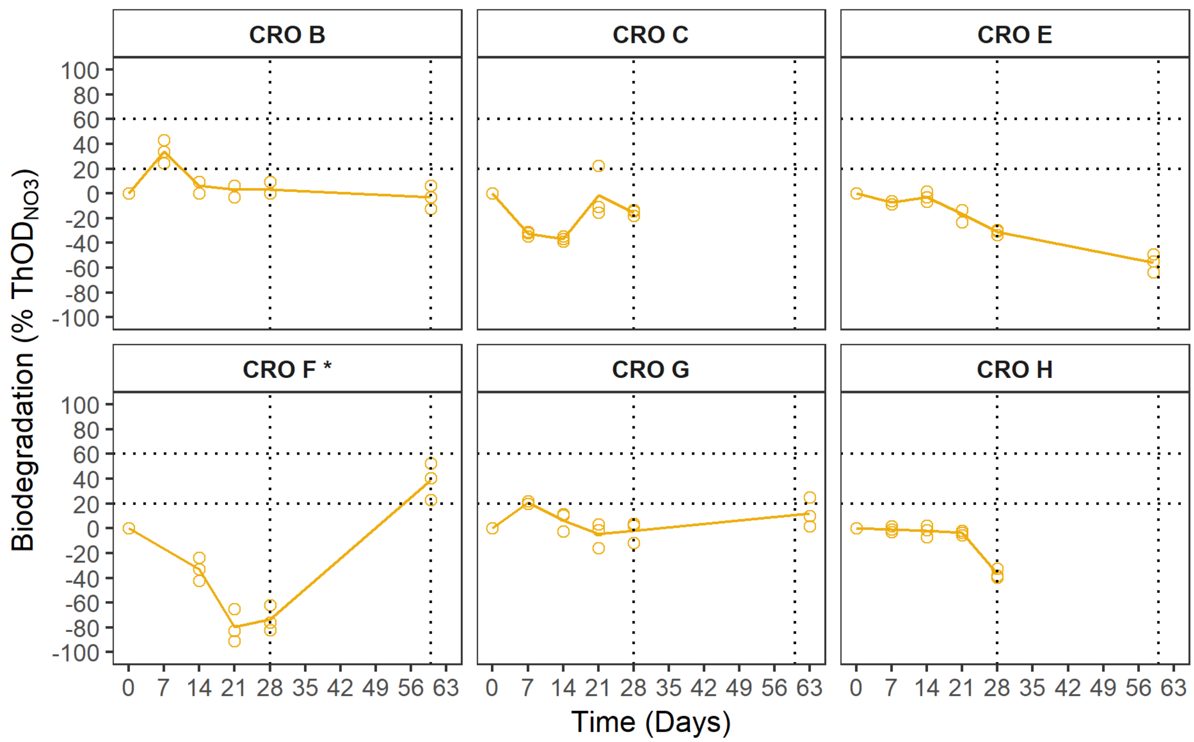


Figure S13. Biodegradation of pentachlorophenol in the $OECD306_{CB}$. * For removed outlier, see Figure S19.

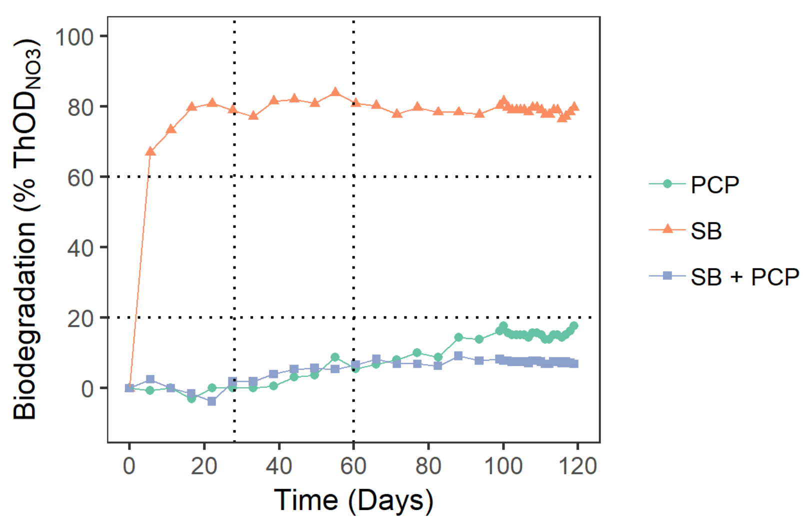


Figure S14. Pentachlorophenol (PCP) toxicity control with sodium benzoate (SB) for the $imBST_{MR}$ by CRO L.

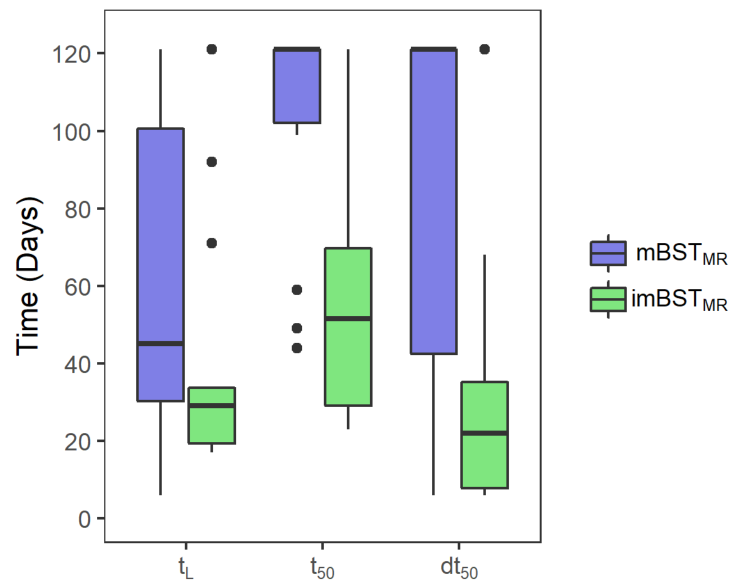


Figure S15. Increased cell numbers in the new test reduce t_L (time to 10% degradation), t_{50} (time to 50% degradation) and dt_{50} (t_{50} – t_L) for triethanolamine. For non-degrading $mBST_{MR}$ and $imBST_{MR}$ replicates, descriptor values were set to 121 days.

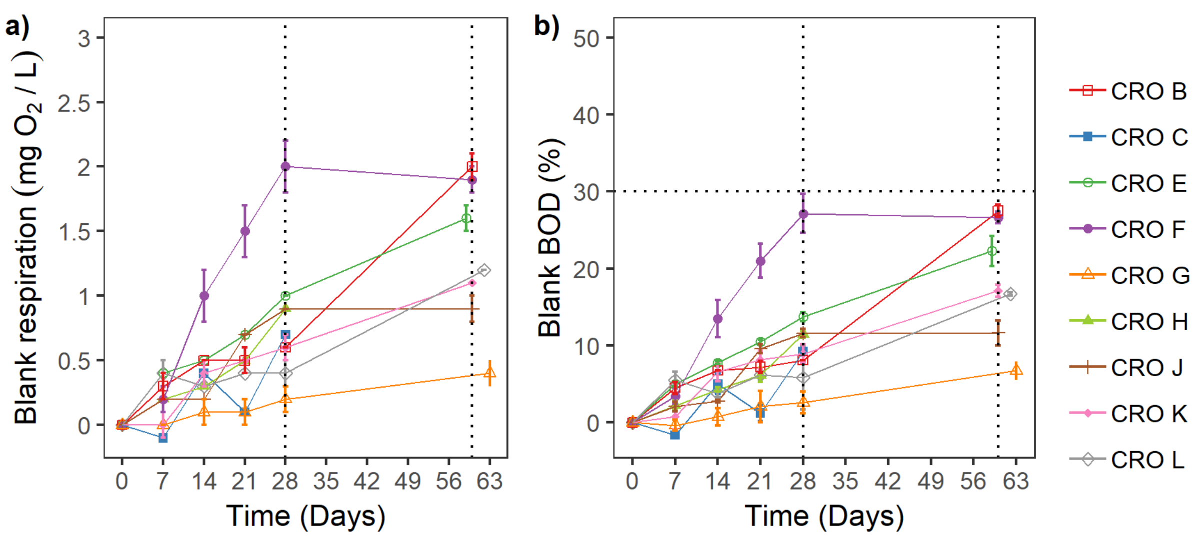


Figure S16. $OECD306_{CB}$ blank respiration over 60 days across CROs expressed in mg O_2 L^{-1} (a) and % (b). Dotted horizontal line at 30% BOD (b) refers to blank threshold defined in test guideline OECD 306.[9]

