Supplementary Information (SI) for Green Chemistry.

Metabolic Channelling in *Rhodopseudomonas palustris* for Hydroxyalkanoate Polymers Diversification and Sustainability Assessment

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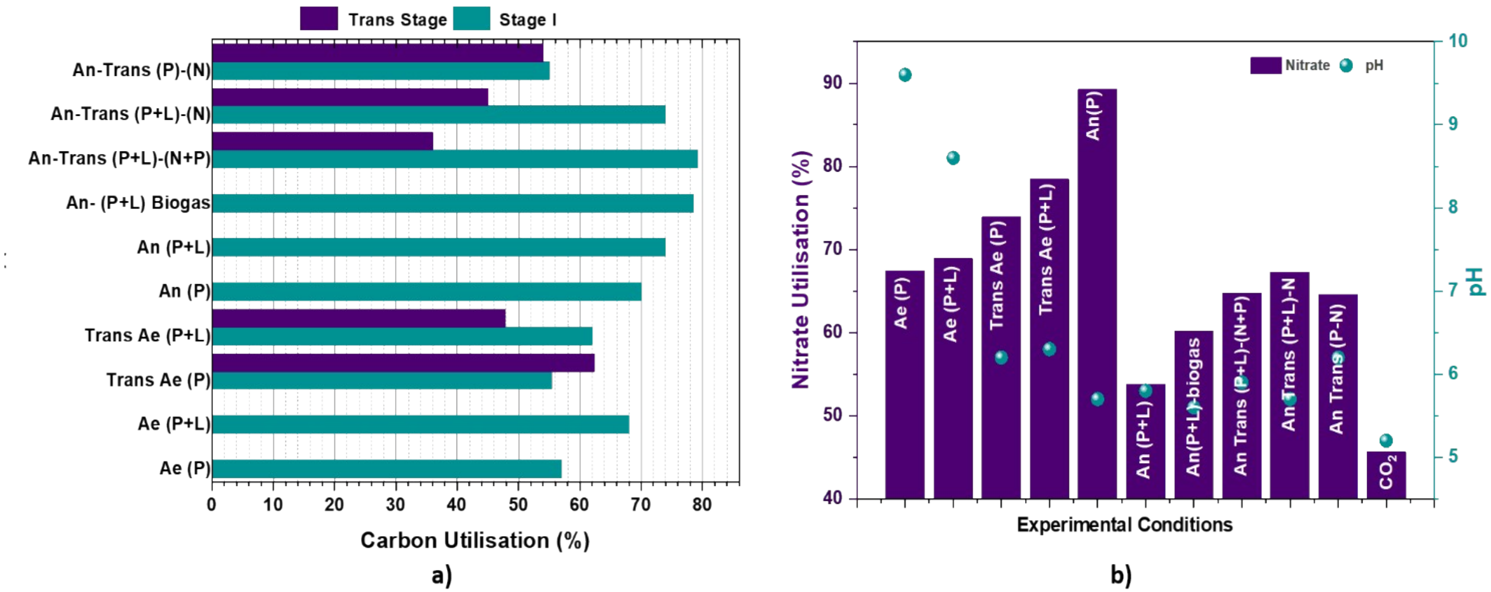
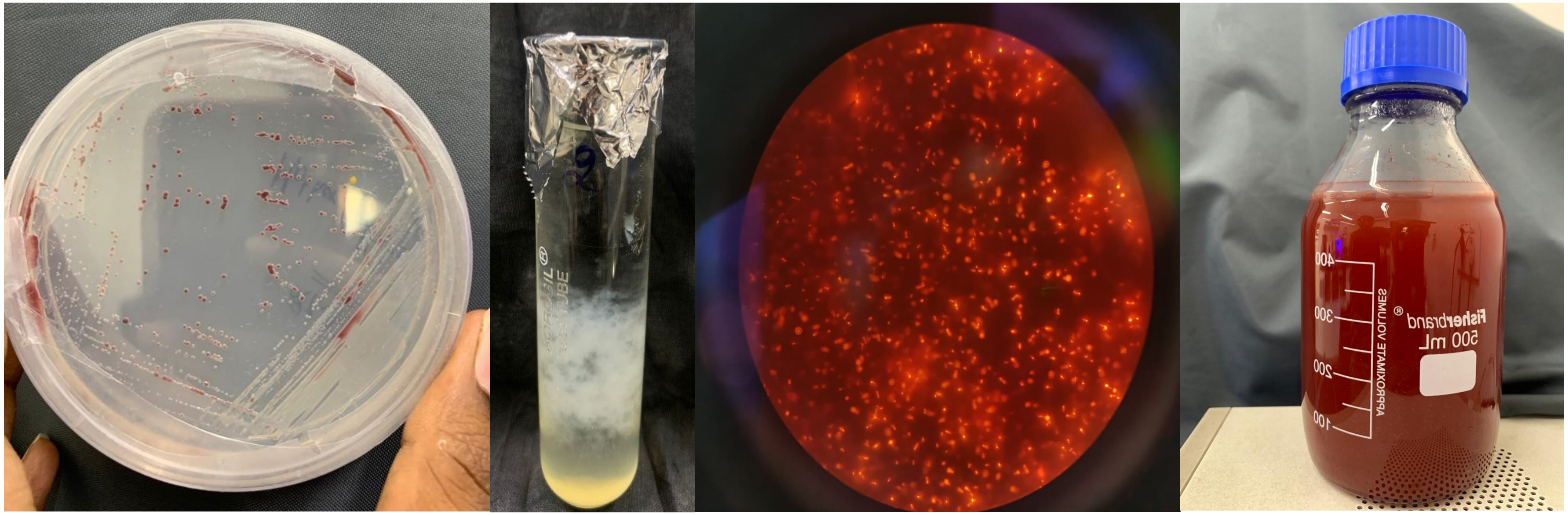


S Fig 1: a) Carbon utilisation in respective experimental conditions at the end of growth phases b) Nitrate concentration and variations at the end of growth phases

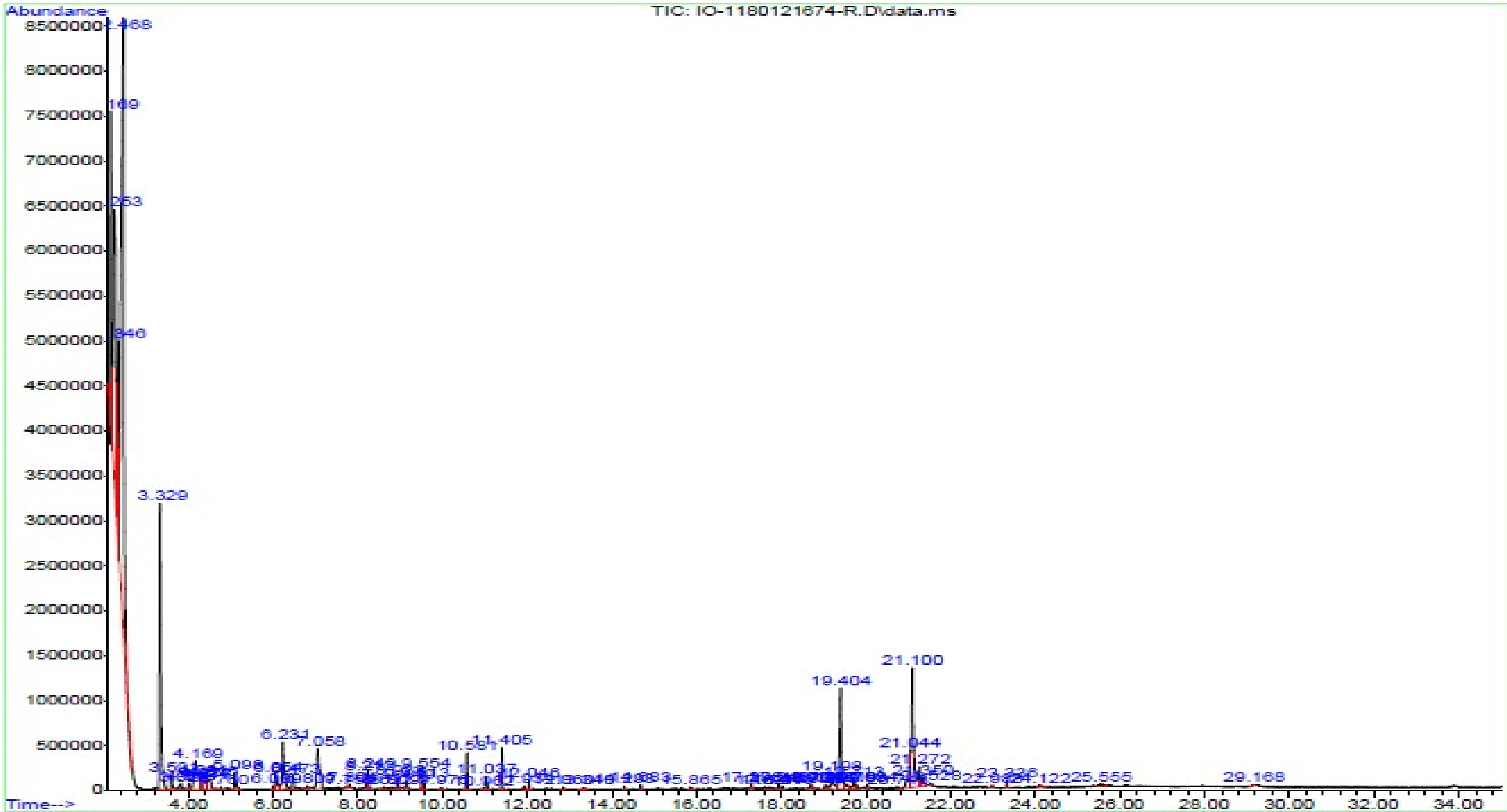


S Fig 2: Isolated *Rhodopseudomonas palustris* SVMIICT10 strain (MTCC deposition no. 16028), PHA Precipitation during Extraction and Fluorescence Imaging

