Supporting Information

Chemical Characterization of Low Molecular Weight (MW < 580 Da) Soluble Microbial Products (SMPs) in an Anaerobic Membrane Bioreactor

Chinagarn Kunacheva[a], Chencheng Le[a,b], Yan Ni Annie Soh[a] and David C. Stuckey[a,c*]

[a]Advanced Environmental Biotechnology Center, Nanyang Environment & Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, Singapore 637141, Singapore.

[b]Division of Environmental and Water Resources Engineering, School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore.

[c]Department of Chemical Engineering, Imperial College London, SW7 2AZ, UK

*Corresponding author

E-mail: d.stuckey@imperial.ac.uk, Tel: +44 207 5945591

E-mail: chinagarn@ntu.edu.sg, C130189@e.ntu.edu.sg, YSOH006@e.ntu.edu.sg

Pages: S1 – S16

Figures: S1 – S9

Tables: S1 – S6

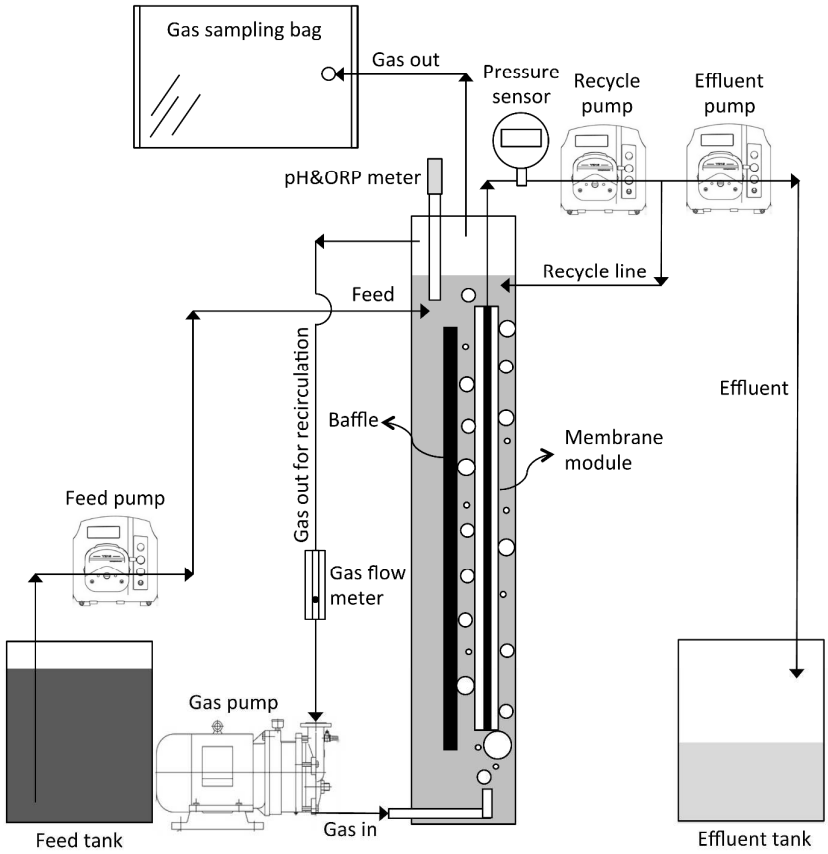

Figure S1. Diagram of the SAMBR.