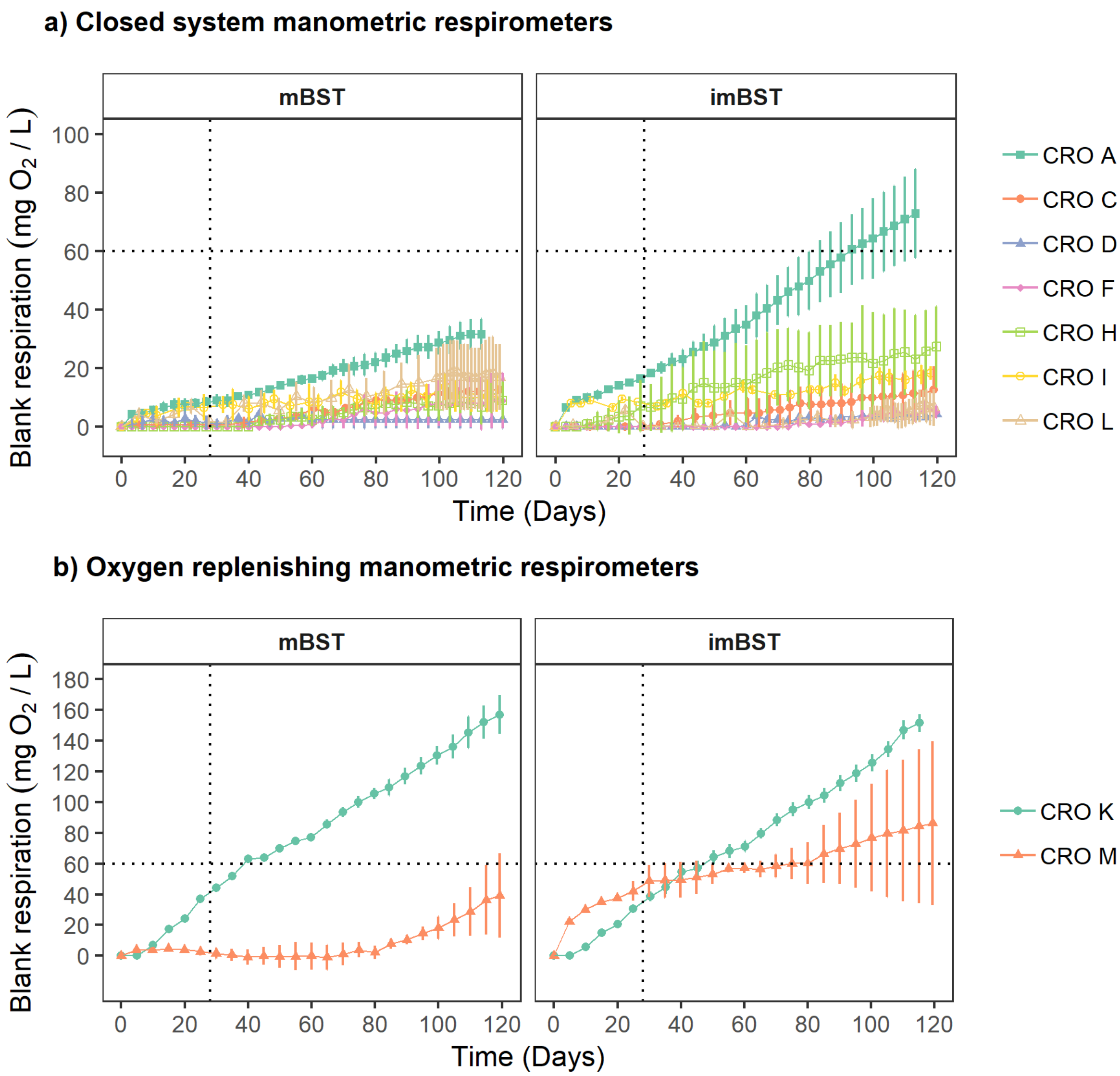


Figure S17. imBST$_{MR}$ and mBST$_{MR}$ blank respiration in closed manometric respirometer systems (a and b) and oxygen replenishing manometric respirometer systems (c and d). Dotted horizontal line at 60 mg O_2 L^{-1} blank respiration and 28 days refers to blank threshold defined in test guideline OECD 301F.[8]

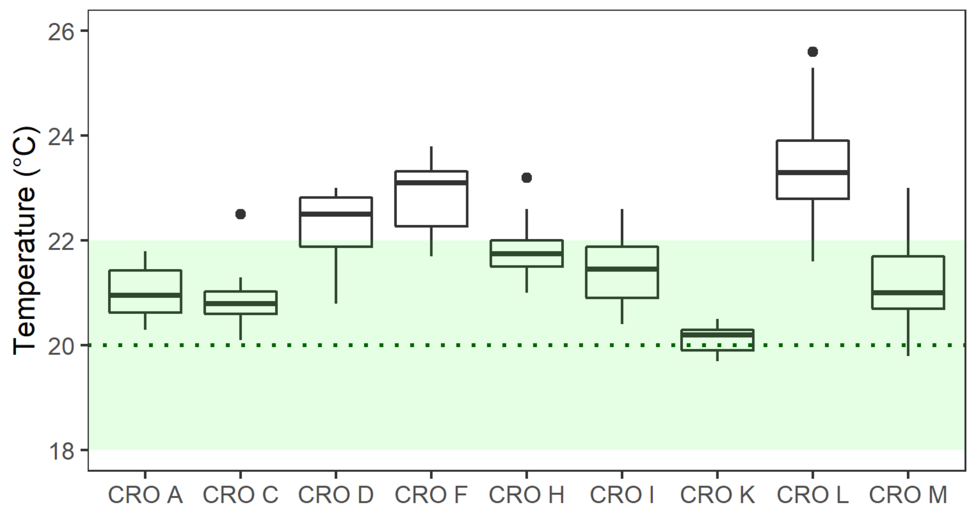


Figure S18. Boxplots showing temperatures measured in mBST$_{MR}$ and imBST$_{MR}$ test media after 120 day incubation period across CROs. Green indicates 20 ± 2°C range.