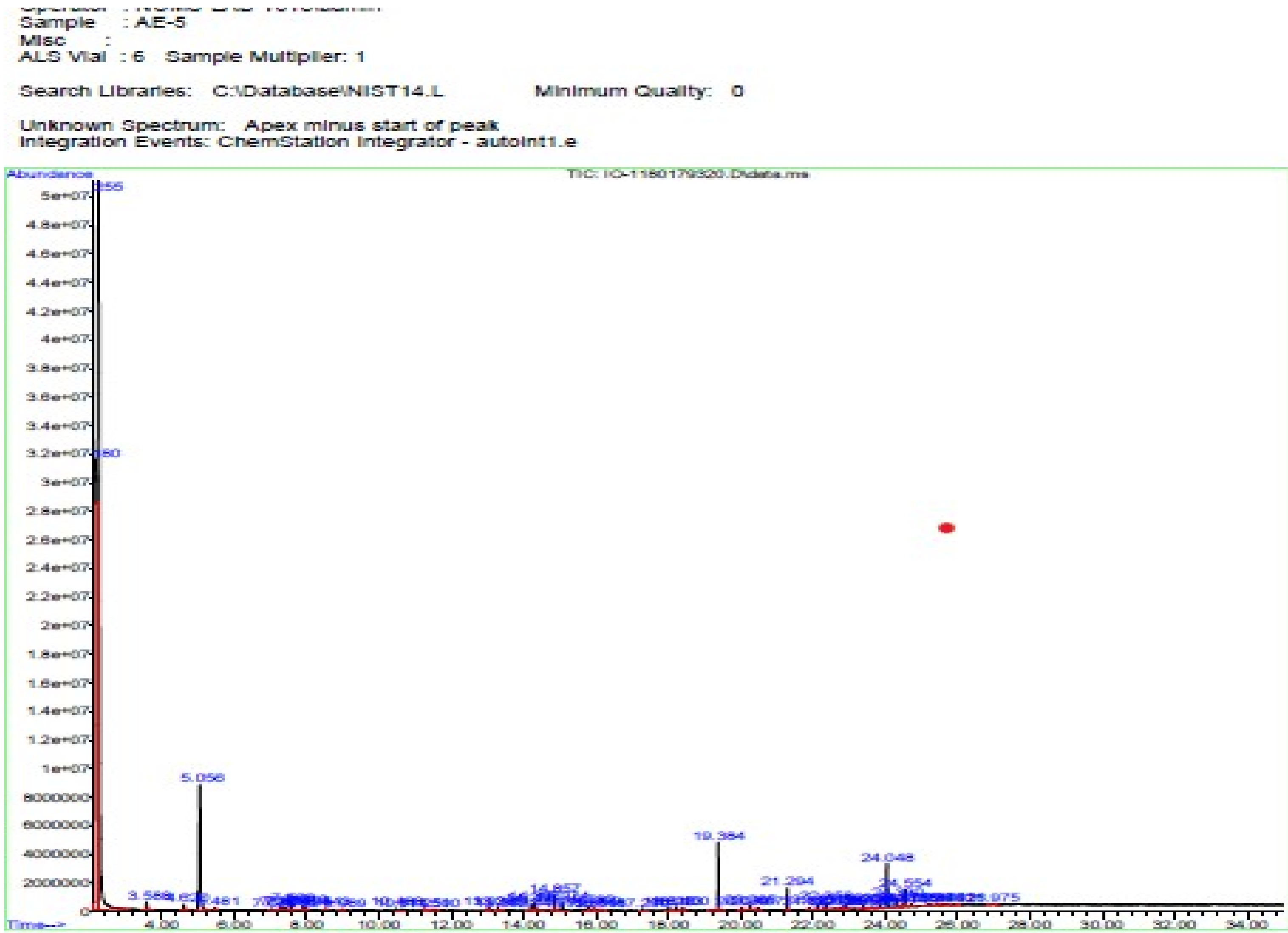
Sample : ST-1
Misc :
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0
C:\Database\NIST14.L Minimum Quality: 0
C:\Database\W9N11.L

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e



S Fig 3a: GC-MS Chromatogram of P-3HB Sigma Aldrich-Natural Origin Standard

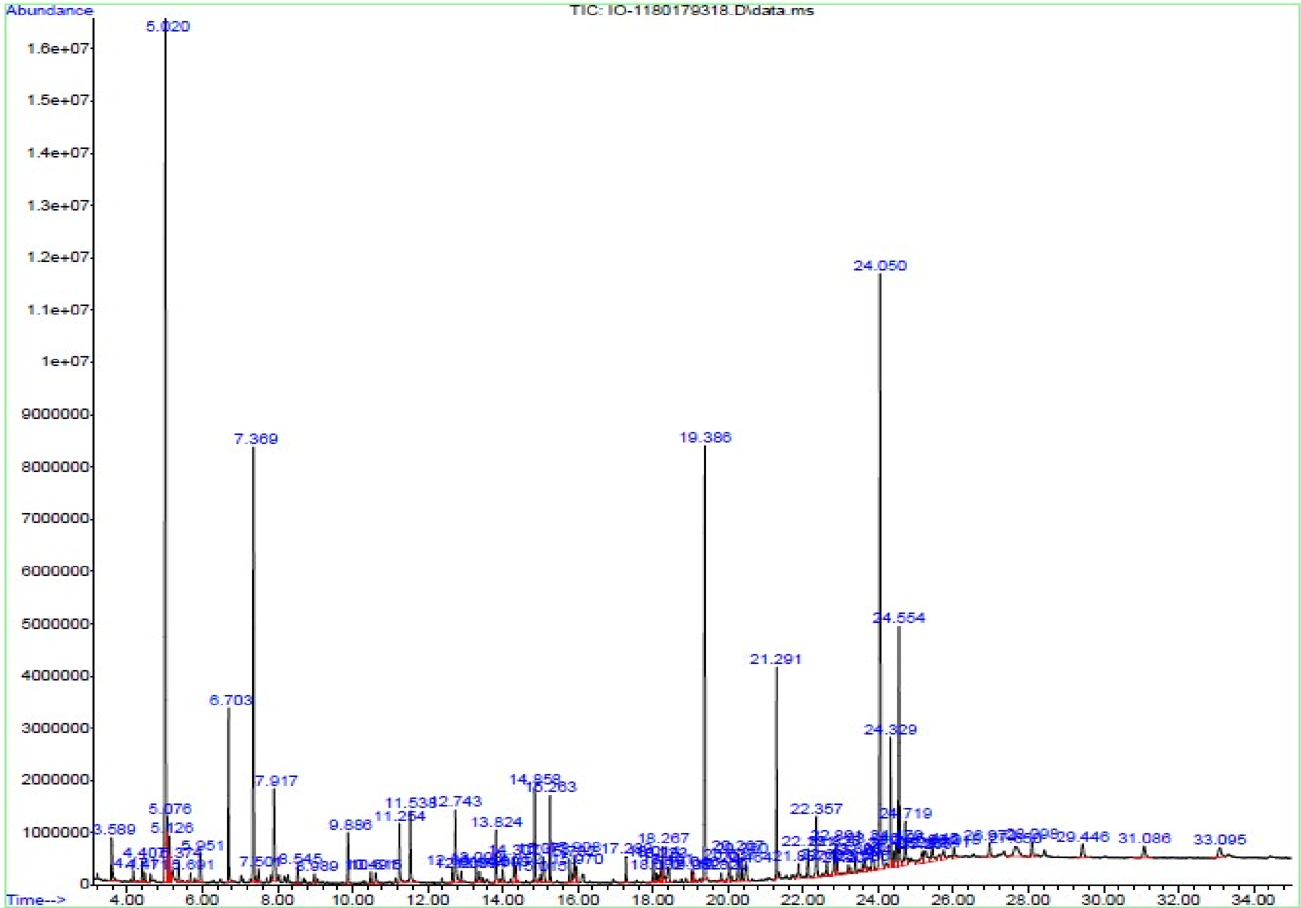


S Fig 3b: GC-MS Raw Chromatogram of Ae (P)

Sample : AE+L-3
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e



S Fig 3c: GC-MS Raw Chromatogram of Ae (P+L)

Sample : AE+L-TRANS-4
Misc :
ALS Vial : 4 Sample Multiplier: 1

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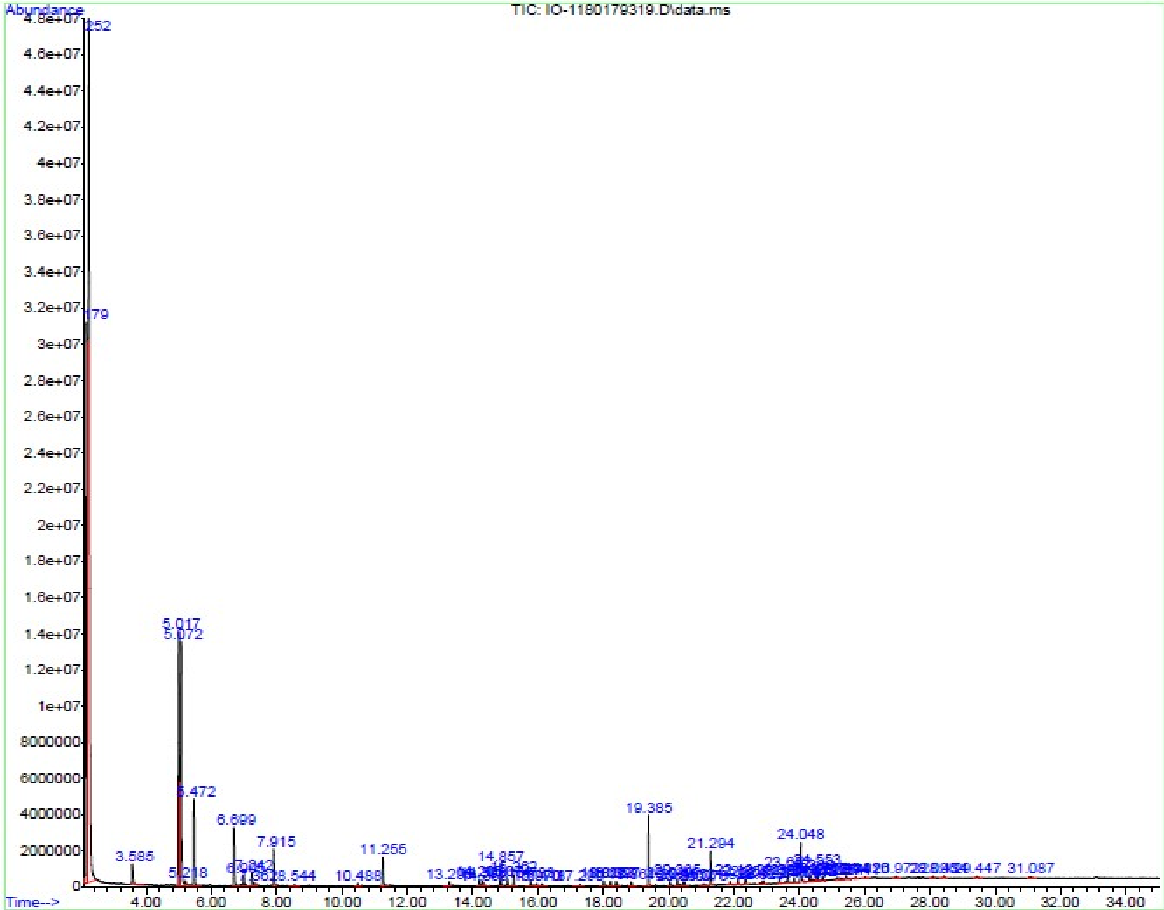
Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e



S Fig 3d: GC-MS Raw Chromatogram of Trans Ae (P+L)