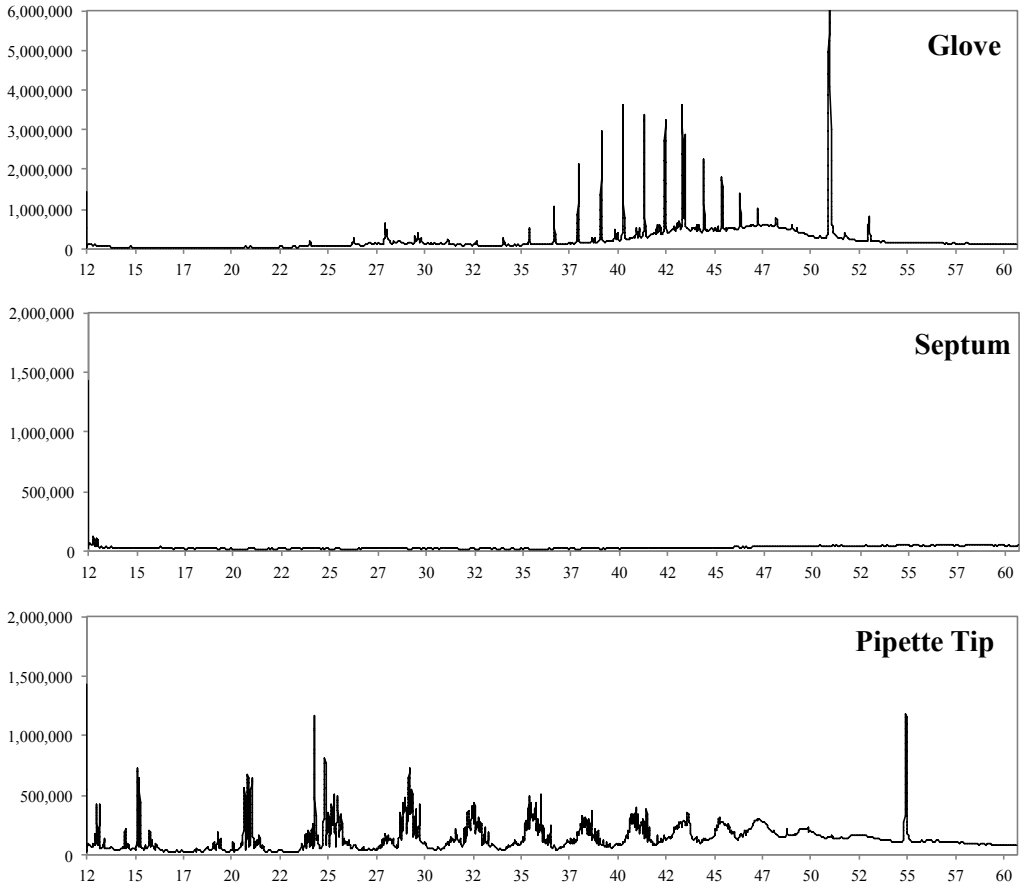

Figure S2. Chromatograms of possible contaminants during the extraction procedure.

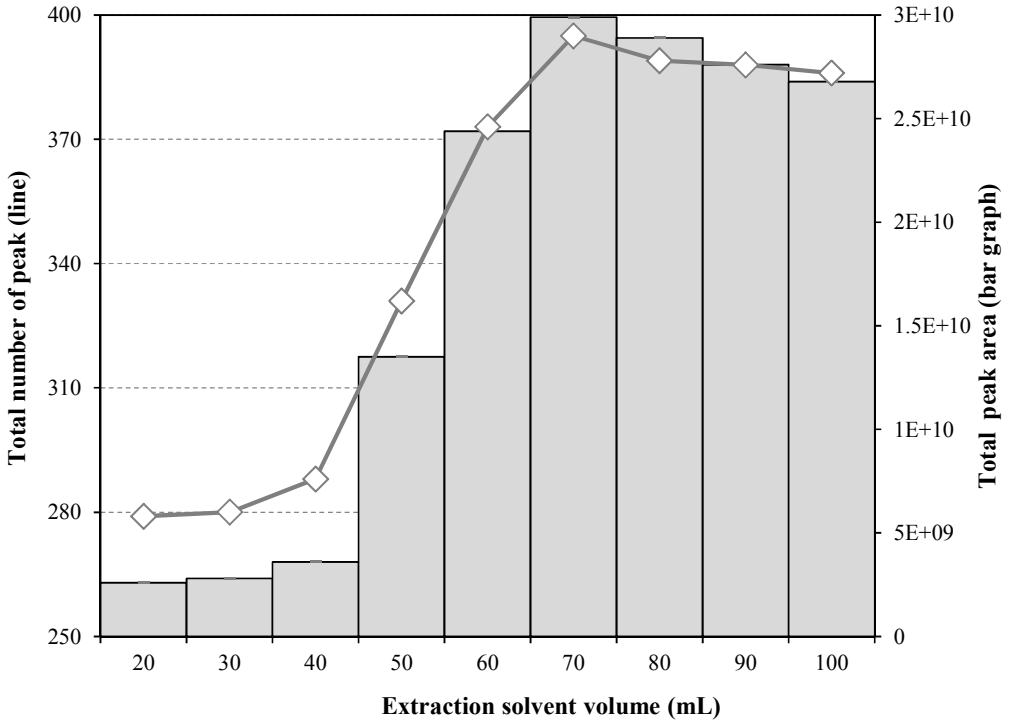

Figure S3. Efficiency of different solvent volumes evaluated for extraction.