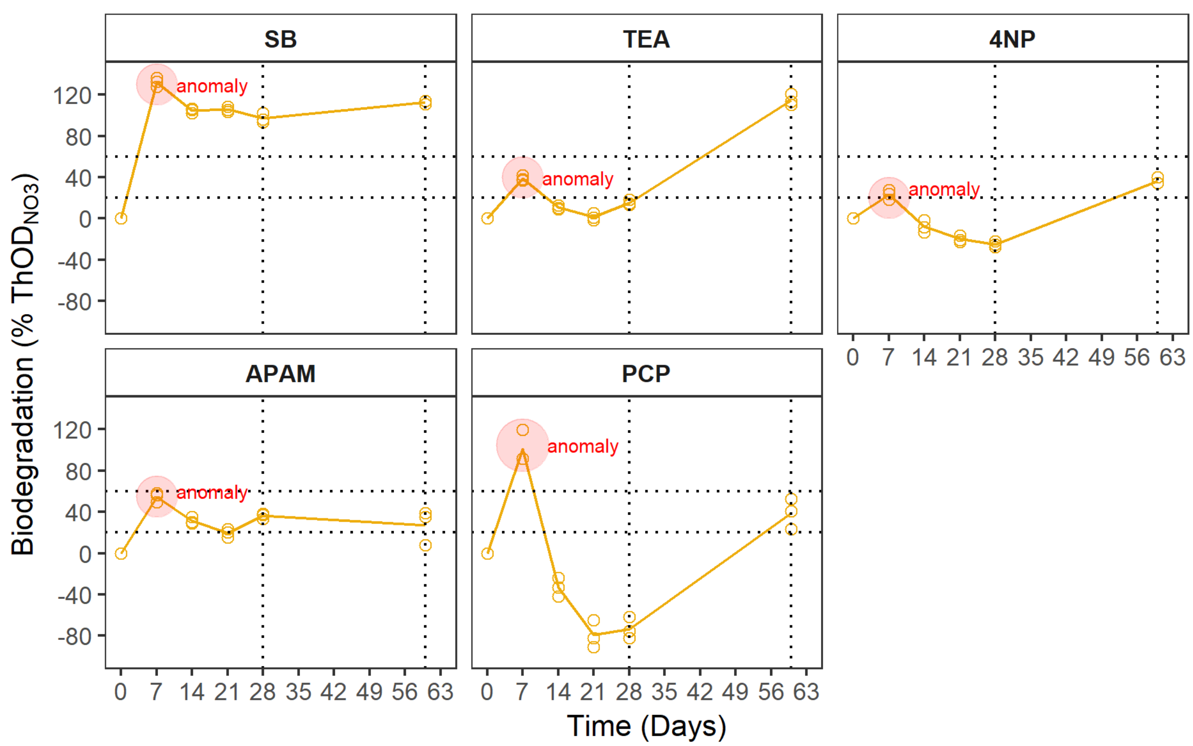


Figure S19. Systematic anomalous results (marked with a red circle) observed in the OECD306$_{CB}$ at CRO F. SB: sodium benzoate. TEA: triethanolamine. 4NP: 4-nitrophenol. APAM: anionic polyacrylamide. PCP: pentachlorophenol.

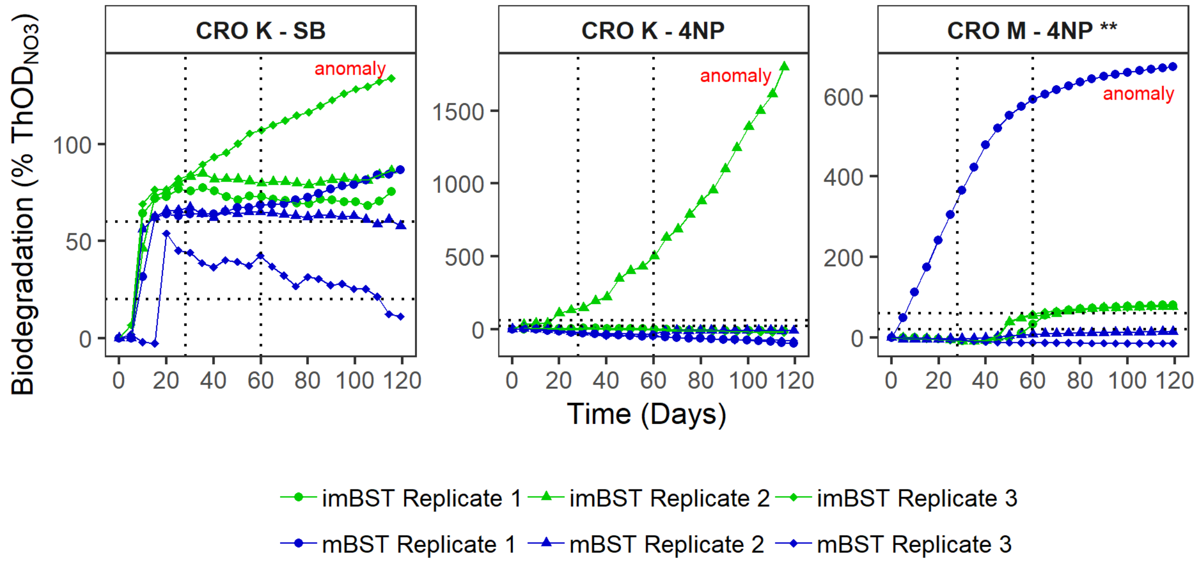
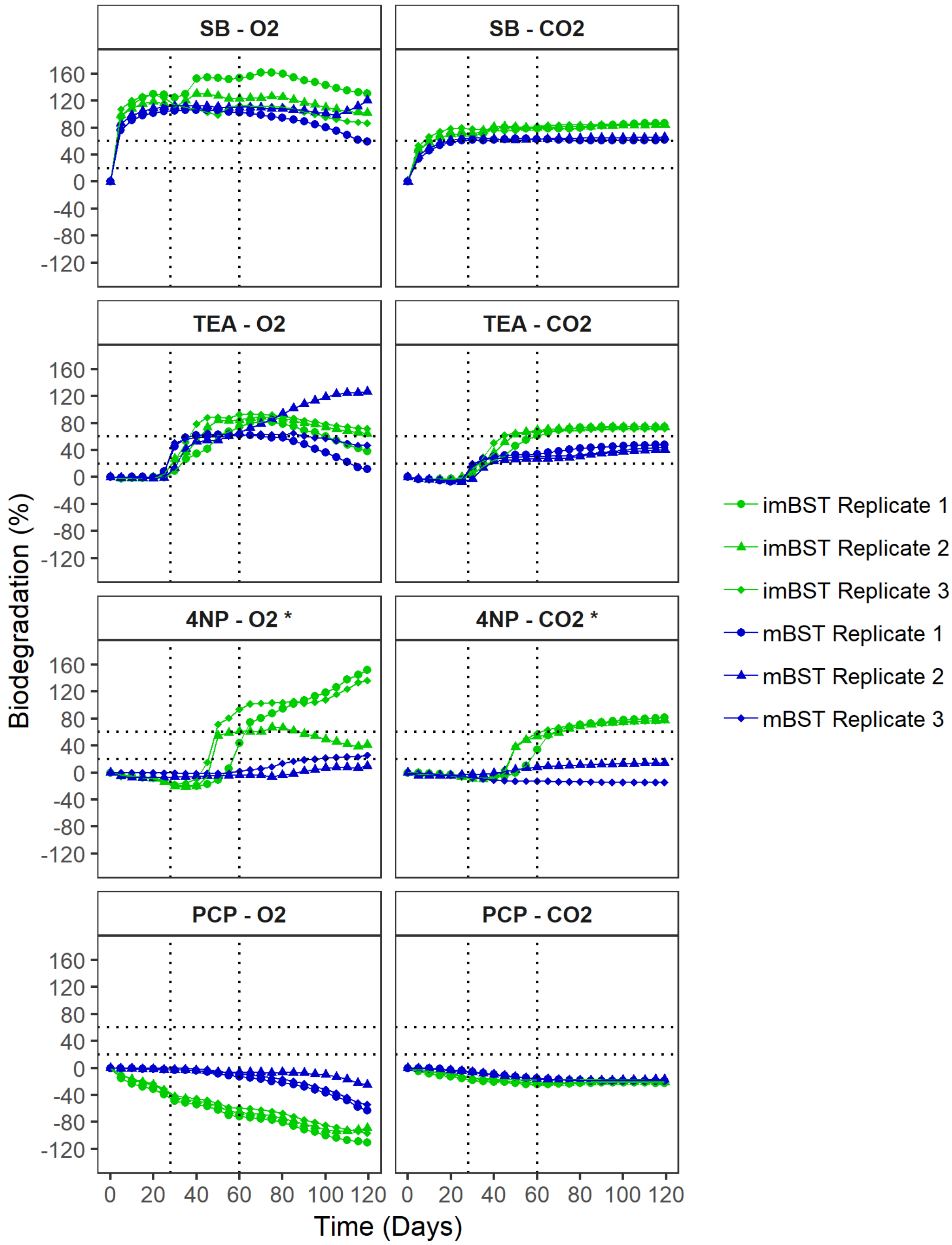


Figure S20. Outliers observed in the mBST$_{MR}$ and imBST$_{MR}$. SB: sodium benzoate. 4NP: 4-nitrophenol. ** Biodegradation based on CO_2 production instead of O_2 consumption.



166

167 **Figure S21.** Comparison of biodegradation values calculated based on O_2 consumption and CO_2 production
168 for CRO M. SB: sodium benzoate. TEA: triethanolamine. 4NP: 4-nitrophenol. PCP: pentachlorophenol. *
169 For removed outlier, see Figure S20.

Table S1. Instruments and methods employed at the CROs for the $mBST_{MR}$, $imBST_{MR}$ and $OECD306_{CB}$.