Sample : AE-TRANS-6
Misc :
ALS Vial : 12 Sample Multiplier: 1

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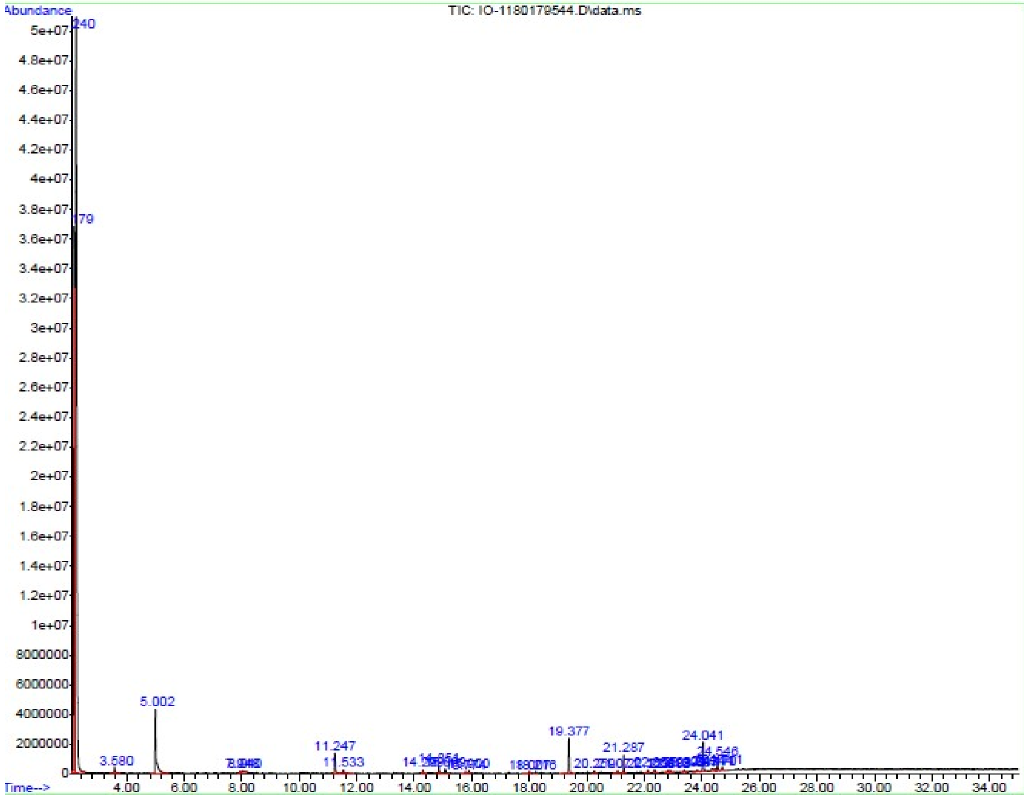
C:\Database\W9N11.L

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e



S Fig 3e: GC-MS Raw Chromatogram of Trans Ae (P)

Sample : AN-7
Misc :
ALS Vial : 13 Sample Multiplier: 1

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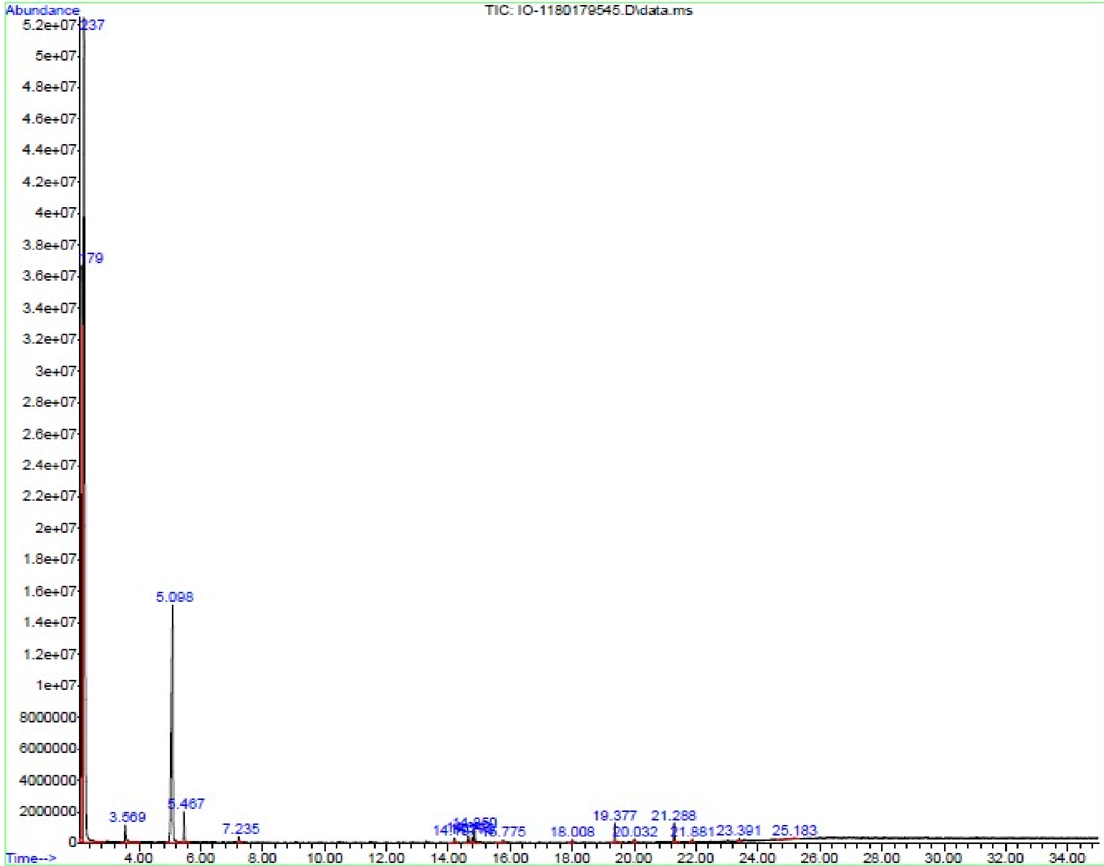
C:\Database\W9N11.L

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e



S Fig 3f: GC-MS Raw Chromatogram of An (P)

Sample : AN+L-8
Misc :
ALS Vial : 14 Sample Multiplier: 1

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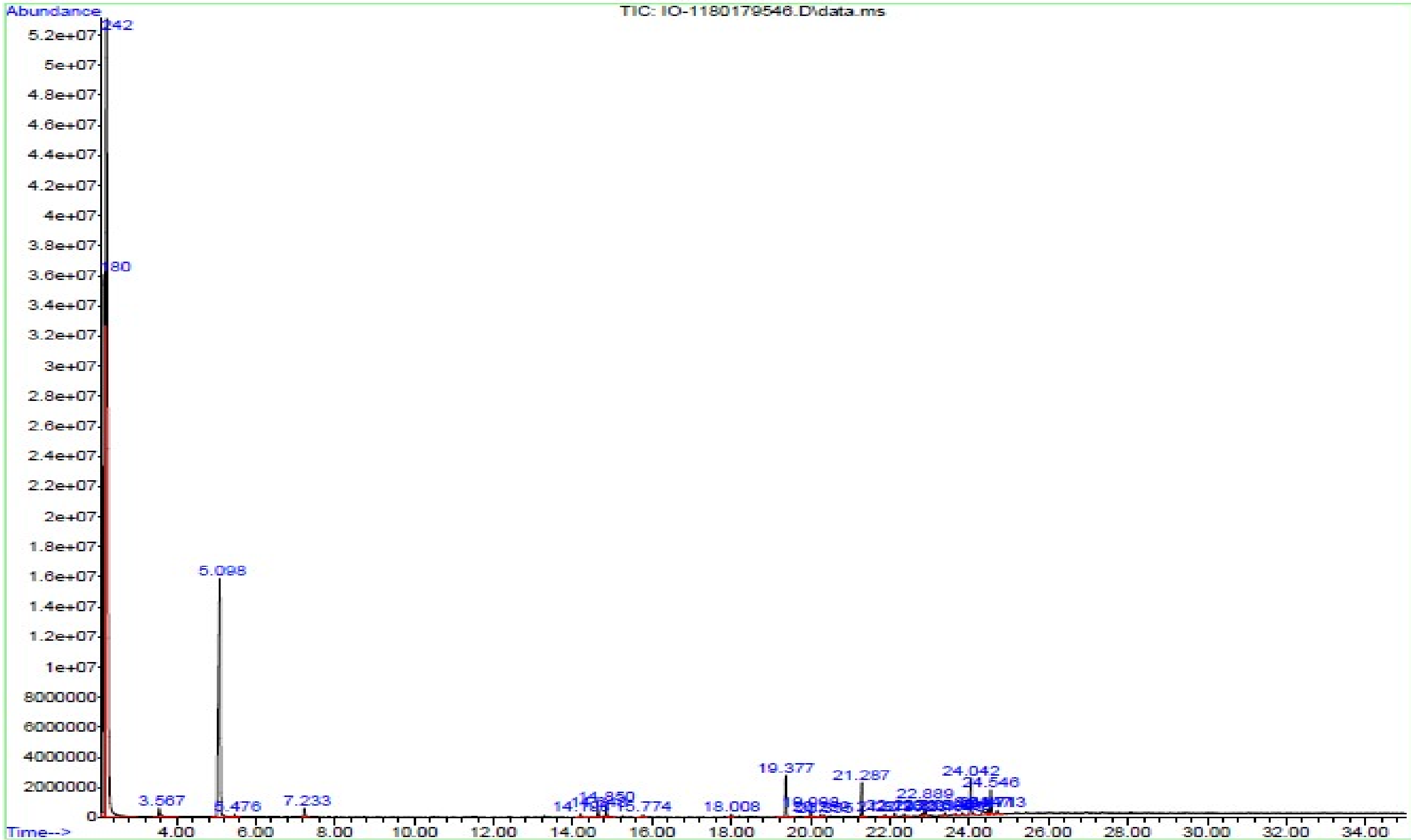
C:\Database\NIST14.L Minimum Quality: 0
C:\Database\W9N11.L

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e



S Fig 3g: GC-MS Raw Chromatogram of An (P+L)