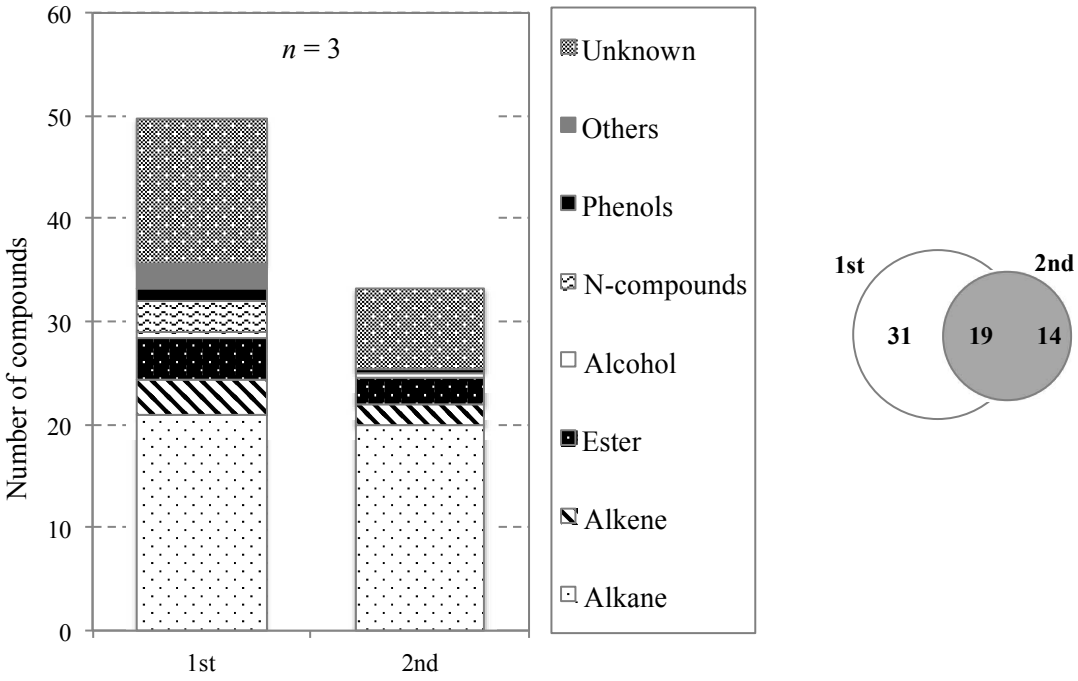

Figure S4. Number of compound identified using the 1st and 2nd OasisHLB cartridges.

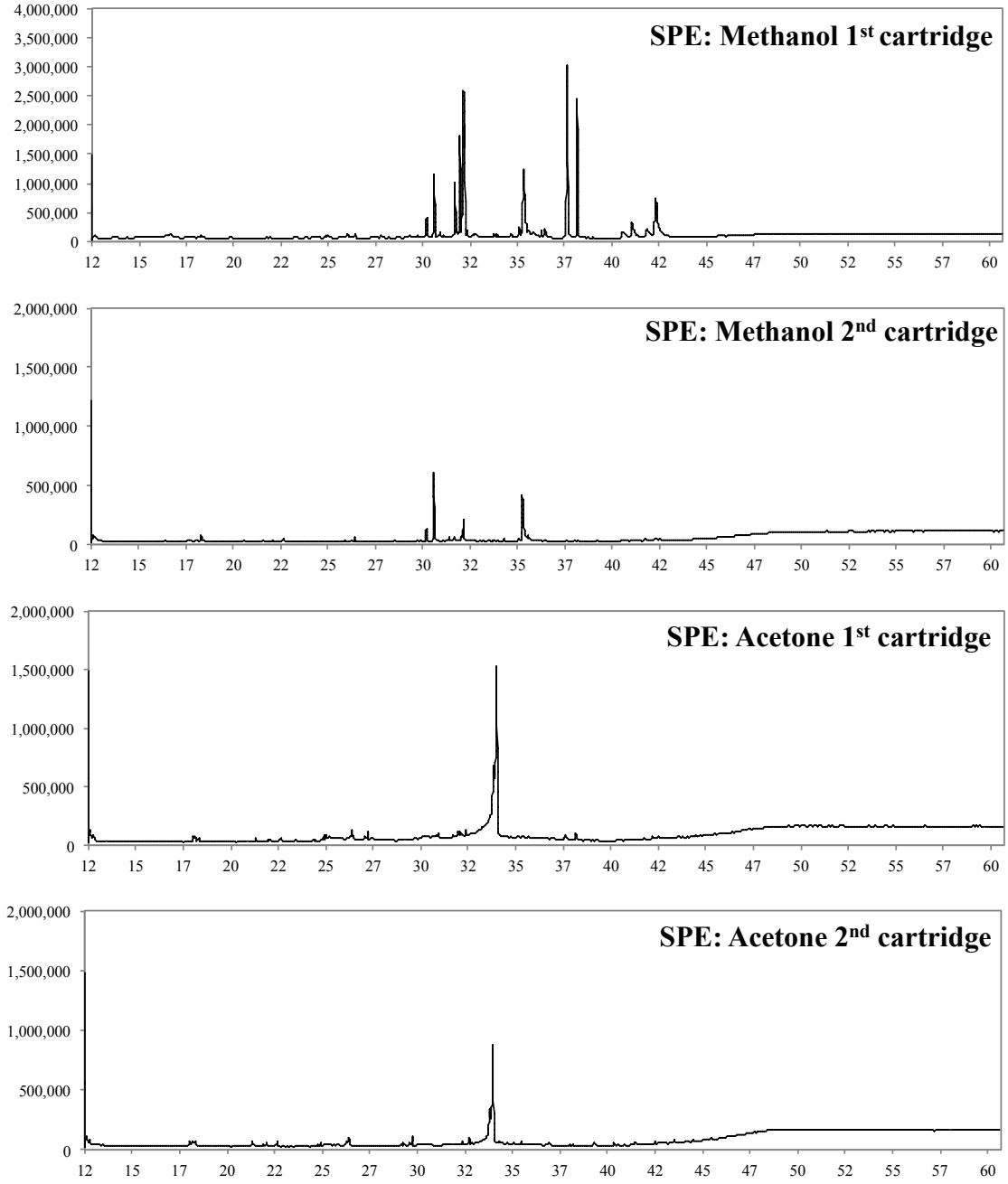

Figure S5. Chromatograms of the sample 2 (SPE-LLE).