CRO →	A	B	C	D	E	F	G	H	I	J	K	L	M
$mBST_{MR}$ and $imBST_{MR}$													
Manometric respirometer	WTW OxiTop Control	—	WTW OxiTop Control	WTW OxiTop Control	—	WTW OxiTop IS	—	WTW OxiTop Control	WTW OxiTop Control	—	CES multi-channel aerobic respire-meter	WTW OxiTop Control	Columbus Instrument Micro-Oxymax Respirometer
$OECD306_{CB}$													
Removing coarse particles		Filtration (11 µm)	Filtration (10 µm)		Filtration	Not performed	Sedimentation	Sedimentation		Sedimentation	Filtration (coarse filter paper)	Sedimentation and siphoning	
Ageing conditions	—	7 days ageing with 3 days aeration; 20°C; dark	6 days with full aeration; 20°C; dark	—	7 days with full aeration; 20°C; dark	7 days with full aeration; 20°C; dark	7 days with full aeration 18°C ± 2°C; dark	7 days with full aeration; 20°C; dark	—	7 days with no aeration; 18.4-19°C; dark	10 days with full aeration; 21°C; dark	6 days with aeration for 2h 15 min; 20°C; dark	—
DO (mg/L)		YSI 58	Days 0-14: YSI DO; Days 21-28: Mettler Toledo SevenGo pro DO		Hach HQ40d LDO101	Winkler Titration Method	YSI Oximeter model 5100	WTW Oxi 1970i		Hach HQ30d	YSI Model 57	WTW inoLab Oxi 7310	

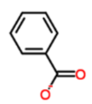
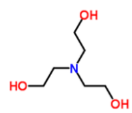
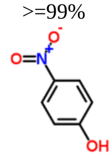
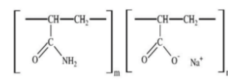
—: test setup not conducted. DO: dissolved oxygen.

Table S2. Instruments and methods employed at the CROs to characterize the seawater.

CRO →	A	B	C	D	E	F	G	H	I	J	K	L	M
pH	WTW Multi 350i	Orion Star A111	Hanna HI113 pH/ mV	HM-25R, DKK-TOA Corpor-ation	Hach PHC101 probe	Fisher Scientific AP 115	WTW InoLab pH 730	Fisher Scientific Meter 0503	See CRO A as same seawater was used	Handylab pH	Hach HQ30D	WTW pH 340i, PHM220 lab pH	Orion Star A221
T (°C)	WTW Multi 350i	YSI Pro 30	Mercury thermo-meter	Alcohol thermo-meter	Hach CDC401 probe	Hach sension5	Total immersion glass thermo-meter	Thermo Scientific Orion Star		Testo 110	Hach HQ30D	WTW Multi 3430, WTW InoLab Oxi 7310	Alcohol thermo-meter
DO (mg/L)	Hach HQ 40d	YSI 58 DO Meter	YSI 55 DO	ID-150, Iijima Electronics	Hach LDO101 probe	Hach sension5	YSI Oximeter 5100	Fisher Scientific Meter 0503		Hach HQ30d	Hach HQ30D	WTW Multi 3430, WTW Inolab Oxi 7310	HQ40d meter LBOD101r
Conduc-tivity (mS/cm)	WTW Multi 340i	YSI Pro 30	Mettler Toledo Seven Multi	CM-31P, DKK-TOA Corpor-ation	Hach CDC401 probe	Hach sension5	Not measured	Fisher Scientific Meter 0503		WTW Conducto-meter	Hach HQ30D	WTW Multi 3430, WTW inoLab Terminal Level 3 Tetracon 325 probe	YSI 3200
Salinity (ppt)	WTW Multi 340i	YSI Pro 30	Mettler Toledo Seven Multi	CM-31P, DKK-TOA Corpor-ation	Hach CDC401 probe	Hach sension5	Thermo-balance Satorius MA35	Fisher Scientific Meter 0503		WTW Conducto-meter	Hach HQ30D	WTW Multi 3430, WTW inoLab Terminal Level 3 Tetracon 325 probe	YSI 3200
HPC/mL	DEV nutrient agar	Serial extinction marine broth bottle test	np.	Trypticase soy agar	Marine Agar	APHA Method 9215	Total viable count	Marine agar		np.	Trypticase soy agar	PCA with seawater	np.

DO: dissolved oxygen. HPC: heterotrophic plate counts. np: not performed. T: temperature.

Table S3. Chemical and physical properties of reference chemicals. All data for APAM provided by chemical supplier SNF. Information for other chemicals obtained from PhysProp [12] , except for calculated $ThCO_2$ and $ThOD_{NH3/NO3}$ values [9] and chemical structures (obtained from ChemSpider [13]). All chemicals except APAM purchased from Sigma Aldrich, St. Louis, USA.

	Positive control: Sodium benzoate (SB)	**Variable degradation:** Triethanolamine (TEA)	4-Nitrophenol (4NP)	Anionic polyacrylamide (APAM)	**Negative control:** Pentachlorophenol (PCP)
CAS	532-32-1	102-71-6	100-02-7	25937-30-8	87-86-5
Formula	$C_7H_5NaO_2$	$C_6H_{15}NO_3$	$C_6H_5NO_3$	$[C_3H_5NO]_m$ $[C_3H_3NaO_2]_l$	$C_6H_5Cl_5O$
Purity	$\geq$ 99.0%	98%	>=99%	/	97%
Structure					
Molecular weight (g/mol)	144.11	149.19	139.11	7.6 M Da	266.34
Water solubility (mg/L)	5.56×10^5 at 25ºC, exp.	1.00×10^6 at 22ºC, exp.	1.16×10^4 at 20ºC, exp.	100%	14 at 25ºC, exp.
Vapour pressure (mm Hg)	3.67×10^{-9} at 25ºC, est.	3.59×10^{-6} at 25ºC, exp.	9.79×10^{-5} at 20ºC, exp.	information not available	1.10×10^{-4} at 25ºC, exp.
Henry's law constant at 25ºC (atm-m^3/mol)	1.09×10^{-7}, est.	7.05×10^{-13}, est.	4.15×10^{-10}, exp.	information not available	2.45×10^{-8}, exp.
Log K_{ow}	-2.27, est.	-1, exp.	1.19, exp.	-2.34, exp.	5.12, exp.
$ThOD_{NH3}$ and $ThOD_{NO3}$ (mg O_2/mg test substance)	1.67 1.67	1.61 2.04	1.15 1.61	1.25 1.88	0.54 0.54
$ThCO_2$ (mg CO_2/mg test substance)	2.14	1.77	1.90	information not available	0.99

est: estimated data. exp: experimental data.

Table S4. Explanation on test chemical selection and assigned "correct" biodegradation classification to compare the results of the standard OECD 306 test, the revised test and the new test. Note that these assigned biodegradation classifications are not definitive as they are restricted by the quality and scope of the evaluated data.[1,14]

