Enhanced removal efficiency of nevirapine by *Tetradesmus obliquus via* co-substrate supplementation: removal mechanisms and relative gene expression

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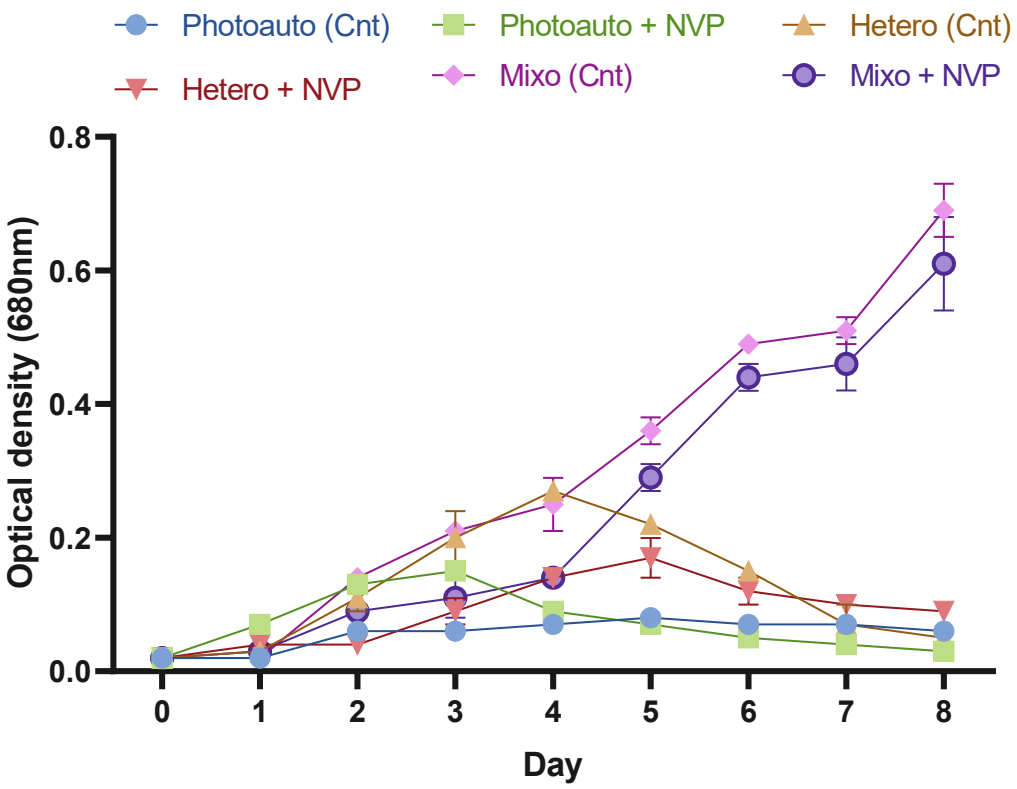
Supplementary Data:

S1. Oligonucleotide sequences of primers used for relative gene expression studies.

Primer	Sequence (5'-3')	Optimized annealing temp. (°C)	Reference
18S rRNA	(F) CGGTCCGCCTATGGTGAGTA (R) CTCCGGTCCTACAGACCAACA	62	[1]
sod1	(F) ACG GCT CCC TGT CGA TCG A (R) CGC CAC GTC CGG CAG CGC GG	50	[2]
gpx1	(F) CGAAGCCGCACATGGTATAGT (R) TGCTCCAATCACGACCTATTTG	50	[2]
cat2	(F) GGAGGCTGCAGGAAAACTGA (R) ATTTCCAGCCTGGGCTACCT	50	[2]

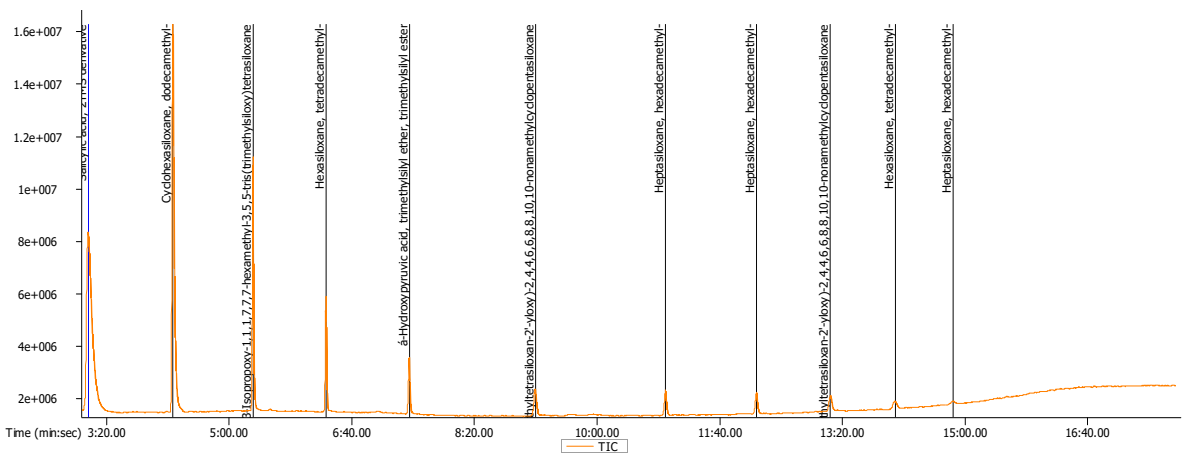
F- Forward; R- Reverse, Temp- temperature (°C)



S2. Effect of different cultivation conditions on growth of *Tetradesmus obliquus* in BG-11 medium supplemented with NVP.