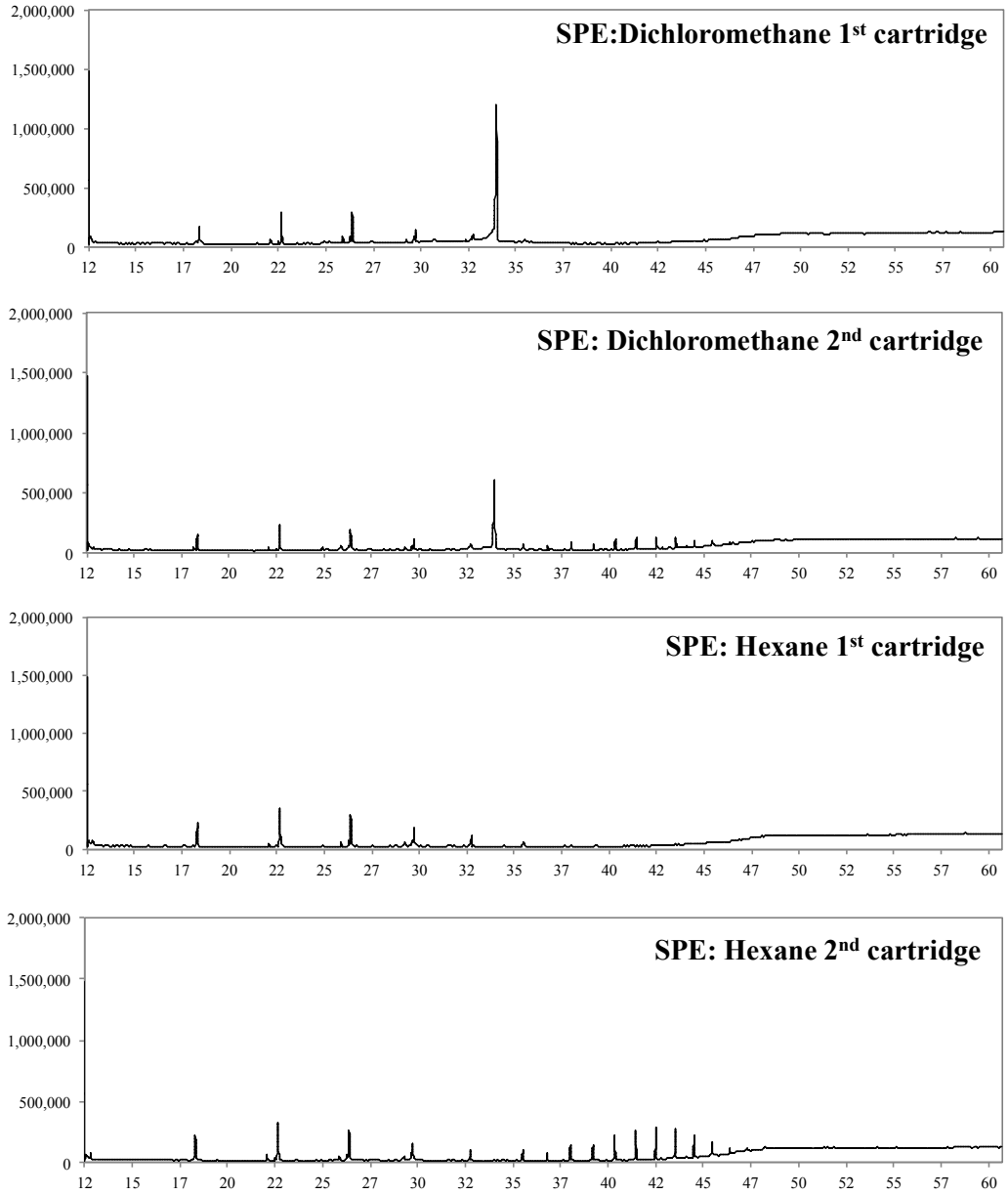

Figure S6. Chromatograms of the sample 2 (SPE-LLE) (*cont.*).