Assigned reference biodegradation classification	Previously reported biodegradation data and explanation on test chemical selection
Sodium benzoate (SB); rapidly biodegradable – non persistent	– ECHA database: Readily biodegradable;[15] – Comber and Holt (2010) grouped SB in bin 1 (would normally pass a BST and enhanced BST);[16] – Positive control in BSTs OECD 301, 306, 310;[4,8,9]
Triethanolamine (TEA); rapidly biodegradable – non persistent	– ECHA database: Readily biodegradable;[17] – Recommended by regulators for testing in ring test; – Variable degradation observed in BSTs ranging from 0-100%: ○ Eide-Haugmo et al. (2012) found TEA to degrade 20% in 28 days in OECD 306 Closed Bottle test;[18] ○ Unpublished results vary from under 20% to over 60% biodegradation after 28 days for OECD 306 Closed Bottle test (Cefas, personal communication, 2016); ○ Gerike and Fisher (1979) found TEA to degrade 91-100% in 28 days in Sturm test, 97% in 42 days in AFNOR test, 96% in 19 days in precursor to OECD 301E test, 0-2% in 14 days in MITI test and 0-9% in 30 days in Closed Bottle test;[19]
4-nitrophenol (4NP); inherently biodegradable – non persistent	– ECHA database: Inherently biodegradable;[20] – Comber and Holt (2010) grouped 4NP in bin 2 (would normally fail a current BST, but pass an enhanced BST);[16] – Previously tested during intra-laboratory activated sludge and marine BST validation;[1,21] – Variable degradation observed in BSTs ranging from 0-100%: ○ Nyholm and Kristensen (1987) found 4NP to degrade in OECD 306 Closed Bottle tests 38% in 28 days and 0-64% in 60 days; 4NP degraded in OECD 306 Shake Flask tests 35-54% in 28 days and 0-100% in 60 days (results from OECD 306 ring test 1984-85);[22,23] ○ Ott et al. (2019) found 4NP to degrade 3-91% in 60 days in marine OECD 301B tests with varying cell concentrations;[1] ○ Martin et al. (2017) found 4NP to degrade 84-91% in 60 days in activated sludge OECD 301B tests with varying cell concentrations;[21] ○ Gerike and Fisher (1979) found 4NP to degrade 90-98% in 28 days in Sturm test, 97% in 42 days in AFNOR test, 100% in 19 days in precursor to OECD 301E test, 1-3% in 14 days in MITI test and 0-60% in Closed Bottle test;[19]
Anionic polyacrylamide (APAM); no reference biodegradation classification assigned	– No information available in ECHA database as polymers are exempt from REACH;[24] – Recommended by industry for testing in ring test: polyacrylamides (PAMs) are widely used in several industrial fields such as for water treatment, agriculture and oil recovery;[25] – Previous research found PAM macromolecules resistant to microbial attack, requiring initial physical-chemical break-down;[26,27] – Unpublished biodegradability data shows no degradation for OECD 306 Closed Bottle test, marine BODIS test or Zahn Wellens test (SNF, personal communication, 2018); – Variable degradation reported in unpublished $imBST_{MR}$-similar industry study with 100-fold increased bacterial cell concentrations from seawater measuring O_2 consumption with MRs and 400 mg/L APAM (Equinor, personal communication, 2016): ○ Study 1, April: over 20% biodegradation measured in 120 days; ○ Study 2, November: no biodegradation detected in 90 days; – Due to a lack of peer-reviewed reference literature for APAM, it was not possible to assign a "correct" biodegradation classification; consequently, APAM results in the ring test were discussed separately to data of SB, TEA, 4NP and PCP;
Pentachlorophenol (PCP); potentially persistent	– Not registered under REACH [28], but the Finish Environment Institute (SYKE) database indicates potential persistence based on BST results;[29] – Comber and Holt (2010) grouped PCP in bin 3 (should normally fail a BST and enhanced BST);[16] – Previously tested during intra-laboratory activated sludge and marine BST validation;[1,21] – Variable degradation observed in different biodegradation test, depending on PCP concentration and adaptation: ○ Ott et al. (2019) found radiolabeled PCP at 10 mg/L to not degrade (0-1%) in 60 days in marine OECD 301B tests with varying cell concentrations;[1] ○ Martin et al. (2017) found radiolabeled PCP at 10 mg/L to not degrade (0-1%) in 60 days in activated sludge OECD 301B tests with varying cell concentrations;[21] ○ Lapertot and Pulgarin (2006) found PCP to not degrade (0%) in 28 days in inherent test OECD 302B, but concluded that this may have been the result of substrate inhibition;[30] ○ Ingerslev et al. (1998) observed PCP degradation in shake flask simulation tests in unadapted systems only after long acclimation phases (14-85 days in river water tests), but PCP degradation rates increased in adapted systems; no or little degradation was observed at inhibitory PCP concentrations above 20 mg/L, but PCP degraded quickly (t_{50} = 3-10 days) at concentrations under 2.5 mg/L;[31] – Toxicity [31,32] and low solubility concerns; however, PCP was most suitable negative control after screening 34 potential compounds proposed from regulators and recommendations from previous report;[16,33]

Table S5. Chemical and test strategy**.** Overview of the test setups and chemicals tested at each anonymised CRO, labelled CRO A-M. The total number of each test method, per chemical, is included in the last row of the table.

CRO	**OECD306$_{CB}$**						**mBST$_{MR}$**						**imBST$_{MR}$**					
	B	**SB**	**TEA**	**4NP**	**APAM**	**PCP**	**B**	**SB**	**TEA**	**4NP**	**APAM**	**PCP**	**B**	**SB**	**TEA**	**4NP**	**APAM**	**PCP**
A							X	X	X			X	X	X	X			X
B	X	X	X	X	X	X												
C	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
D							X	X	X	X	X	X	X	X	X	X	X	X
E	X	X	X	X	X	X												
F	X	X	X	X	X	X	X	X		X		X	X	X		X		X
G	X	X	X	X	X	X												
H	X	X	X	X		X	X	X		X		X	X	X		X		X
I							X	X	X	X	X	X	X	X	X	X	X	X
J	X	X	X	X	X													
K	X	X	X	X	X	X	X	X		X	X	X	X	X		X	X	X
L	X	X	X	X	X		X		X			X	X	X	X			X
M							X	X	X	X		X	X	X	X	X		X
Total:	9	9	9	9	8	7	9	8	6	7	4	9	9	9	6	7	4	9

B: blank. SB: sodium benzoate. TEA: triethanolamine. 4NP: 4-nitrophenol. APAM: anionic polyacrylamide. PCP: pentachlorophenol.

Table S6. Oxygen available in the $OECD306_{CB}$ and closed system MR systems.

<table>
<tr><th colspan="2">Assumptions</th></tr>
<tr><td colspan="2">
– "At 15°C and 20°C and 32 parts per thousand salinity (ocean water), the solubility of dissolved oxygen is about 8.1 and 7.4 mg/l, respectively." [9]
– $OECD306_{CB}$: fill volume 300 mL, no headspace, incubation temperature 20°C;
– $mBST_{MR}$ and $imBST_{MR}$: fill volume 250 mL, headspace 260 mL, incubation temperature 20°C;
– For the $imBST_{MR}$ and $mBST_{MR}$, calculations are only relevant for closed MR systems (OxiTop), as the other MR systems (CES respirometer and Micro-Oxymax) replenish oxygen immediately after consumption;
– Molecular mass O_2: 32 g/mol; 21% O_2 in air; ideal gas at 20°C, 1 atm: 24.04 L/ mol;
</td></tr>
<tr><th>$OECD306_{CB}$:</th><th>$mBST_{MR}$ and $imBST_{MR}$</th></tr>
<tr><td>
<u>O_2 in liquid phase:</u>
0.3 L x 7.4 mg O_2/L = 2.22 mg O_2

<u>O_2 in headspace:</u> /

Total O_2 in $OECD306_{CB}$ bottle:
2.22 mg O_2 + 0 mg O_2 =
2.22 mg O_2
</td><td>
<u>O_2 in liquid phase:</u>
0.25 L x 7.4 mg O_2/L = 1.85 mg O_2

<u>O_2 in headspace:</u>
Volume O_2 in headspace: 0.26 L x 0.21 = 0.055 L O_2;
$n(O_2)$ = 0.055 L O_2 ÷ 24.04 L/mol = 2.29×10^{-3} mol O_2
m(O2) = 32 g/mol x 2.29×10^{-3} mol O_2 = 7.33×10^{-2} g = 73.28 mg O_2

Total O_2 in $imBST_{MR}$ or $mBST_{MR}$ bottle:
1.85 mg O_2 + 73.28 mg O_2 =
75.13 mg O_2
</td></tr>
<tr><td colspan="2">
75.13 mg O_2 ÷ 2.22 mg O_2 = 33.84
In this study, MR test setups provide at least 34-times more O_2 than the $OECD306_{CB}$ test setup.
</td></tr>
</table>

Table S7. Raw and processed seawater characterization. CRO A and I used seawater collected and processed from the same source. All analysis except TCC performed by CROs (methods see Table S2). Temperature measurement S1 does not always represent original seawater temperature.

