**Supplemental Information**

**Identification of Enantiomeric Byproducts During Microalgae-mediated Transformation of Metoprolol by MS/MS Spectrum Based Networking**

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**Supplemental Information**

24 pages, including 8 tables and 6 figures.

Page 3 provides details on the sampling and analytical methods.

Pages 4 includes Table S1, which is referred to directly in the Materials and methods portion.

Pages 5-10 include Figure S1-S6, which are referred to directly in the Results and discussion portion of the text.

Pages 11-24 include Table S2-S8, which are the detailed MS/MS peaks annotation of MPL standard and TPs.

**Material and methods**

**Analysis of water quality parameters**

Samples were ﬁltered through 0.45 μm pore-size cellulose ﬁlters (Millipore, Billerica, MA, USA) and frozen until analysis within a week. PO43-- P in the ﬁltrate was processed following an alkaline potassium persulfate digestion. Ammonium (NH4+), nitrate (NO3- -N) in the filtrate as well as PO43-- P in the digest were analyzed by a ﬂow injection analyzer (QC8500, Lachat®, Loveland, Co., USA).

**Sample preparation for identification of transformation products**

Oasis HLB cartridge (500 mg, 6 mL, Waters) were preconditioned with 20 mL methanol, 6 mL water, and 6 mL water under pH 2.0. The samples were passed through the cartridges at a flow rate around 3 mL/min. After the cartridges were dried under an airstream for 10 min, analytes were eluted with 12 mL of methanol and 6 mL of acetone-methanol 50/50 (v/v). The elutes were collected, evaporated to dryness with nitrogen gas and reconstituted to 1.0 mL with methanol.

**TABLE S1**│Component of synthetic wastewater for MPL degradation

|  |  |  |  |
| --- | --- | --- | --- |
| Compound | Concentration (mM) | Compound (trace metal) | Concentration (mM) |
| NaNO3 | 0.86 | FeCl2.4H2O | Fe2+ = 0.01 |
| K2HPO4 | 0.19 | MnSO4.H2O | Mn2+ = 0.02 |
| NaCl | 0.43 | (NH4)6 Mo7O24.4H2O | Mo7O246- = 0.001 |
| NaHCO3 | 1 |  | NH4+ = 0.006 |
| MgSO4 | 0.3 | CuSO4 | Cu2+ = 0.005 |
| CaCl2.2H2O | 0.17 | ZnSO4.7H2O | Zn2+ = 0.006 |
| EDTA disodium salt | 0.01 | NiSO4.6H2O | Ni2+ = 0.001 |



**FIGURE S1│** Liquid chromatogram for a 20 d sample during metoprolol degradation by *Haematococcus pluvialis*.



**FIGURE S2**│ The removal of nutrient and metoprolol by *Selenastrum capricornutum* and *Chlorella vulgaris* and their growth in the synthetic effluent. The MPL concentrations were the sum of (S)-MPL and (R)-MPL concentrations.



**FIGURE S3│** (S)-MPL and (R)-MPL concentrations changes versus time during MPL biodegradation by *Scenedesmus quadricauda* Breb (a) and Rayleigh representation of the enantiomeric enrichment of MPL versus MPL biodegradation (b). Insets: Kinetics of first-order biodegradation of the two MPL enantiomers.



**FIGURE S4│** EF changes against time during MPL biodegradation by *Haematococcus pluvialis* and *Scenedesmus quadricauda* Breb.



(a)



(b)



(e)

(d)

(c)





(f)

**FIGURE S5│** LC-HRMS chromatogram of six pairs of transformation products with (a), (b), (c), (d), (e), (f) representing chiral DMPLD, DMPLA, O-DMPL, MPLA, α-HMPLA, α-HMPL, respectively. The extracted chromatogram of the control is shown in the upper window of (a), (b), (c), (d), (e) and (f).