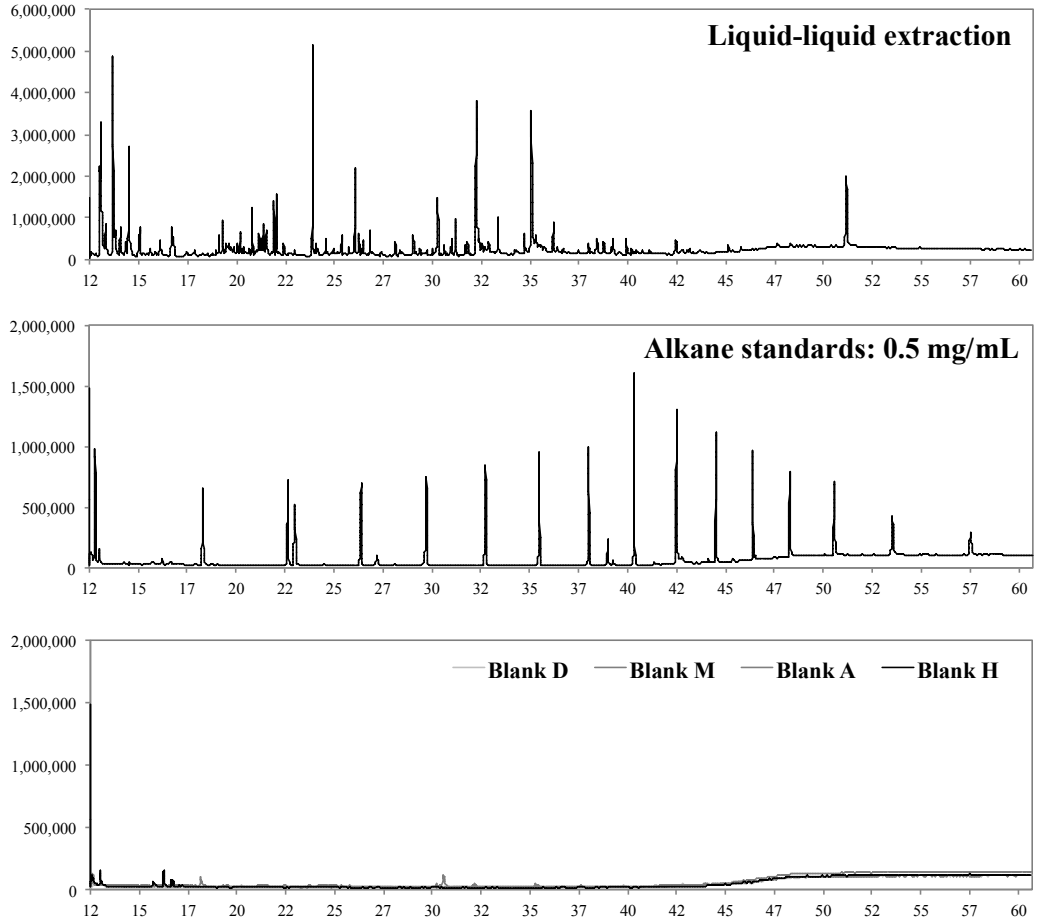

Figure S7. Chromatograms of the sample 2 (SPE-LLE) (*cont.*).

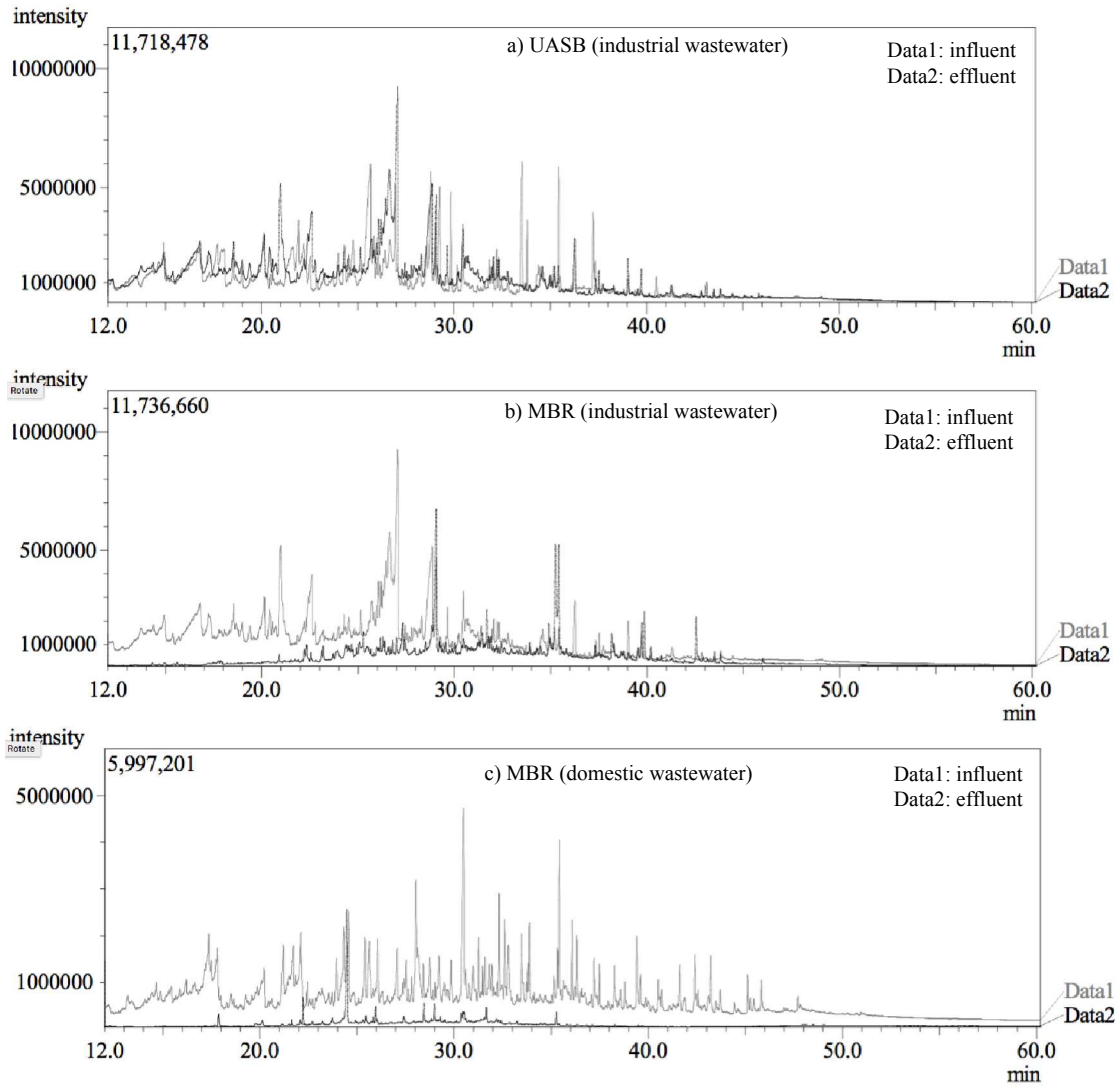

Figure S8. Comparison between chromatograms (LLE) of influent and effluent from a UASB (industrial wastewater), an MBR (industrial wastewater), and an MBR (domestic wastewater).