Description		CRO A	CRO B	CRO C	CRO D	CRO E	CRO F	CRO G	CRO H	CRO I	CRO J	CRO K	CRO L	CRO M
Seawater collection	Collection date $OECD306_{CB}$	—	01.06.17	09.03.17	—	30.05.17	01.05.17	23.05.17	06.04.17	See CRO A	02.06.17	24.04.17	14.03.17	—
	Collection date MR tests	27.03.17	—	07.03.17	08.05.17	—	01.05.17	—	04.04.17			24.04.17	14.03.17	14.08.17
	Depth (m)	6	3	nr.	10	2	10	50	nr.		10	nr.	60	0.5
	Distance offshore (m)	40-50	45	67	300	100	250	5000	nr.		100	nr.	nr.	200
	Water appearance	Clear	Clear	Clear	Clear	Clear	Slightly turbid	Clear	Clear		Clear	Clear	Clear	Clear
	Date setup $OECD306_{CB}$	—	14.06.17	15.03.17	—	06.06.17	11.05.17	30.05.17	13.04.17	—	09.06.17	04.05.17	21.03.17	—
	Date setup MR tests	31.03.17	—	08.03.17	13.05.17	—	04.05.17	—	06.04.17	31.03.17	—	26.04.17	15.03.17	17.08.17
Raw seawater (S1)	pH	8.0	7.8	8	8.1	7.9	7.40	8	7.70	See CRO A	8.2	7.8	8	7.9
	T (°C)	10.4	24.9	18.7	17.8	19.2	9.0	22.0	15.6		12.0	10.4	14.9	2.8
	DO (mg/L)	10.3	6.0	8	9.5	9.19	7.9	7.4	7.85		9.6	11.1	7.9	12.
	Conductivity (mS/cm)	24.0	44.1	45.3	44.3	46.7	45.0	np.	48.10		43.8	45.8	53.3	42.7
	Salinity (ppt)	16.1	28.7	32.2	27.5	34.7	28.0	34.1	30.60		31.1	29.6	34.6	27.5
	HPC x 10^3/ mL	82	10	np.	0.92	0.48	0.5	2	4.5		np.	2	Not countable	np.
	TCC x 10^5/ mL	5.4 ± 0.4	—	2 ± 0.094	2.8 ± 0.21	—	3.1 ± 0.49	—	0.6 ± 0.034		—	0.7 ± 0.07	1.1 ± 0.04	7.5 ± 0.21
10 µm filtered seawater for $mBST_{MR}$ (S2)	pH	8.7	—	8	np.	—	np.	—	7.80	See CRO A	—	6.76	8.	8
	T (°C)	19.1		18.7					16.00			19.7	10.7	0.9
	DO (mg/L)	8.8		8					7.72			9.3	8.3	13.4
	Conductivity (mS/cm)	24.5		45.3					48.10			48.4	53.5	41.1
	Salinity (ppt)	16.7		32.2					30.60			30.3	34.4	26.3
	HPC x 10^3/ mL	Not countable		np.	0.39		0.2		2.1			0.65	4	np.
	TCC x 10^5/ mL	4.8 ± 0.36		2.4 ± 0.37	1.6 ± 0.12		1.2 ± 0.0094		0.4 ± 0.02			0.86 ± 0.12	1.87± 0.12	5.4 ± 0.15
TFF processed seawater for	pH	8.8	—	7.9	np.	—	np.	—	7.80	See CRO A	—	7.1	8	7.6
	T (°C)	19.0		18.9					16.10			19.8	12.6	6.0
	DO (mg/L)	8.5		8.1					7.23			9	8.5	11.4

Description		CRO A	CRO B	CRO C	CRO D	CRO E	CRO F	CRO G	CRO H	CRO I	CRO J	CRO K	CRO L	CRO M
imBST$_{MR}$ (S3)	Conductivity (mS/cm)	24.4		45.7					48.00			48.1	53.6	42.6
	Salinity (ppt)	16.6		33.2					30.60			31.3	34.6	27.4
	HPC x 10^4/ mL	140		np.	0.19		0.37		49			Not countable	20	np.
	TCC x 10^7/ mL	7.6 ± 0.14		0.37 ± 0.041	2.4 ± 0.096		1.3 ± 0.035		0.71 ± 0.0036			0.16 ± 0.0054	0.26 ± 0.013	12 ± 0.99
Aged seawater for OECD306$_{CB}$ (S4)	pH		8.00	8		7.9	7.3	8	8.2		8.2	8.3	7.8	
	T (°C)		19.80	19.6		20.0	20.3	19.0	19.7		18.6	21.2	19.7	
	DO (mg/L)		7.40	7.5		9.0	7.7	6.4	7.6		7.8	9	7.6	
	Conductivity (mS/cm)	—	44.1	49.0	—	46.1	44.6	np.	48.8	—	43.6	44.6	52.5	—
	Salinity (ppt)		28.50	34.7		33.1	31.0	34.1	31.6		31.4	31.5	34.4	
	HPC x 10^4/ mL		10	np.		6.8	0.012	0.06	0.3		np.	0.33	10	

—: test setup not conducted. HPC: heterotrophic plate counts. nr: not recorded. np: not performed. T: temperature. TCC: total cell counts.

Table S8. Effect of pretreatment on bacteria concentrations in OECD306$_{CB}$ and imBST$_{MR}$. Coloring indicates fold cell increase (green) and fold cell reduction (red) between treatment steps. CRO A and I used the same seawater.

Test	Fold change	CRO A/I	CRO B	CRO C	CRO D	CRO E	CRO F	CRO G	CRO H	CRO J	CRO K	CRO L	CRO M
OECD306$_{CB}$	S1→S4	—	94	np.	—	141.7	0.2	0.3	0.7	np.	1.7	25	—
imBST$_{MR}$	S1→S3	140.4	—	18.8	88	—	42	—	118.9	—	23.3	23.7	160.2
	S2→S3	156.2	—	14.8	148	—	103	—	180.6	—	19.1	14.1	221.8

—: test setup not conducted. S1: raw seawater. S2: 10 µm filtered seawater. S3: 10 µm filtered and TFF treated seawater to increase bacteria concentrations 100-fold nominally. S4: seawater after OECD 306 pretreatment (filtered/sedimented and aged). np: analysis not performed.

Table S9. Chemical degradation of reference compounds in the three test systems in respect to CROs as evaluated against two regulatory persistence thresholds. Cursive brackets state the number of CROs out of all CROs where the reference compound degraded in at least 2/3 replicates to pass the stated persistence criteria and classify as non-persistent.

	Current test: OECD306$_{CB}$		**Revised test: mBST$_{MR}$**		**New test: imBST$_{MR}$**	
	Not persistent under OSPAR [a]	Not persistent under REACH [b]	Not persistent under OSPAR [a]	Not persistent under REACH [b]	Not persistent under OSPAR [a]	Not persistent under REACH [b]
SB	**100%** *(9/9)*	**100%** *(7/7)*	**100%** *(8/8)*	**100%** *(8/8)*	**100%** *(9/9)*	**100%** *(9/9)*
TEA	**0%** *(0/9)*	**14%** *(1/7)*	**0%** *(0/6)*	**17%** *(1/6)*	**33%** *(2/6)*	**50%** *(3/6)*
4NP	**11%** *(1/9)*	**0%** *(0/7)*	**0%** *(0/7)*	**0%** *(0/7)*	**0%** *(0/7)*	**14%** *(1/7)*
APAM	**25%** *(2/8)*	**0%** *(0/7)*	**0%** *(0/4)*	**0%** *(0/4)*	**0%** *(0/3)*	**0%** *(0/3)*
PCP	**33%** *(2/6)*	**0%** *(0/4)*	**0%** *(0/8)*	**0%** *(0/8)*	**0%** *(0/8)*	**0%** *(0/8)*

[a] OSPAR: Biodegradation ≥ 20% over 28 days = non-persistent; biodegradation < 20% over 28 days = persistent [34]

[b] REACH: Biodegradation ≥ 60% over 60 days = non-persistent; biodegradation < 60% over 60 days = potentially persistent [35]

Table S10. Overview of sodium benzoate (SB) degradation in the three test systems based on replicates. The mean biodegradation values recorded on day 28, 60 and 120 are stated. Lag phase (t_L), time to reach 50% degradation (t_{50}) and dt_{50} (t_{50}-t_L) were only determined for the $mBST_{MR}$ and $imBST_{MR}$ tests. Cursive values state the number of SB replicates out of all performed SB replicates, which were used to calculate the respective benchmark criteria.