**FIGURE S6│** The HRMS spectrum corresponding to extracted chromatogram peak of [M+H]+ of 284.1858 Da during degradation of MPL by *Chlorella vulgaris*.

**TABLE S2**│ Fragmentation structures of **MPL**

|  |  |  |  |
| --- | --- | --- | --- |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M+H]+**(­parent ion)** | 268.1907 | 268.1910 | 1.12 |
| [M-H2O+H]+ | 250.1802 | 250.1800 | -0.80 |
| [M-C3H7+H+H]+ | 226.1438 | 226.1437 | -0.44 |
| [M-H2O-CH3OH+H]+ | 218.1539 | 218.1539 | -0.09 |
| [M-C3H6-CH3OH+H]+ | 194.1176 | 194.1174 | -1.03 |
| [M-H2O-C3H7NH]+ | 191.1067 | 191.1066 | -0.52 |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M-H2O-C3H6-CH3OH+H]+ | 176.1070 | 176.1069 | -0.57 |
| [M-H2O-CH3OH-C3H7NH]+ | 159.0804 | 159.0804 | -0.57 |
| [M-CH3OC2H4C6H4OH+H]+ | 116.1070 | 116.1069 | -0.86 |
| [M- CH3OC2H4C6H4O-H2O]+ | 98.0964 | 98.0963 | -1.02 |

**TABLE S3**│ Fragmentation structures of **α-HMPL**

|  |  |  |  |
| --- | --- | --- | --- |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M+H]+ **(­parent ion)** | 284.1856 | 284.1858 | 0.70 |
| [M-H2O+H]+ | 266.1751 | 266.1753 | 0.75 |
| [M-2H2O+H]+ | 248.1645 | 248.1646 | 0.40 |
| [M-C3H7+H+H]+ | 242.1387 | 242.1390 | 1.24 |
| [M-H2O -C3H7 +H+H]+ | 224.1281 | 224.1282 | 0.45 |
| [M-H2O-C3H7NH]+ | 207.1016 | 207.1017 | 0.48 |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M-H2O-C3H7NH-CH3OH]+ | 175.0754 | 175.0753 | -0.57 |
| [M-H2O-C3H7NH-CH3OCH2CHOH+H]+ | 133.0648 | 133.0648 | 0.00 |
| [M-CH3OCH2CHOHC6H4OH+H]+ | 116.1070 | 116.1070 | 0.00 |
| [M-CH3OCH2CHOHC6H4O-H2O]+ | 98.0964 | 98.0964 | -0.41 |

**TABLE S4**│ Fragmentation structures of **α-HMPLA**

|  |  |  |  |
| --- | --- | --- | --- |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M+H]+ **(­parent ion)** | 284.1493 | 284.1494 | 0.35 |
| [M-H2O+H]+ | 266.1387 | 266.1384 | -1.13 |
| [M-2H2O+H]+ | 248.1281 | 248.1281 | -0.20 |
| [M-C3H7+H+H]+ | 242.1023 | 242.1021 | -0.83 |
| [M-CO2H]+ | 238.1438 | 238.1434 | -1.68 |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M-H2O-C3H7NH]+ | 207.0652 | 207.0652 | 0.19 |
| [M-2H2O-C3H7+H+H] | 206.0812 | 206.0812 | 0.24 |
| [M-H2O-C3H7NH-CO2HCHOH+H]+ | 133.0648 | 133.0650 | 1.50 |
| [M-CO2HCHOHC6H4OH+H]+ | 116.1070 | 116.1070 | 0.00 |
| [M-H2O-CO2HCHOHC6H4O]+ | 98.0964 | 98.0964 | -0.20 |