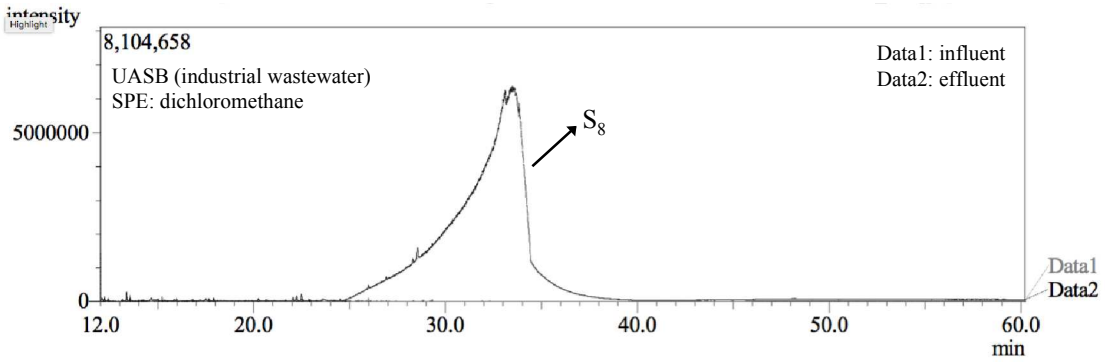

Figure S9. Chromatogram of influent and effluent from an UASB (industrial wastewater), (SPE: dichloromethane).

Table S1. Alkane standards for quantification.

Compound name	Formula	Molecular weight	Retention time (min)	R^2	*IDL* (mg/L)	*IQL* (mg/L)	Retention Index
Decane	$C_{10}H_{22}$	142	12.15	0.999	0.026	0.087	1015
Dodecane	$C_{12}H_{26}$	170	17.16	0.999	0.017	0.056	1214
Tetradecane	$C_{14}H_{30}$	198	21.96	0.999	0.019	0.064	1413
Hexadecane	$C_{16}H_{34}$	226	25.70	0.997	0.020	0.066	1612
Octadecane	$C_{18}H_{38}$	254	29.03	0.997	0.015	0.050	1810
Eicosane	$C_{20}H_{42}$	282	32.04	0.996	0.020	0.068	2009
Docosane	$C_{22}H_{46}$	310	34.78	0.997	0.022	0.073	2208
Tetracosane	$C_{24}H_{50}$	338	37.30	0.991	0.017	0.055	2407
Hexacosane	$C_{26}H_{54}$	366	39.62	0.995	0.013	0.042	2606
Octacosane	$C_{28}H_{58}$	394	41.77	0.993	0.014	0.046	2804
Triacontane	$C_{30}H_{62}$	422	43.78	0.994	0.016	0.054	3003
Dotriacontane	$C_{32}H_{66}$	450	45.66	0.990	0.015	0.051	3202
Tetratriacontane	$C_{34}H_{70}$	478	47.51	0.991	0.010	0.033	3401
Hexatriacontane	$C_{36}H_{74}$	506	49.72	0.990	0.014	0.046	3600
Octatriacontane	$C_{38}H_{78}$	534	52.59	0.981	0.011	0.038	3800
Tetracontane	$C_{40}H_{82}$	562	56.45	0.984	0.054	0.182	3997

Note: IDL= Instrumental Detection Limit, IQL= Instrumental Quantification Limit

Table S2. Efficiency of different additives evaluated for extraction.

Additives	pH	Total peak area (Mean ($\times 10^9$) ± SD)	Total number of peaks (Mean ± SD)
Nil	7	2.99 ± 0.06	395 ± 1.1
HCl	< 2	2.24 ± 0.05	388 ± 0.4
NaOH	> 11	2.91 ± 0.36	372 ± 0.8
NaCl	7	2.98 ± 0.22	393 ± 1.3
$MgSO_4$	7	2.78 ± 0.25	383 ± 1.4

Note: SD = standard deviation

222 Table S3. Efficiency of different sample sizes evaluated for extraction.
223

Sample size (mL)	Total peak area (Mean ($\times 10^9$) ± SD)	Total number of peak (Mean ± SD)
400	5.82 ± 0.02	396 ± 2.7
200	2.99 ± 0.06	395 ± 1.1
100	0.55 ± 0.33	326 ± 1.2
50	0.46 ± 0.39	290 ± 1.0

Note: SD = standard deviation

224

225

226

227

228

229

230

231

232

233

234

235

236

237

238

239

240

241

242

243

Table S4. List of 50 compounds that have the highest peak area (SPE as a pretreatment).