	$OECD306_{CB}$		**$mBST_{MR}$**		**$imBST_{MR}$**	
	Mean ± SD	*R*	Mean ± SD	*R*	Mean ± SD	*R*
Day 28	73 ± 15 %	*27/27*	73 ± 14 %	*22/22*	77 ± 9 %	*26/26*
Day 60	82 ± 15 %	*21/21*	77 ± 15 %	*22/22*	80 ± 9 %	*26/26*
Day 120	ND		76 ± 20 %	*22/22*	81 ± 16 %	*26/26*
t_L	ND		4 ± 3 d	*22/22*	2 ± 1 d	*26/26*
t_{50}	ND		7 ± 4 d	*22/22*	4 ± 2 d	*26/26*
dt_{50}	ND		3 ± 3 d	*22/22*	2 ± 1 d	*26/26*

ND: not defined. R: replicate numbers. SD: standard deviation.

Table S11. Overview of triethanolamine (TEA) degradation in the three test systems in respect to replicates. The mean biodegradation values recorded on day 28, 60 and 120 are stated. Lag phase (t_L), time to reach 50% degradation (t_{50}) and dt_{50} (t_{50}-t_L) were only determined for the $mBST_{MR}$ and $imBST_{MR}$ tests. Cursive values state the number of TEA replicates out of all performed TEA replicates, which were used to calculate the respective benchmark criteria.

	$OECD306_{CB}$		**$mBST_{MR}$**		**$imBST_{MR}$**	
	Mean ± SD	*R*	Mean ± SD	*R*	Mean ± SD	*R*
Day 28	6 ± 7 %	*27/27*	4 ± 6 %	*18/18*	20 ± 24 %	*18/18*
Day 60	28 ± 33 %	*20/20*	24 ± 25 %	*18/18*	51 ± 28 %	*18/18*
Day 120	ND		43 ± 31 %	*18/18*	61 ± 24 %	*18/18*
t_L	ND		42 ± 19 d	*14/18*	32 ± 20 d	*17/18*
t_{50}	ND		82 ± 30 d	*7/18*	50 ± 26 d	*16/18*
dt_{50}	ND		30 ± 21 d	*7/18*	21 ± 17 d	*16/18*

ND: not defined. R: replicate numbers. SD: standard deviation.

Table S12. Overview of 4-nitrophenol (4NP) degradation in the three test systems in respect to replicates. The mean biodegradation values recorded on day 28, 60 and 120 are stated. Lag phase (t_L), time to reach 50% degradation (t_{50}) and dt_{50} (t_{50}-t_L) were only determined for the $mBST_{MR}$ and $imBST_{MR}$ tests. Cursive values state the number of 4NP replicates out of all performed 4NP replicates, which were used to calculate the respective benchmark criteria.

	$OECD306_{CB}$		**$mBST_{MR}$**		**$imBST_{MR}$**	
	Mean ± SD	*R*	Mean ± SD	*R*	Mean ± SD	*R*
Day 28	3 ± 4 %	*27/27*	0 ± 1 %	*20/20*	6 ± 18 %	*20/20*
Day 60	8 ± 12 %	*21/21*	4 ± 13 %	*20/20*	21 ± 30 %	*20/20*
Day 120	ND		5 ± 13%	*20/20*	38 ± 36 %	*20/20*
t_L	ND		73 ± 38 d	*3/20*	53 ± 25 d	*11/20*
t_{50}	ND		39 d	*1/20*	56 ± 23 d	*10/20*
dt_{50}	ND		3 d	*1/20*	6 ± 3 d	*10/20*

ND: not defined. R: replicate numbers. SD: standard deviation.

Table S13. Overview of anionic polyacrylamide (APAM) degradation in the three test systems in respect to replicates. The mean biodegradation values recorded on day 28, 60 and 120 are stated. Lag phase (t_L), time to reach 50% degradation (t_{50}) and dt_{50} (t_{50}-t_L) were only determined for the $mBST_{MR}$ and $imBST_{MR}$ tests. Cursive values state the number of APAM replicates out of all performed APAM replicates, which were used to calculate the respective benchmark criteria.

	$OECD306_{CB}$		**$mBST_{MR}$**		**$imBST_{MR}$**	
	Mean ± SD	*R*	Mean ± SD	*R*	Mean ± SD	*R*
Day 28	9 ± 13 %	*24/24*	0 ± 0 %	*12/12*	3 ± 4 %	*10/10*
Day 60	10 ± 11 %	*21/21*	0 ± 1 %	*12/12*	6 ± 6 %	*10/10*
Day 120	ND		2 ± 2 %	*12/12*	8 ± 8 %	*10/10*
t_L	ND		ND	*0/12*	62 ± 30 d	*5/10*
t_{50}	ND		ND	*0/12*	ND	*0/10*
dt_{50}	ND		ND	*0/12*	ND	*0/10*

ND: not defined. R: replicate numbers. SD: standard deviation.

Table S14. Overview of pentachlorophenol (PCP) degradation in the three test systems in respect to replicates. The mean biodegradation values recorded on day 28, 60 and 120 are stated. Lag phase (t_L), time to reach 50% degradation (t_{50}) and dt_{50} (t_{50}-t_L) were only determined for the $mBST_{MR}$ and $imBST_{MR}$ tests. Cursive values state the number of PCP replicates out of all performed PCP replicates, which were used to calculate the respective benchmark criteria.

	$OECD306_{CB}$		$mBST_{MR}$		$imBST_{MR}$	
	Mean ± SD	*R*	Mean ± SD	*R*	Mean ± SD	*R*
Day 28	1 ± 2 %	*18/18*	0 ± 0 %	*24/24*	1 ± 4 %	*24/24*
Day 60	13 ± 18 %	*12/12*	0 ± 0 %	*24/24*	3 ± 8 %	*24/24*
Day 120	ND		0 ± 0 %	*24/24*	6 ± 14 %	*24/24*
t_L	ND		ND	*0/24*	35 ± 29 d	*6/24*
t_{50}	ND		ND	*0/24*	ND	*0/24*
dt_{50}	ND		ND	*0/24*	ND	*0/24*

ND: not defined. R: replicate number. SD: standard deviation.

Table S15. Chemical degradation of reference compounds in the three test systems in respect to replicates as evaluated against two regulatory persistence thresholds. Cursive brackets state the number of replicates out of all replicates where the reference compound degraded to pass the stated persistence criteria and classify as non-persistent.

	Current test: $OECD306_{CB}$				**Revised test: $mBST_{MR}$**				**New test: $imBST_{MR}$**			
	Not persistent under OSPAR [a]		Not persistent under REACH [b]		Not persistent under OSPAR [a]		Not persistent under REACH [b]		Not persistent under OSPAR [a]		Not persistent under REACH [b]	
SB	**100%**	*27/27*	**100%**	*21/21*	**100%**	*22/22*	**95%**	*21/22*	**100%**	*26/26*	**100%**	*26/26*
TEA	**4%**	*1/26*	**11%**	*2/19*	**0%**	*0/18*	**11%**	*2/18*	**33%**	*6/18*	**50%**	*9/18*
4NP	**7%**	*2/27*	**0%**	*0/21*	**0%**	*0/21*	**0%**	*0/21*	**10%**	*2/20*	**15%**	*3/20*
APAM	**25%**	*6/24*	**0%**	*0/21*	**0%**	*0/12*	**0%**	*0/12*	**0%**	*0/10*	**0%**	*0/10*
PCP	**39%**	*7/18*	**0%**	*0/12*	**0%**	*0/24*	**0%**	*0/24*	**4%**	*1/24*	**0%**	*0/24*

[a] OSPAR: Biodegradation ≥ 20% over 28 days = non-persistent; biodegradation < 20% over 28 days = persistent [34]

[b] REACH: Biodegradation ≥ 60% over 60 days = non-persistent; biodegradation < 60% over 60 days = potentially persistent [35]

260 **Table S16.** Test variation per chemical across tests described by the coefficient of variation.

	Current test: $OECD306_{CB}$	Revised test: $mBST_{MR}$	New test: $imBST_{MR}$
SB	5%	11%	9%
TEA	55%	51%	25%
4NP	75%	69%	50%
APAM	57%	57%	36%
PCP	52%	21%	56%
Mean	49%	42%	35%
Mean excl. PCP	48%	47%	30%

REFERENCES.

(1) Ott, A.; Martin, T. J.; Snape, J. R.; Davenport, R. J. Increased Cell Numbers Improve Marine Biodegradation Tests for Persistence Assessment. *Sci. Total Environ.* **2020**, *706*, 135621. https://doi.org/10.1016/j.scitotenv.2019.135621.