**TABLE S5**│ Fragmentation structures of **MPLA**

|  |  |  |  |
| --- | --- | --- | --- |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M+H]+ **(­parent ion)** | 268.1543 | 268.1544 | 0.37 |
| [M-H2O+H]+ | 250.1438 | 250.1439 | 0.40 |
| [M-C3H7+H+H]+ | 226.1074 | 226.1075 | 0.44 |
| [M-H2O-C3H7+H+H]+ | 208.0968 | 208.0971 | 1.44 |
| [M-H2O-C3H7NH]+ | 191.0703 | 191.0704 | 0.53 |
| [M-H2O-C3H7NH2-CO2H]+ | 145.0648 | 145.0649 | 0.69 |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M-CO2HCH2C6H4OH+H]+ | 116.1070 | 116.1070 | 0.00 |
| [M-H2O -CO2HCH2C6H4O]+ | 98.0964 | 98.0964 | -0.20 |

**TABLE S6**│ Fragmentation structures of **O-DMPL**

|  |  |  |  |
| --- | --- | --- | --- |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M+H]+ **(­parent ion)** | 254.1751 | 254.1753 | 0.79 |
| [M-H2O+H]+ | 236.1645 | 236.1649 | 1.69 |
| [M-2H2O+H]+ | 218.1539 | 218.1543 | 1.83 |
| [M-C3H7+H+H]+ | 212.1281 | 212.1285 | 1.88 |
| [M-C3H7-H2O+H]+ | 194.1176 | 194.1183 | 3.61 |
| [M-H2O-C3H7NH]+ | 177.0910 | 177.0913 | 1.69 |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M-2H2O-C3H6+H]+ | 176.1070 | 176.1073 | 1.70 |
| [M-2H2O-C3H7NH]+ | 159.0804 | 159.0807 | 1.89 |
| [M-H2O-CH2OHCH2-C3H7NH+H]+ | 133.0648 | 133.0650 | 1.50 |
| [M-CH2OHCH2C6H4OH+H]+ | 116.1070 | 116.1071 | 0.86 |
| [M-H2O-CH2OHCH2C6H4O]+ | 98.0964 | 98.0963 | -1.02 |

**TABLE S7**│ Fragmentation structures of **DMPLA**

|  |  |  |  |
| --- | --- | --- | --- |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M+H]+ **(­parent ion)** | 254.1387 | 254.1389 | 0.79 |
| [M-H2O+H]+ | 236.1281 | 236.1282 | 0.42 |
| [M-C3H7+H+H]+ | 212.0917 | 212.0918 | 0.47 |
| [M-H2O-C3H7+H+H]+ | 194.0812 | 194.0812 | 0.05 |
| [M-H2O-C3H7NH]+ | 177.0546 | 177.0546 | 0.06 |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M-H2O-C3H6-OH]+ | 176.0706 | 176.0706 | 0.00 |
| [M-H2O-CO2-C3H7NH]+ | 133.0648 | 133.0648 | 0.23 |
| [M-CO2HC6H4OH+H]+ | 116.1070 | 116.1070 | -0.09 |
| [M-H2O-CO2HC6H4O]+ | 98.0964 | 98.0964 | -0.41 |

**TABLE S8**│ Fragmentation structures of **DMPLD**

|  |  |  |  |
| --- | --- | --- | --- |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M+H]+ **(­parent ion)** | 238.1438 | 238.1439 | 0.37 |
| [M-H2O+H]+ | 220.1332 | 220.1335 | 1.36 |
| [M-C3H7+H+H]+ | 196.0968 | 196.0969 | 0.51 |
| [M-H2O-C3H7+H+H]+. | 178.0863 | 178.0864 | 0.56 |
| [M-H2O -C3H7NH]+ | 161.0597 | 161.0598 | 0.62 |
| [M-H2O-CO2-C3H7NH]+ | 133.0648 | 133.0647 | -0.75 |
| Structure | Exact mass | Experiment | Error(ppm) |
| [M-CH2OC6H4OH+H]+ | 116.1070 | 116.1070 | -0.06 |
| [M-H2O-CH2OC6H4O]+ | 98.0964 | 98.0962 | -2.04 |