RT	Compound name	Chemical formula	Mass	Retention Index	Compound Group	Peak Area	Similarity Index
24.38	Phenol, 3,5-bis(1,1-dimethylethyl)-	C14H22O	206	1555	Phenol	4805680	92
22.27	Tetradecane	C14H30	198	1413	Alkane	1893471	96
17.77	Dodecane	C12H26	170	1214	Alkane	1749187	96
26.04	Hexadecane	C16H34	226	1612	Alkane	1542929	96
35.82	Tributyl acetylcitrate	C20H34O8	402	2594	Ester	1371280	94
29.18	Octadecane	C18H38	254	1810	Alkane	1172653	93
12.19	Decane	C10H22	142	1015	Alkane	1075648	91
32.18	Eicosane	C20H42	282	2009	Alkane	816872	95
32.01	9H-Pyrido[3,4-b]indole, 1-methyl-	C12H10N2	182	1746	N-compound	669681	84
16.52	5-Undecene, (E)-	C11H22	154	1123	Alkene	636746	83
25.91	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	C16H30O4	286	1605	Ester	448224	87
34.49	1-Propene-1,2,3-tricarboxylic acid, tributyl ester	C18H30O6	342	2297	Ester	448127	88
35.05	Docosane	C22H46	310	2208	Alkane	446279	91
37.52	Tetracosane	C24H50	338	2407	Alkane	409996	88
39.79	Hexacosane	C26H54	366	2606	Alkane	376296	84
38.64	Eicosane, 7-hexyl-	C26H54	366	2542	Alkane	343980	94
28.78	Heptadecane, 3-methyl-	C18H38	254	1746	Alkane	295922	91
41.72	Octacosane	C28H58	394	2804	Alkane	264170	85
24.64	Benzoic acid, 4-ethoxy-, ethyl ester	C11H14O3	194	1448	Ester	239171	81
16.51	Camphor	C10H16O	152	1121	Others	226671	92
25.49	Pentadecane, 3-methyl-	C16H34	226	1548	Alkane	226363	94
29.06	1-Octadecene	C18H36	252	1801	Alkene	195662	89
25.76	Cetene	C16H32	224	1602	Alkene	190939	96
31.36	1H-Pyrido[3,4-b]indole, 2,3,4,9-tetrahydro-1-methyl-	C12H14N2	186	1820	N-compound	188408	83
33.82	Eicosane, 10-methyl-	C21H44	296	2045	Alkane	183125	83
20.14	3-Methyl-2,3-dihydro-benzofuran	C9H10O	134	1097	Others	172360	84
42.93	2-methyloctacosane	C29H60	408	2840	Alkane	160679	91
28.89	Tryptophol	C10H11NO	161	1606	Alcohol	155851	80
31.82	Dibutyl phthalate	C16H22O4	278	2037	Ester	148063	87
36.32	Heptadecane, 9-octyl-	C25H52	352	2442	Alkane	147424	86
38.57	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	C14H16N2O	228	2138	N-compound	145538	91
31.70	Nonadecane, 2-methyl-	C20H42	282	1945	Alkane	145244	92
26.65	Benzophenone	C13H10O	182	1603	Others	133971	80
31.91	2-(Hexyloxycarbonyl)benzoic acid	C14H18O4	250	2027	Ester	132798	80
29.13	Benzenesulfonamide, N-butyl-	C10H15NO2S	213	1797	N-compound	130013	84
31.22	Pentadecanoic acid, 14-methyl-, methyl ester	C17H34O2	270	1814	Ester	121465	86
31.37	1-Naphthalenemethanamine	C11H11N	157	1588	N-compound	111091	80
23.26	4-Hydroxyphenylacetamide	C8H9NO2	151	1523	N-compound	103739	84
34.58	Heneicosane, 3-methyl-	C22H46	310	2144	Alkane	99344	87
37.85	Triphenyl phosphate	C18H15O4P	326	-	Others	95462	84
33.70	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296	2085	Ester	87321	83
31.19	Hexadecanoic acid, 15-methyl-, methyl ester	C18H36O2	284	1914	Ester	75783	80
23.03	Ethanone, 1-(4-ethoxyphenyl)-	C10H12O2	164	1318	Others	74947	83
28.72	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	C7H10N2O2	154	1404	N-compound	74614	94
43.93	Triacontane	C30H62	422	3003	Alkane	66715	87
29.26	Octadecane, 2-methyl-	C19H40	268	1846	Alkane	64001	84
27.13	[1,1'-Biphenyl]-2,2'-diol	C12H10O2	186	1808	Others	61775	84
30.84	9-Hexadecenoic acid, methyl ester, (Z)-	C17H32O2	268	1886	Ester	60770	87
26.21	Benzothiazole, 2-(methylthio)-	C8H7NS2	181	1548	Others	43624	82
40.88	2-methylhexacosane	C27H56	380	2641	Alkane	40676	92
28.69	Heptadecane, 2,6-dimethyl-	C19H40	268	1782	Alkane	36068	92
40.87	Docosane, 7-hexyl-	C28H58	394	2740	Alkane	30692	87