(2) Martin, T. J.; Goodhead, A. K.; Snape, J. R.; Davenport, R. J. Improving the Ecological Relevance of Aquatic Bacterial Communities in Biodegradability Screening Assessments. *Sci. Total Environ.* **2018**, *627*, 1552–1559. https://doi.org/10.1016/j.scitotenv.2018.01.264.

(3) Ott, A.; Martin, T. J.; Whale, G. F.; Snape, J. R.; Rowles, B.; Galay-Burgos, M.; Davenport, R. J. Improving the Biodegradability in Seawater Test (OECD 306). *Sci. Total Environ.* **2019**, *666*, 399–404. https://doi.org/10.1016/j.scitotenv.2019.02.167.

(4) OECD. *OECD 310 - Ready Biodegradability - CO2 in Sealed Vessels (Headspace Test)*; Paris, 2014. https://doi.org/10.1787/9789264016316-en.

(5) Weytjens, D.; Van Ginneken, I.; Painter, H. The Recovery of Carbon Dioxide in the Sturm Test for Ready Biodegradability. *Chemosphere* **1994**, *28* (4), 801–812. https://doi.org/10.1016/0045-6535(94)90232-1.

(6) Fiebig, S.; Maischak, H.; Maeß, C. *Investigation of Alternative Test Designs for Testing the Biodegradability in Seawater - Poster Presented at SETAC Europe 27th Annual Meeting, Brussels, Belgium*; 2017.

(7) Xie, W. H.; Shiu, W. Y.; Mackay, D. A Review of the Effect of Salts on the Solubility of Organic Compounds in Seawater. *Mar. Environ. Res.* **1997**, *44* (4), 429–444. https://doi.org/10.1016/S0141-1136(97)00017-2.

(8) OECD. *OECD Guideline for Testing of Chemicals (301): Ready Biodegradability*; Paris, 1992.

(9) OECD. *OECD Guideline for Testing of Chemicals (306): Biodegradability in Seawater*; Paris, 1992. https://doi.org/http://dx.doi.org/10.1787/9789264070486-en.

(10) Painter, H. A. Detailed Review Paper on Biodegradability Testing. *OECD Environ. Monogr.* **1995**, *98*, 1–162.

(11) Courtes, R.; Bahlaoui, a; Rambaud, a; Deschamps, F.; Sunde, E.; Dutrieux, E. Ready Biodegradability Test in Seawater: A New Methodological Approach. *Ecotoxicology and environmental safety*. 1995, pp 142–148. https://doi.org/10.1006/eesa.1995.1054.

(12) Syracuse Research Corporation. Physprop http://esc.syrres.com/fatepointer/search.asp (accessed Jul 23, 2019).

(13) Royal Society of Chemistry. ChemSpider https://www.chemspider.com/Default.aspx (accessed Jul 23, 2019).

(14) Ingre-Khans, E.; Ågerstrand, M.; Beronius, A.; Rudén, C. Reliability and Relevance Evaluations of REACH Data. *Toxicol. Res. (Camb).* **2019**, *8* (1), 46–56. https://doi.org/10.1039/c8tx00216a.

(15) ECHA. Brief profile sodium benzoate https://echa.europa.eu/brief-profile/-/briefprofile/100.007.760 (accessed Nov 5, 2019).

(16) Comber, M.; Holt, M. *Developing a Set of Reference Chemicals for Use in Biodegradability Tests for Assessing the Persistency of Chemicals*; 2010.

(17) ECHA. Brief profile triethanolamine https://echa.europa.eu/fr/brief-profile/-/briefprofile/100.002.773 (accessed Nov 5, 2019).

(18) Eide-Haugmo, I.; Brakstad, O. G.; Hoff, K. A.; da Silva, E. F.; Svendsen, H. F. Marine Biodegradability and Ecotoxicity of Solvents for CO2-Capture of Natural Gas. *Int. J. Greenh. Gas Control* **2012**, *9*, 184–192. https://doi.org/10.1016/j.ijggc.2012.03.006.

(19) Gerike, P.; Fischer, W. K. A Correlation Study of Biodegradability Determinations with Various Chemicals in Various Tests. *Ecotoxicol. Environ. Saf.* **1979**, *3* (1), 159–173. https://doi.org/10.1016/0147-6513(81)90045-2.

(20) ECHA. Brief profile 4-nitrophenol https://echa.europa.eu/fr/brief-profile/-/briefprofile/100.002.556 (accessed Nov 5, 2019).

(21) Martin, T. J.; Snape, J. R.; Bartram, A.; Robson, A.; Acharya, K.; Davenport, R. J. Environmentally Relevant Inoculum Concentrations Improve the Reliability of Persistent Assessments in Biodegradation Screening Tests. *Environ. Sci. Technol.* **2017**, *51* (5), 3065–3073. https://doi.org/10.1021/acs.est.6b05717.

(22) Nyholm, N.; Kristensen, P. *Screening Test Methods for Assessment of Biodegradability of Chemical Substances in Sea Water: Ring Test Programme 1984- 1985. Final Report*; Hørsholm, Denmark, 1987.

(23) Nyholm, N.; Kristensen, P. Screening Methods for Assessment of Biodegradability of Chemicals in Seawater - Results from a Ring Test. *Ecotoxicol. Environ. Saf.* **1992**, *23* (2), 161–172. https://doi.org/10.1016/0147-6513(92)90056-9.

(24) ECHA. Guidance for Monomers and Polymers - Guidance for the Implementation of REACH. **2012**, No. April, 1–25.

(25) Caulfield, M. J.; Qiao, G. G.; Solomon, D. H. Some Aspects of the Properties and Degradation of Polyacrylamides. *Chem. Rev.* **2002**, *102* (9), 3067–3084. https://doi.org/10.1021/cr010439p.

(26) Seybold, C. A. Polyacrylamide Review: Soil Conditioning and Environmental Fate. *Commun. Soil Sci. Plant Anal.* **1994**, *25* (11–12), 2171–2185.

(27) El-Mamouni, R.; Frigon, J. C.; Hawari, J.; Marroni, D.; Guiot, S. R. Combining Photolysis and Bioprocesses for Mineralization of High Molecular Weight Polyacrylamides. *Biodegradation* **2002**, *13* (4), 221–227. https://doi.org/10.1023/A:1021272519714.

(28) ECHA. Substance information pentachlorophenol https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.001.617 (accessed Nov 5, 2019).

(29) SYKE. Data bank of environmental chemicals - Pentachlorophenol http://wwwp.ymparisto.fi/scripts/Kemrek/Kemrek_uk.asp?Method=MAKECHEMdetailsform&txtChemId=245 (accessed Nov 5, 2019).

(30) Lapertot, M. E.; Pulgarin, C. Biodegradability Assessment of Several Priority Hazardous Substances: Choice, Application and Relevance Regarding Toxicity and Bacterial Activity. *Chemosphere* **2006**, *65* (4), 682–690. https://doi.org/10.1016/j.chemosphere.2006.01.046.

(31) Ingerslev, F.; Baun, A.; Nyholm, N. Aquatic Biodegradation Behavior of Pentachlorophenol Assessed through a Battery of Shake Flask Die-Away Tests. *Environ. Toxicol. Chem.* **1998**, *17* (9), 1712–1719. https://doi.org/10.1897/1551-5028(1998)017<1712:ABBOPA>2.3.CO;2.

(32) King, E. F.; Painter, H. A. The Effect of Acclimatisation on the Toxicity of Chemicals to Activated Sludge Microorganisms. *Environ. Pollut.* **1985**, *39* (3), 267–280. https://doi.org/10.1016/0143-1471(85)90101-1.

(33) ECETOC. *Refined Approaches for Risk Assessment of PBT/VPvB Chemicals Technical Report No.112*; Brussels, 2011.

(34) OSPAR. *Guidelines for Completing the Harmonised Offshore Chemical Notification Format (HOCNF) (OSPAR Agreement: 2012/05. Update 2015)*; 2015.

(35) Regulation (EC) No 1907. *Of the European Parliament and of the Council of 18 December 2006 Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals*

(REACH), Establishing a European Chemicals Agency, Amending Directive 1999/4; 2006.