Sample : AN-H2-2
Misc :
ALS Vial : 5 Sample Multiplier: 1

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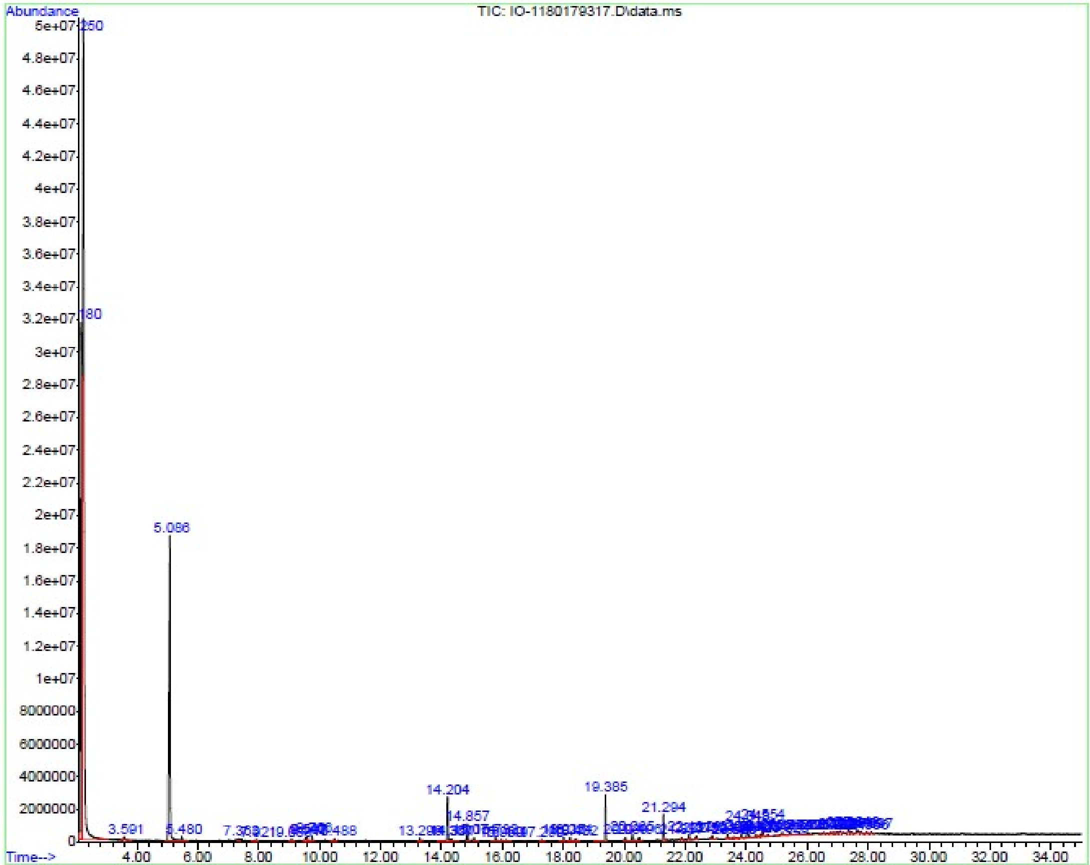
C:\Database\NIST14.L Minimum Quality: 0
C:\Database\W9N11.L

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e

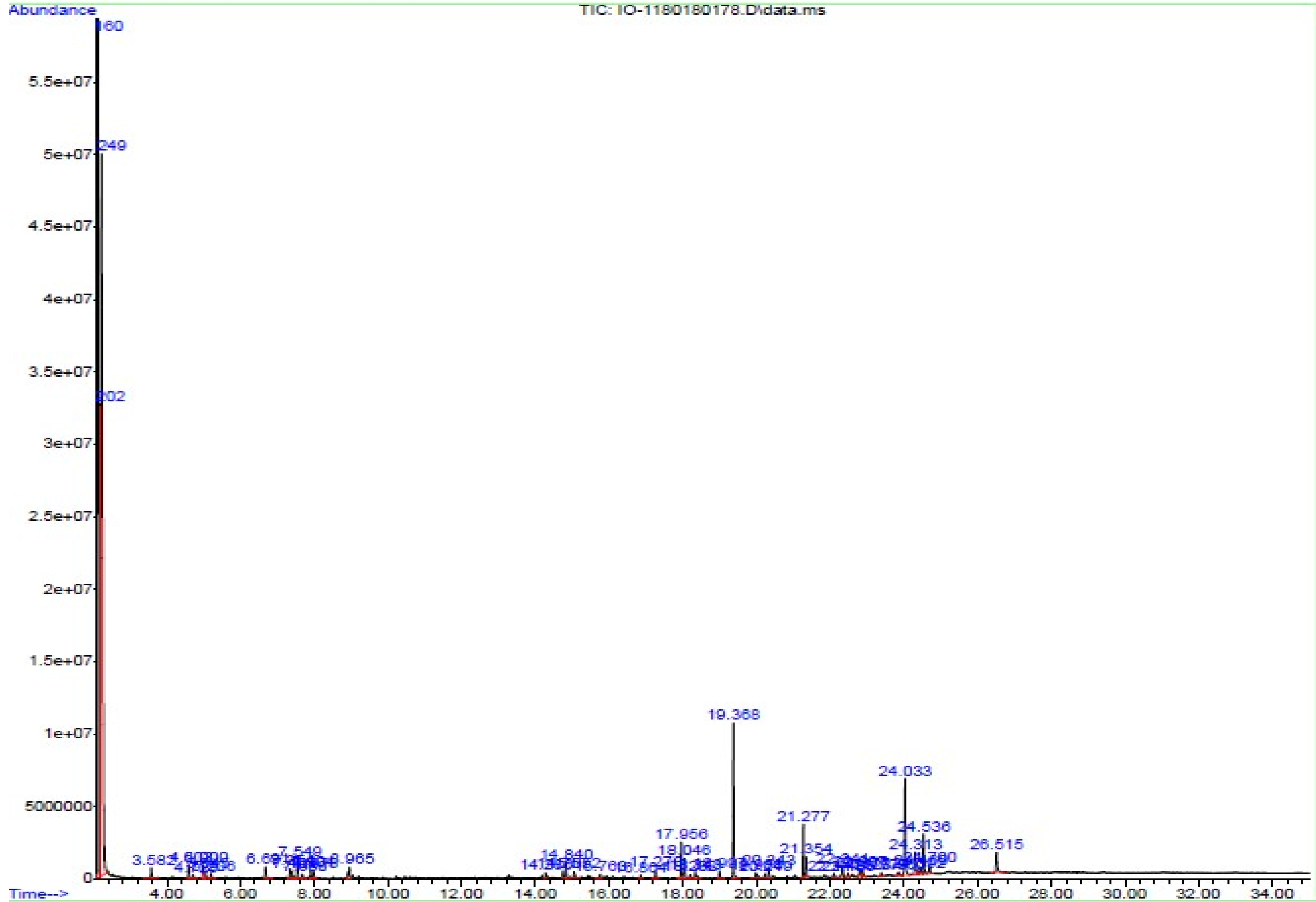


S Fig 3h: GC-MS Raw Chromatogram of An- (P+L) Biogas

Sample : 11-TRANSL-N+P
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e

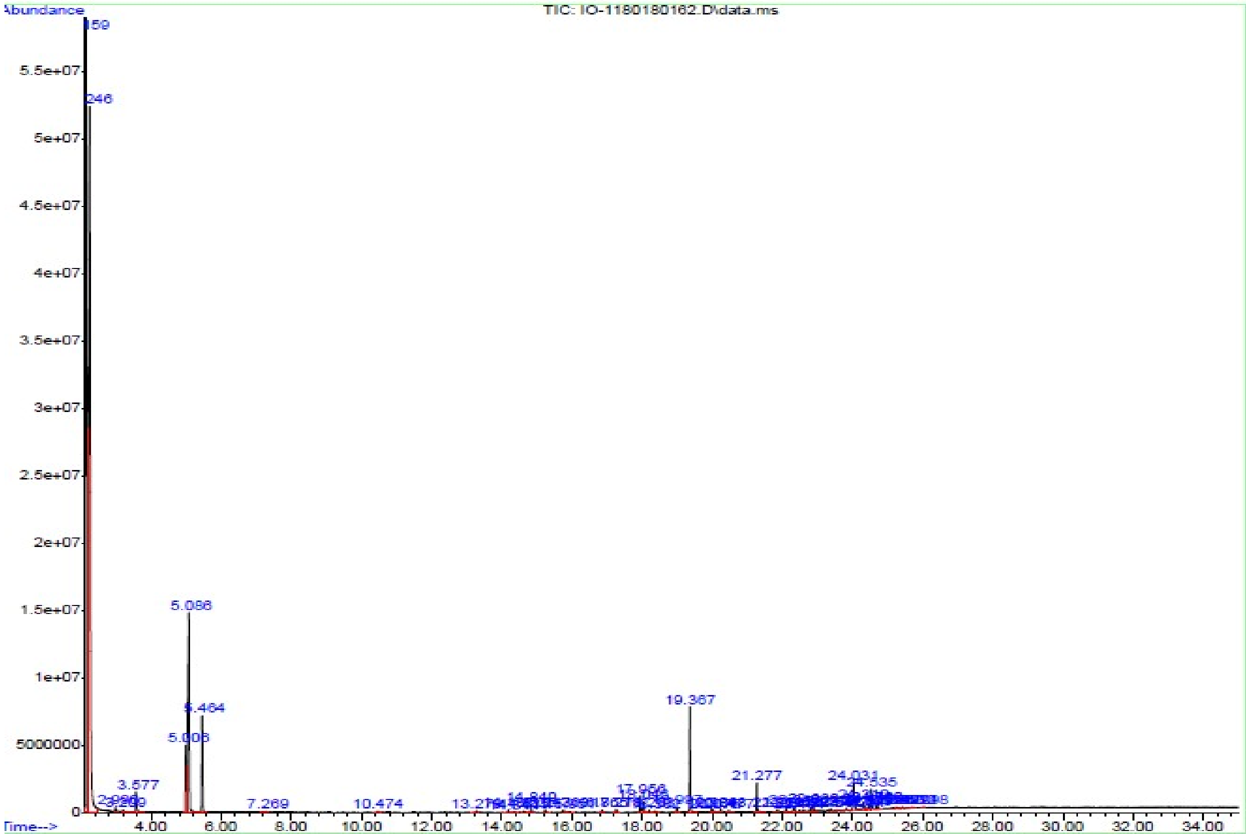


S Fig 3i: GC-MS Raw Chromatogram of **An-Trans (P+L)-(N+Ph)**

Sample : 10-TRANS-N
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e

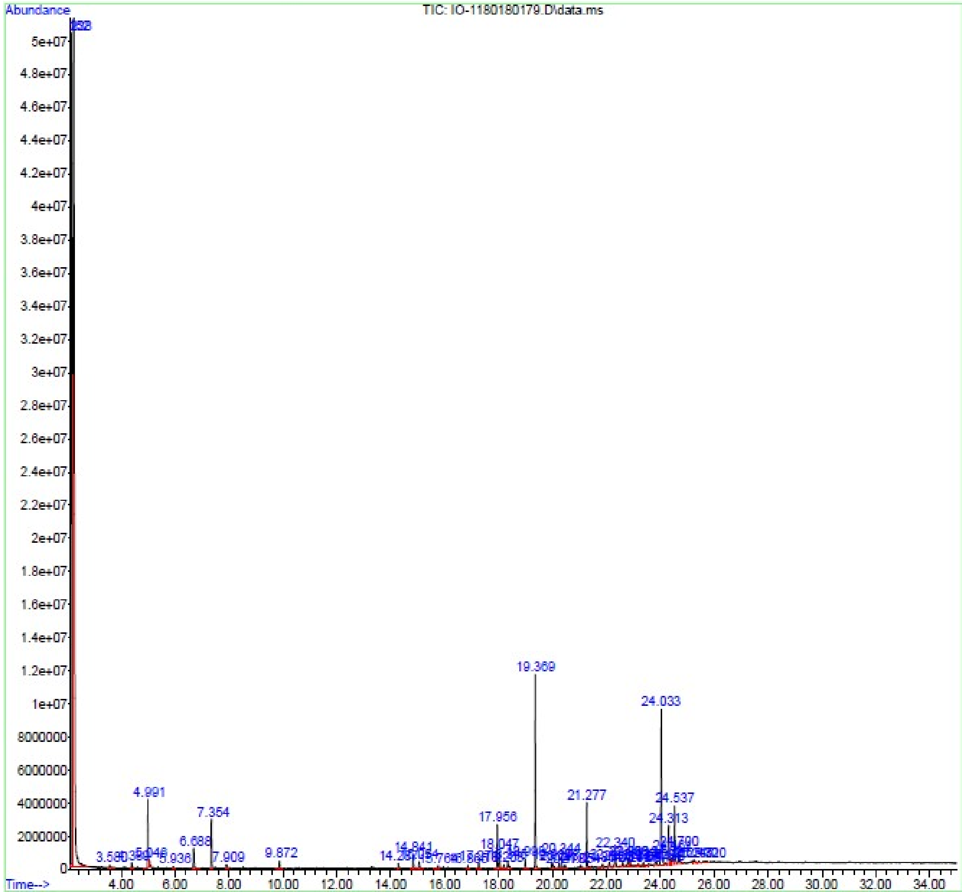


S Fig 3j: GC-MS Raw Chromatogram of An-Trans (P)-(N)