247

248 Table S5. List of 50 compounds that have the highest peak area (LLE as a pretreatment).

RT	Compound name	Chemical formula	Mass	Retention Index	Compound Group	Peak Area (×1000)	Similarity Index
11.20	2,3-Dimethylpentanol	C7H16O	116	832	Alcohol	11961	82
36.24	13-methylpentacosane	C26H54	366	2534	Alkane	5523	93
30.48	Palmitic acid	C16H32O2	256	1968	Others	4890	92
29.91	n-Nonadecane	C19H40	268	1910	Alkane	3805	93
36.15	11-pentyl-heneicosane	C26H54	366	2453	Alkane	3510	89
33.46	10-Methylhenicosane	C22H46	310	2144	Alkane	3183	90
29.16	Di-iso-Butyl phthalate	C16H22O4	278	1908	Ester	2950	90
33.26	Stearic acid	C18H36O2	284	2167	Others	2787	95
32.91	n-Octadecanol	C18H38O	270	2053	Alcohol	2746	92
38.08	Diisooctyl phthalate	C24H38O4	390	2704	Ester	2535	89
18.35	Allyl 4-oxopentanoate	C8H12O3	156	1110	Ester	2309	88
26.57	2-Methylheptadecane	C18H38	254	1746	Alkane	2309	89
30.56	5-methylhex-2-yl butyl phthalate	C19H28O4	320	2207	Ester	2300	95
27.28	8-Methylheptadecane	C18H39	254	1746	Alkane	2108	91
35.66	2-methyltetracosane	C25H52	352	2442	Alkane	2065	90
40.60	(Z)-13-Docosenamide	C22H43NO	337	2625	N-compound	2061	90
19.05	(E)-2-Dodecene	C12H24	168	1222	Alkene	1838	92
38.61	9-methylheptacosane	C28H58	394	2735	Alkane	1779	92
11.43	(E)-Oct-2-enal	C8H14O	126	1013	Others	1616	81
41.07	Squalene	C30H50	410	2914	Alkene	1559	95
15.02	5-Chloro-1-pentyl acetate	C7H13ClO2	164	1110	Others	1526	91
33.53	(Z)-9-Octadecenamide	C18H35NO	281	2228	N-compound	1509	80
35.86	Nonadecanamide	C19H39NO	297	2319	N-compound	1467	91
24.72	2,2,4-Trimethylpentanediol-1,3-diisobutyrate	C16H30O4	286	1605	Others	1344	92
28.14	Tris(2-chloro-1-methylethyl) phosphate	C9H18Cl3O4P	326	-	Others	1282	95
18.48	5-Sec-butylnonane	C13H28	184	1185	Alkane	1130	91
40.89	n-Octacosane	C28H58	394	2804	Alkane	1106	91
23.83	7,9-Dimethylhexadecane	C18H38	254	1682	Alkane	1061	80
38.16	2-methylhexacosane	C27H56	380	2641	Alkane	1061	91
22.83	n-Tetradecane	C14H30	198	1413	Alkane	966	93
19.47	2-Methyldodecane	C13H28	184	1249	Alkane	956	93
19.53	4,6-Dimethyldodecane	C14H30	198	1285	Alkane	918	95
33.35	2-Methyl-1-hexadecanol	C17H36O	256	1890	Alcohol	837	94
12.54	4-Methyl-2-heptanone	C8H16O	128	888	Others	759	90
38.68	2-methyloctacosane	C29H60	408	2840	Alkane	741	89
30.76	8-Hexylpentadecane	C21H44	296	2045	Alkane	717	92
30.89	10-Methylnonadecane	C20H42	282	1945	Alkane	717	96
33.70	5-methyl-henicosane	C22H46	310	2151	Alkane	716	92
12.76	4,4-Dimethylheptane	C9H20	128	831	Alkane	581	93
12.80	3,7-Dimethyldecane	C12H26	170	1086	Alkane	567	94
43.01	11-Decyltetracosane	C34H70	478	3337	Alkane	560	92
14.12	2,6-Dimethyldecane	C12H26	170	1086	Alkane	555	92
26.38	n-Hexadecane	C16H34	226	1612	Alkane	493	91
40.67	2-Octyl-1-dodecanol	C20H42O	298	2188	Alcohol	490	92
30.64	5-methylnonadecane	C20H42	282	1953	Alkane	478	91
30.52	5,5,7,7-Tetraethylundecane	C19H40	268	1741	Alkane	464	88
27.42	2,6,10,15-Tetramethylheptadecane	C21H44	296	1852	Alkane	432	96
33.94	8-butyl-9-ethyloctadencane	C24H50	338	2140	Alkane	428	89
12.18	4-Ethyl-4-methyl-1-hexene	C9H18	126	821	Alkene	418	82
29.98	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	C17H24O3	276	2081	Others	416	87

249

250

251

252

253

254 Table S6. List of compounds found in the feed sample.

RT	Compound name	Chemical formula	Mass	Retention Index	Compound Group	Peak Area
30.65	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C11H18N2O	194	1699	N-compound	6008180
29.65	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	C7H10N2O2	154	1404	N-compound	2174238
38.57	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	C14H16N2O	228	2138	N-compound	1727397
28.92	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione, N-acetyl-	C10H14N2O	178	1858	N-compound	1573576
32.61	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine	C14H22N2O	234	1898	N-compound	429462
29.12	dl-Alanyl-l-leucine	C9H18N2O3	202	1723	N-compound	112331
27.57	2,5-Piperazinedione, 3-methyl-6-(1-methylethyl)-	C8H14N2O2	170	1303	N-compound	105497

255

256

257