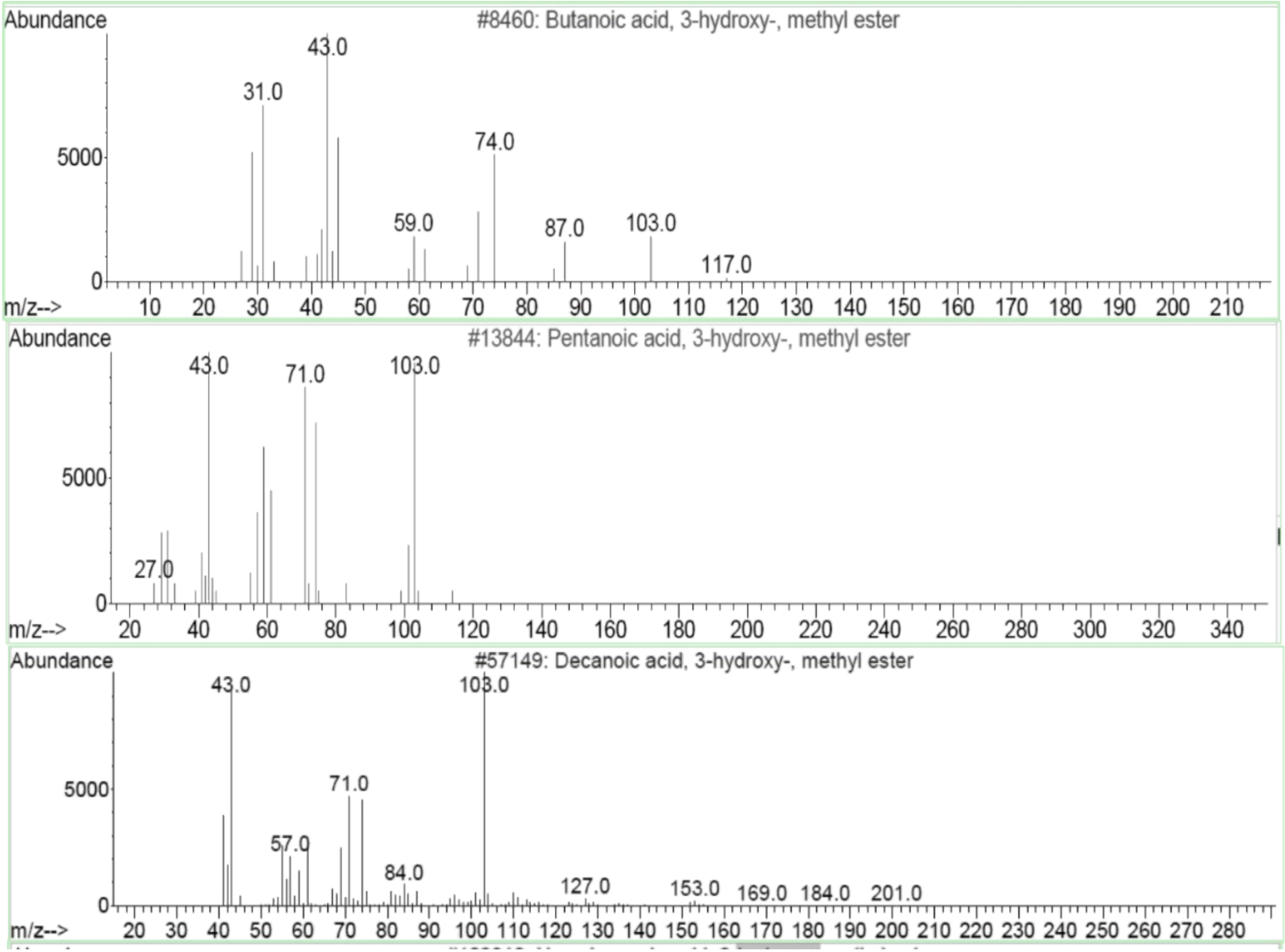
Sample : 12-TRANS-N
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e



S Fig 3k: GC-MS Raw Chromatogram of An-Trans (P+L)-(N)



S Fig 4: Fragmentogram of the identified Alkyl groups as corresponding alkyl methyl esters in GCMS analysis

Table S 1: Back calculations for the relative absorbance of key functional group from FT-IR analysis

Condition	Transmittance % (C-H)	Transmittance % (C=O)	$A_{(C\text{-}H)} = -\log_{10}(T)$	$A_{(C=O)} = -\log_{10}(T)$
Ae (P)	90	41	0.045757491	0.387216
Ae (P+L)	82.007	58	0.086149075	0.236572
Trans Ae (P)	98.95	92.8	0.004584201	0.032452
Trans Ae (P+L)	92	75	0.036212173	0.124939
An (P)	93	85	0.031517051	0.070581
An (P+L)	98.7	94	0.005682847	0.026872
An- (P+L) -Biogas	96.5	92	0.015472687	0.036212
An-Trans (P+L)-(N+Ph)	97.74	92	0.009927665	0.036212
An-Trans (P+L)-(N)	96.29	82	0.016418813	0.086186
An-Trans (P)-(N)	90	65	0.045757491	0.187087
CO_2	97.83	99.7	0.009527946	0.001

Table S 2: Prospective up-scale scenario of 25 l, with the assumptions

Process	Lab Scale Production	Process scale-up
Sterilisation	Auto-clave	In-tank Autoclaving (utilisation of the maximal efficiency)
Batch Cultivation	Bottles/Conical Flask	bio-reactor
Agitation	Magnetic stirrers	In-tank stirring
Illumination	Tube light	In tank LED lights with uniform Illumination
Biomass Harvesting	Centrifuge	Filtration/Centrifuge
Drying	sunlight/oven	Vacuum drying/oven
Solvents	Evaporation	Recovery through distillation
Spent Biomass	Disposed	Multiproduct valorisation
Cell Disruption	Sonication	Sonication

Table S 3: Energy distribution for the lab scale and scale up production

Energy	Lab Scale Production (KWh)	Process scale-up 25 L (KWh)
Sterilisation	0.5	0.02 Kwh/L
Mixing	0.28	0.011
Light	1.108	0.044
Centrifuge	0.0583	0.0097
Sonicator	0.048	0.0016

The energy was calculated according to the power specifications of the instruments when used for its full efficiency for 25 L, Sterilisation (autoclave with 2kwh); Mixing (Magnetic stirrers with 2W); CFL lights (220 V and 80 mA specification for 6 days); Centrifuge (700 W); Sonicator (120 W).

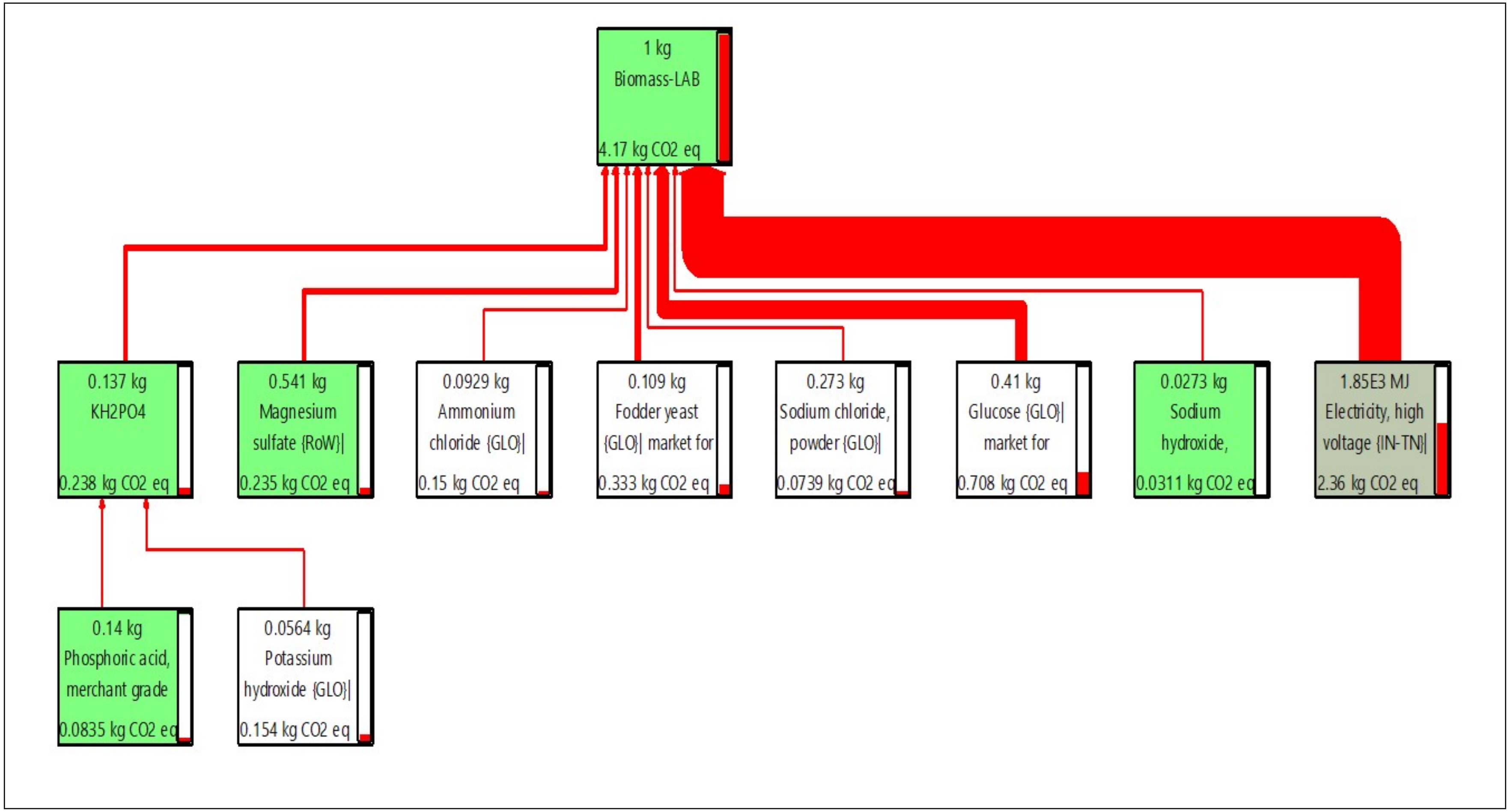
Table S 4: Unit specific Life Cycle Inventory for Lab scale and upscale production

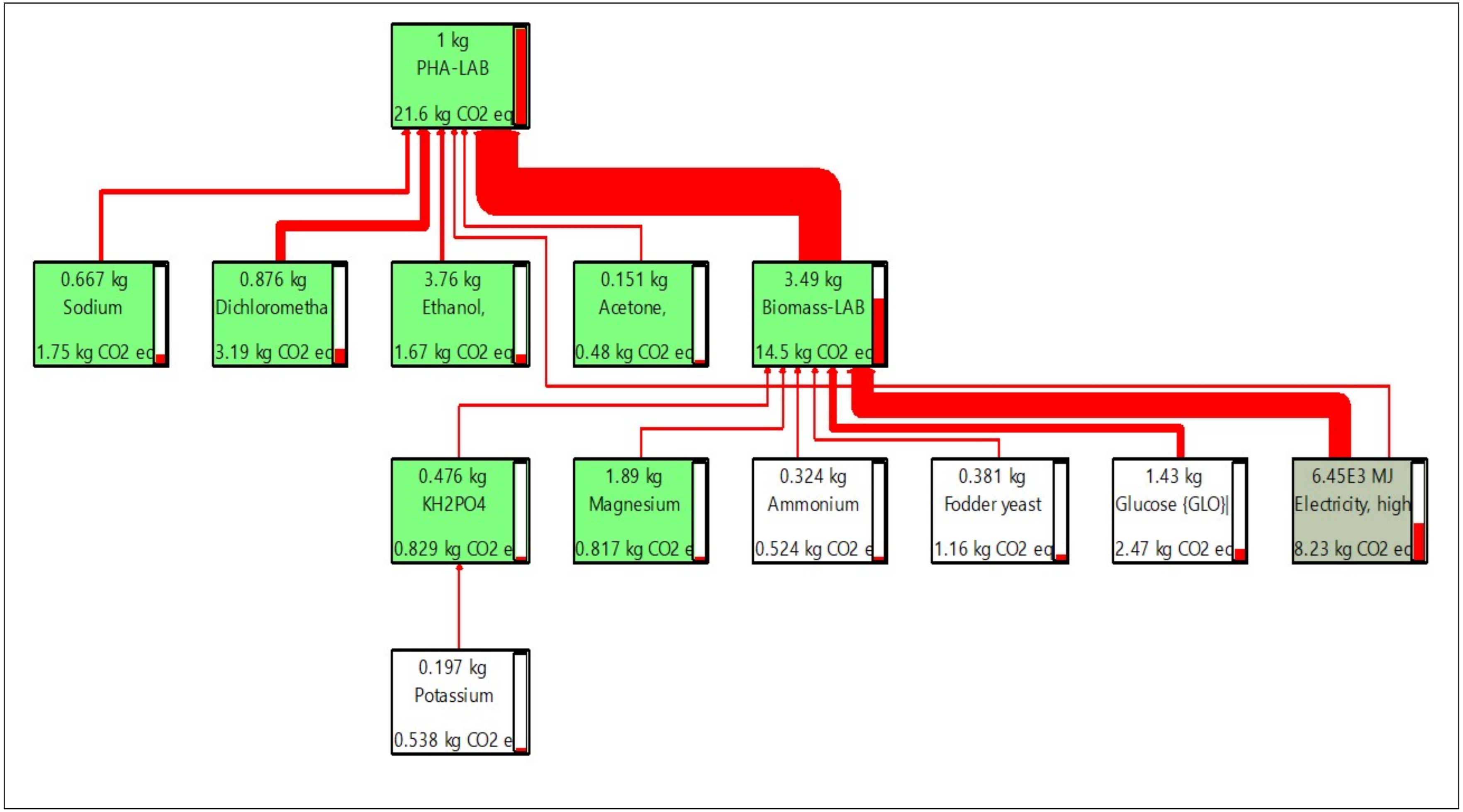
Lab Scale			Up-Scaled[1]		
BIOMASS PRODUCTION UNIT					
INPUTS	**Unit**	**Value**	**INPUTS**	**Unit**	**Value**
KH_2PO_4	g	0.5	KH_2PO_4	g	0.5
$CaCl_2.2H_2O$	g	0.15	$CaCl_2.2H_2O$	g	0.15
$MgSO_4.7H_2O$	g	2	$MgSO_4.7H_2O$	g	2
NH_4Cl	g	0.34	NH_4Cl	g	0.34
Yeast Extract	g	0.4	Yeast Extract	g	0.4
NaCl	g	1	NaCl	g	1
Ferric Citrate	g	0.005	Ferric Citrate	g	0.005
Sodium Pyruvate	g	3	Sodium Pyruvate	g	3

[1] The upscaled condition was assumed to be more efficient than a lab scale study under full sophisticated environmental conditions resulting to the biomass and PHA productivity in range of ± 0.4; the energy requirements are calculated based on the table S 3; the solvent recovery strategy is considered using distillation.

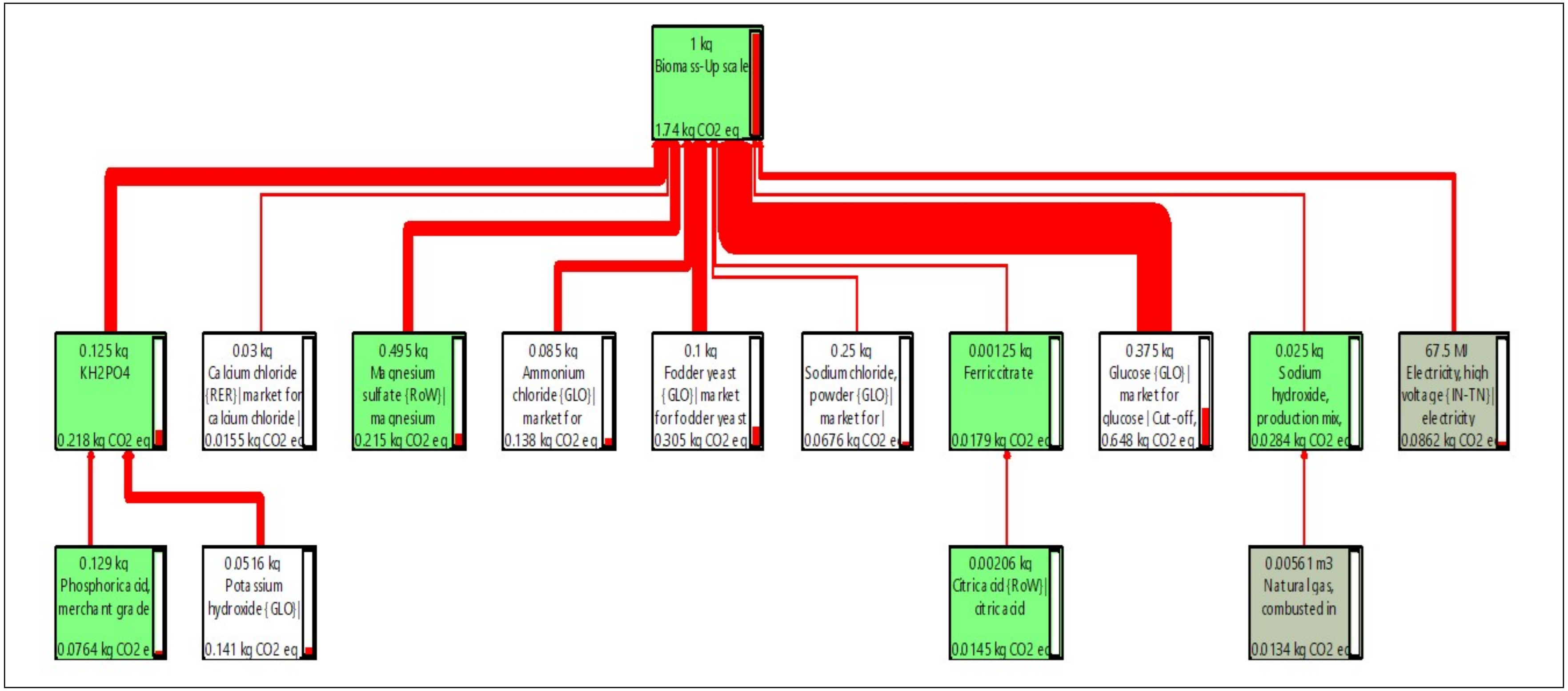
Water	L	1	Water	L	1
NaOH	g	1	NaOH	g	1
Energy	kWh	1.88	Energy	kWh	0.075
OUTPUTS	**Unit**	**Value**	**OUTPUTS**	**Unit**	**Value**
Biomass	g	3.66	Biomass	g	4
Wastewater	L	0.8	Wastewater	L	0
POLYMER DOWNSTREAMING UNIT					
INPUTS	**Unit**	**Value**	**INPUTS**	**Unit**	**Value**
Biomass	g	3.66	Biomass	g	4
NaOCl	g	0.7	NaOCl	g	0.7
DCM	g	0.92	DCM	g	0.01
Ethanol	g	3.945	Ethanol	g	0.1
Acetone	g	0.158	Acetone	g	0.01
Energy	kWh	0.106	Energy	kWh	0.0113
OUTPUTS	**Unit**	**Value**	**OUTPUTS**	**Unit**	**Value**
PHA	g	1.05	PHA	g	1.5
Wastewater	L	0.01	Wastewater	L	0.01
DCM Vapour	g	0.92	DCM Vapour	g	0.01
Ethanol Vapour	g	3.945	Ethanol Vapour	g	0.001

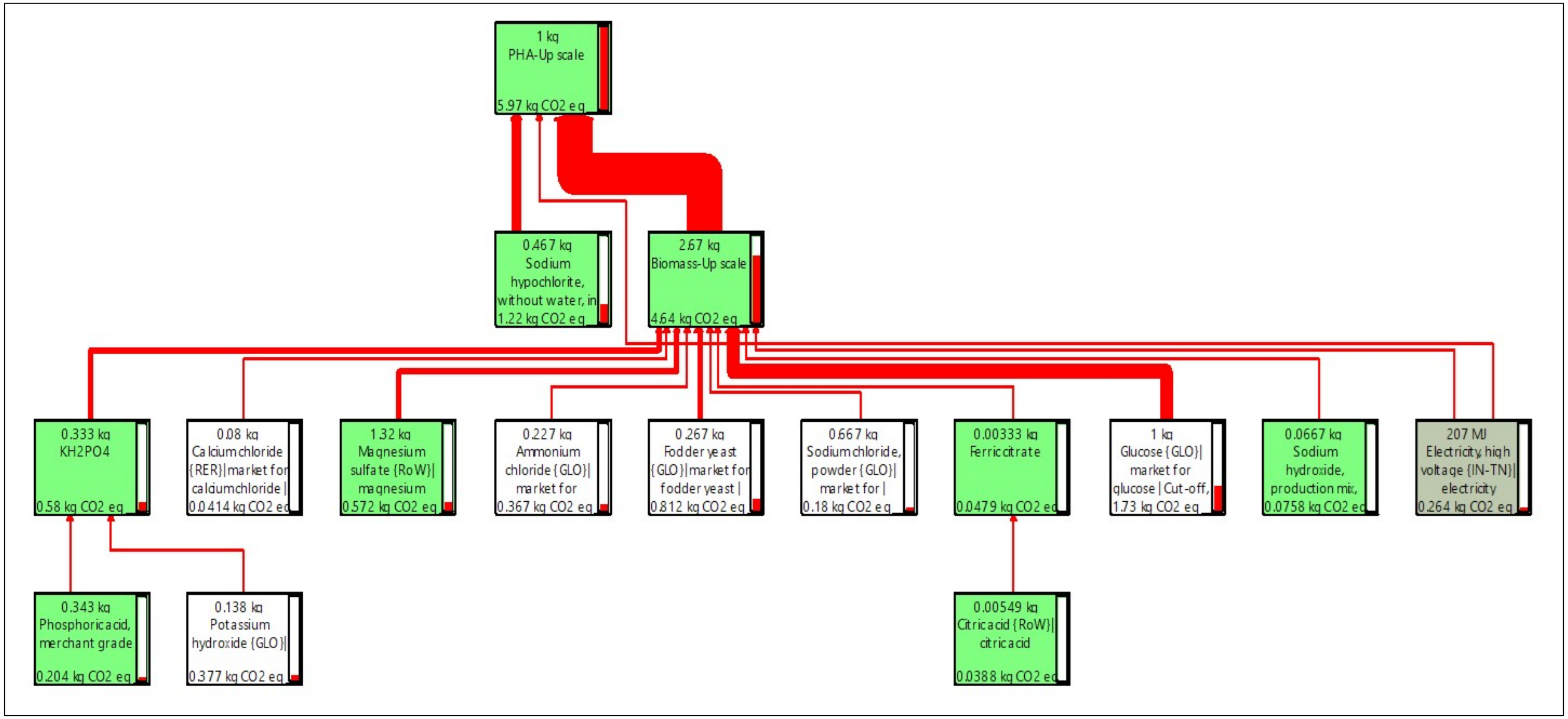
Table S5: CO_2 eq. emission from each unit process, Sankey representation of the contributory elements



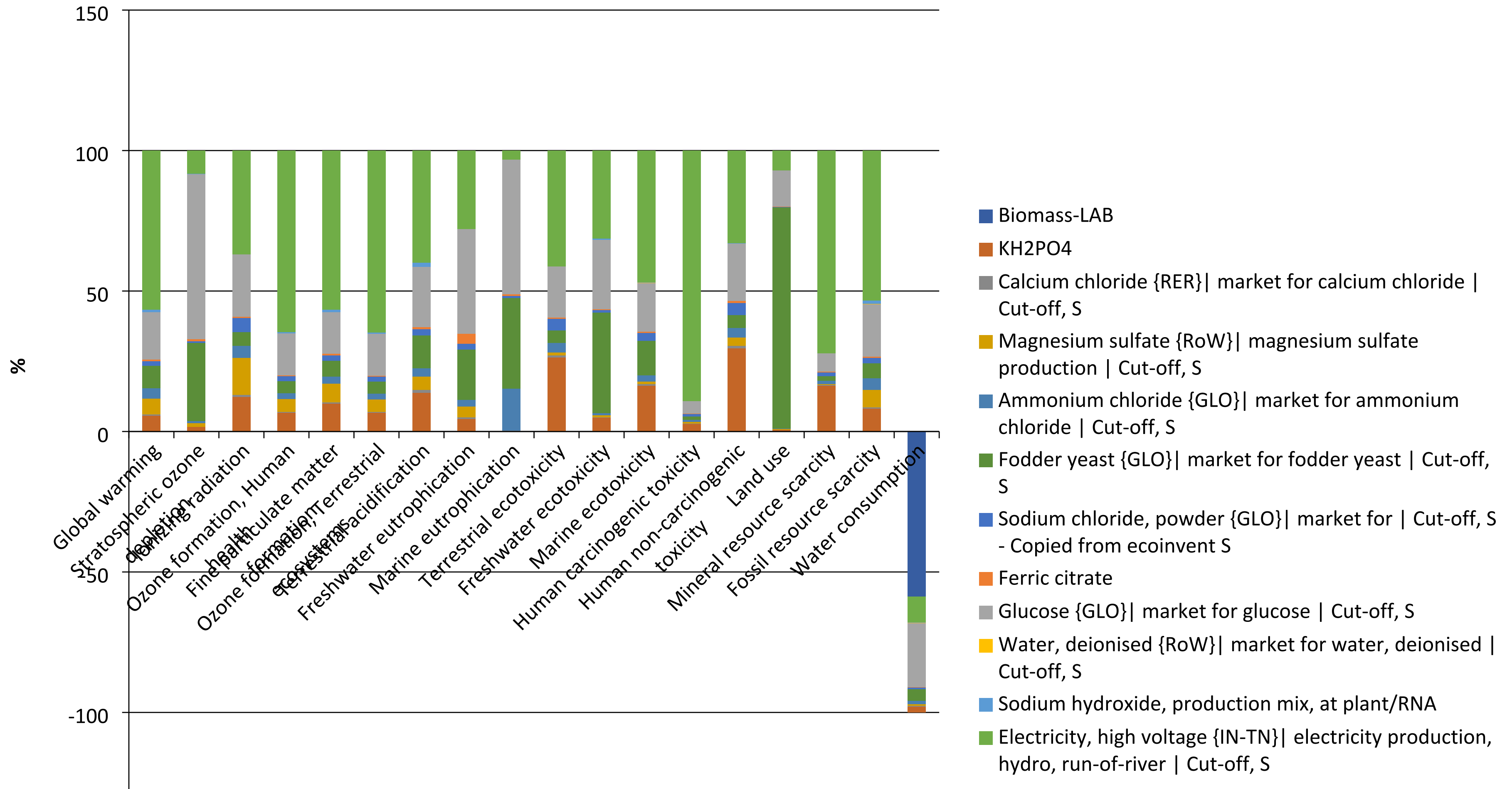


1 kg
PHA-LAB
21.6 kg CO2 eq
0.667 kg
Sodium
1.75 kg CO2 eq
0.876 kg
Dichlorometha
3.19 kg CO2 eq
3.76 kg
Ethanol,
1.67 kg CO2 eq
0.151 kg
Acetone,
0.48 kg CO2 eq
3.49 kg
Biomass-LAB
14.5 kg CO2 eq
0.476 kg
KH2PO4
0.829 kg CO2 e
1.89 kg
Magnesium
0.817 kg CO2 e
0.324 kg
Ammonium
0.524 kg CO2 e
0.381 kg
Fodder yeast
1.16 kg CO2 eq
1.43 kg
Glucose {GLO}|
2.47 kg CO2 eq
6.45E3 MJ
Electricity, high
8.23 kg CO2 eq
0.197 kg
Potassium
0.538 kg CO2 e

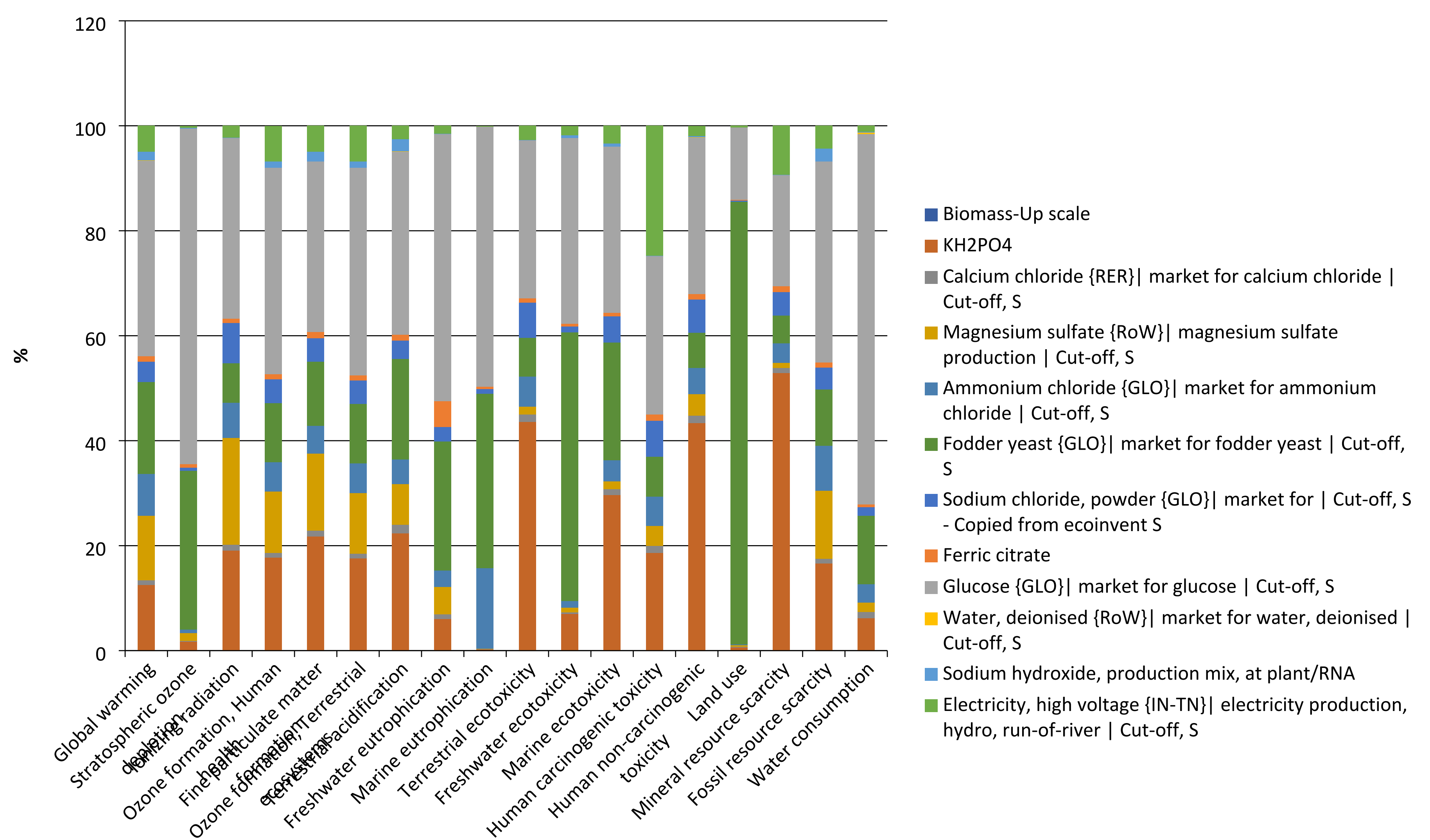

1 kg
Biomass-Up scale
1.74 kg CO2 eq
0.125 kg
KH2PO4
0.218 kg CO2 eq
0.03 kg
Calcium chloride {RER}| market for calcium chloride |
0.0155 kg CO2 eq
0.495 kg
Magnesium sulfate {RoW}| magnesium
0.215 kg CO2 eq
0.085 kg
Ammonium chloride {GLO}| market for
0.138 kg CO2 eq
0.1 kg
Fodder yeast {GLO}| market for fodder yeast
0.305 kg CO2 eq
0.25 kg
Sodium chloride, powder {GLO}| market for |
0.0676 kg CO2 e
0.00125 kg
Ferric citrate
0.0179 kg CO2 eq
0.375 kg
Glucose {GLO}| market for glucose | Cut-off,
0.648 kg CO2 eq
0.025 kg
Sodium hydroxide, production mix,
0.0284 kg CO2 eq
67.5 MJ
Electricity, high voltage {IN-TN}| electricity
0.0862 kg CO2 e
0.129 kg
Phosphoric acid, merchant grade
0.0764 kg CO2 e
0.0516 kg
Potassium hydroxide {GLO}|
0.141 kg CO2 eq
0.00206 kg
Citric acid {RoW}| citric acid
0.0145 kg CO2 eq
0.00561 m3
Natural gas, combusted in
0.0134 kg CO2 eq



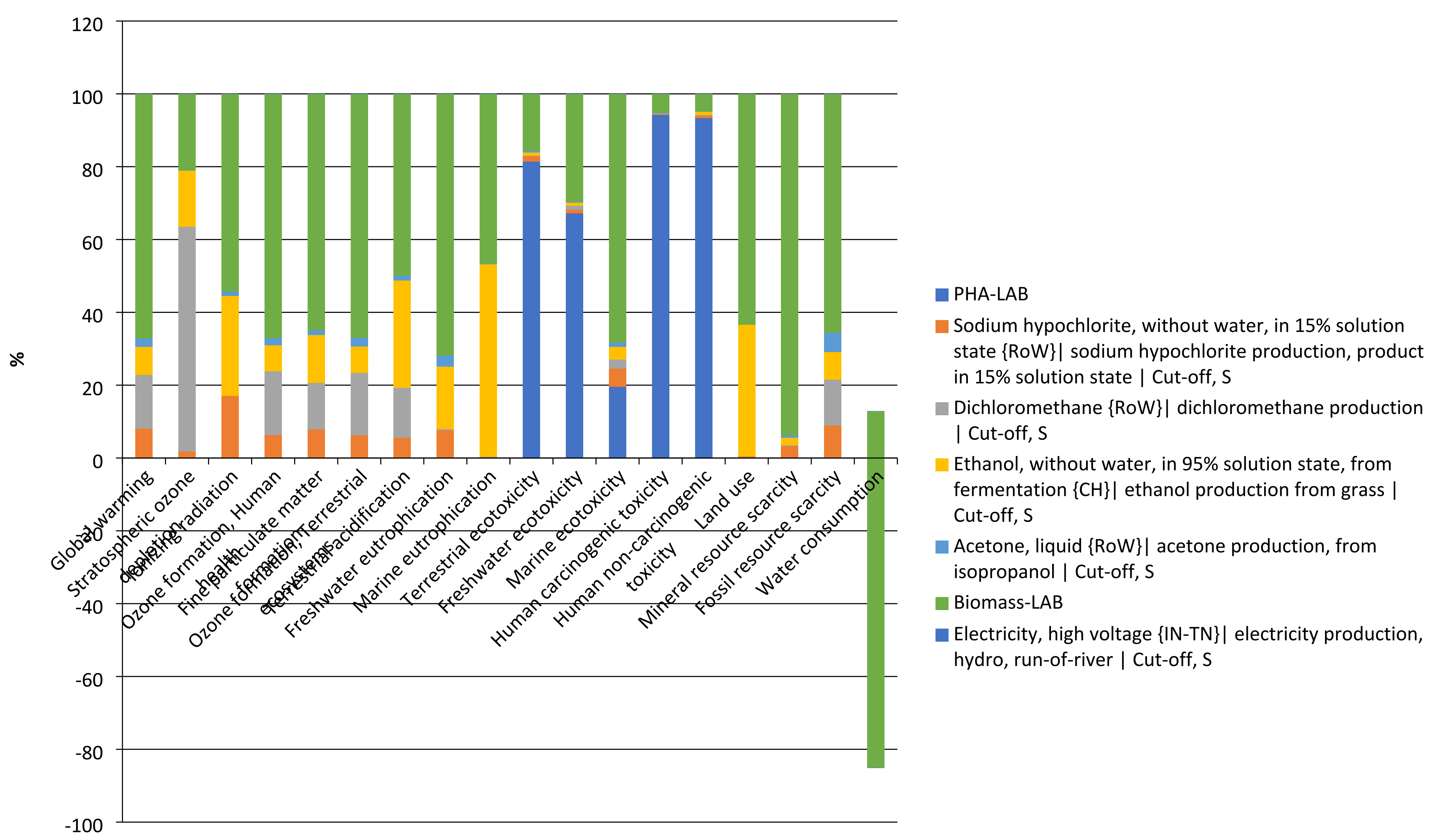
1 kg
PHA-Up scale
5.97 kg CO2 eq
0.467 kg
Sodium hypochlorite, without water, in
1.22 kg CO2 eq
2.67 kg
Biomass-Up scale
4.64 kg CO2 eq
0.333 kg
KH2PO4
0.58 kg CO2 eq
0.08 kg
Calcium chloride {RER}| market for calcium chloride |
0.0414 kg CO2 eq
1.32 kg
Magnesium sulfate {RoW}| magnesium
0.572 kg CO2 eq
0.227 kg
Ammonium chloride {GLO}| market for
0.367 kg CO2 eq
0.267 kg
Fodder yeast {GLO}| market for fodder yeast |
0.812 kg CO2 eq
0.667 kg
Sodium chloride, powder {GLO}| market for |
0.18 kg CO2 eq
0.00333 kg
Ferric citrate
0.0479 kg CO2 eq
1 kg
Glucose {GLO}| market for glucose | Cut-off,
1.73 kg CO2 eq
0.0667 kg
Sodium hydroxide, production mix,
0.0758 kg CO2 eq
207 MJ
Electricity, high voltage {IN-TN}| electricity
0.264 kg CO2 eq
0.343 kg
Phosphoric acid, merchant grade
0.204 kg CO2 eq
0.138 kg
Potassium hydroxide {GLO}|
0.377 kg CO2 eq
0.00549 kg
Citric acid {RoW}| citric acid
0.0388 kg CO2 eq



Analyzing 1 kg 'Biomass-LAB';
Method: ReCiPe 2016 Midpoint (H) V1.08 / World (2010) H / Characterization / Excluding infrastructure processes / Excluding long-term emissions



Analyzing 1 kg 'Biomass-Up scale';
Method: ReCiPe 2016 Midpoint (H) V1.08 / World (2010) H / Characterization / Excluding infrastructure processes / Excluding long-term emissions



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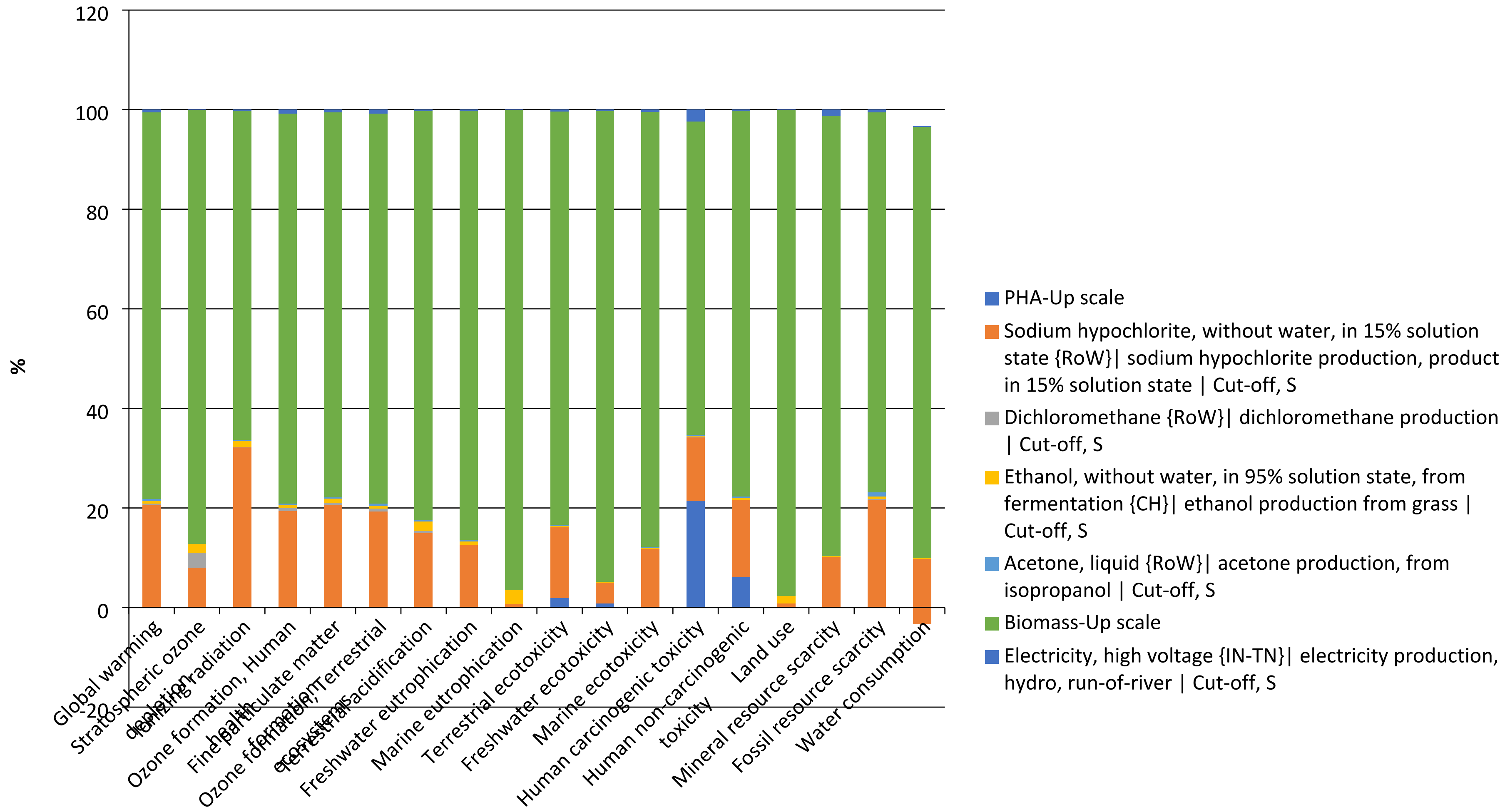

Analyzing 1 kg 'PHA-Up scale';
Method: ReCiPe 2016 Midpoint (H) V1.08 / World (2010) H / Characterization / Excluding infrastructure processes / Excluding long-term emissions