S3. GC-MS total ion current chromatogram (TIC) of *T. obliquus* biomass obtained from (a) Control (Day 4); (b) Treated (Day 4); (c) Control (Day 8); (d) Treated (Day 8).

a



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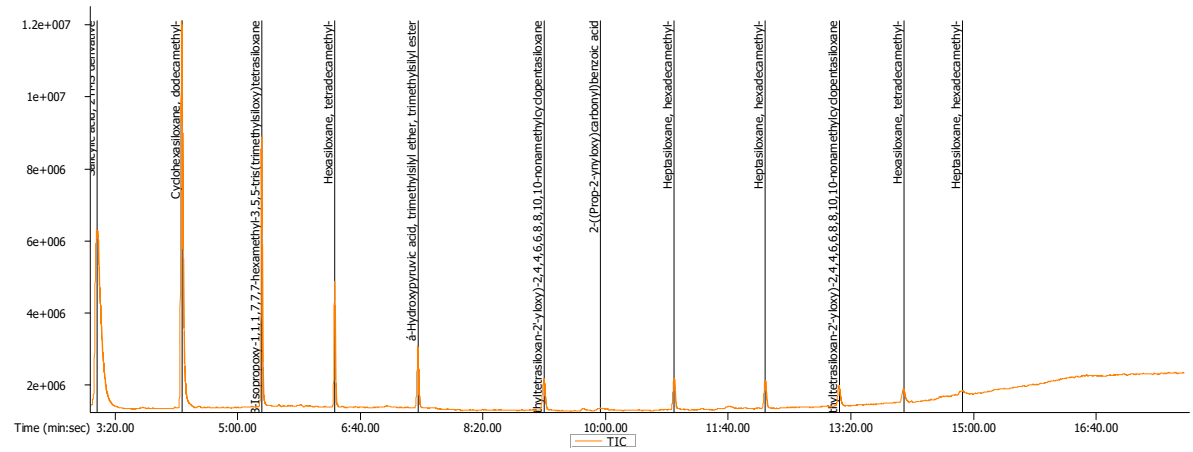

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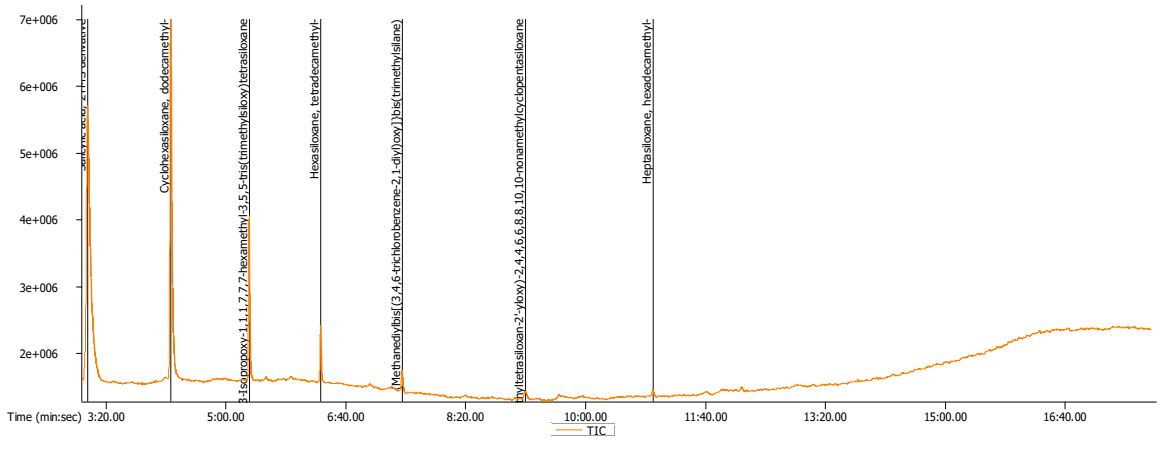

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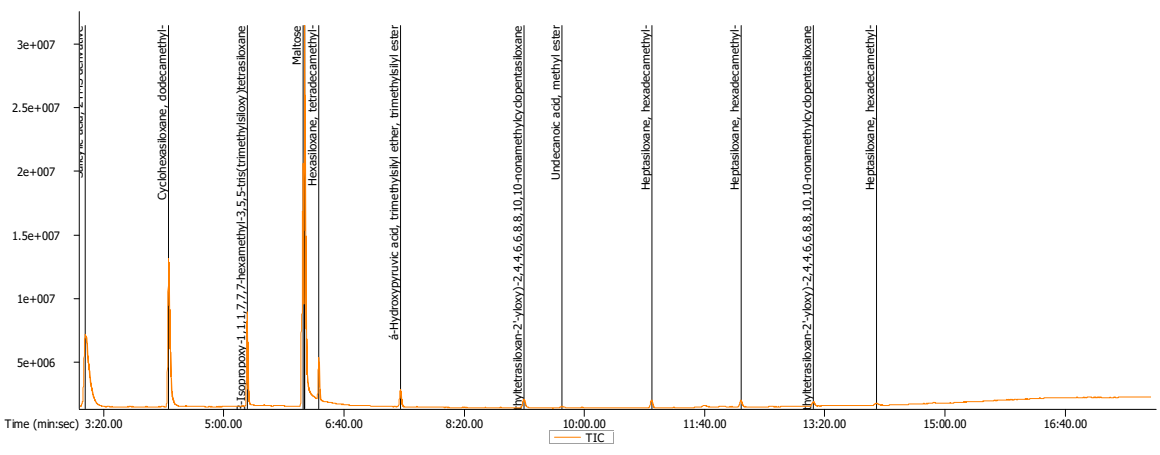
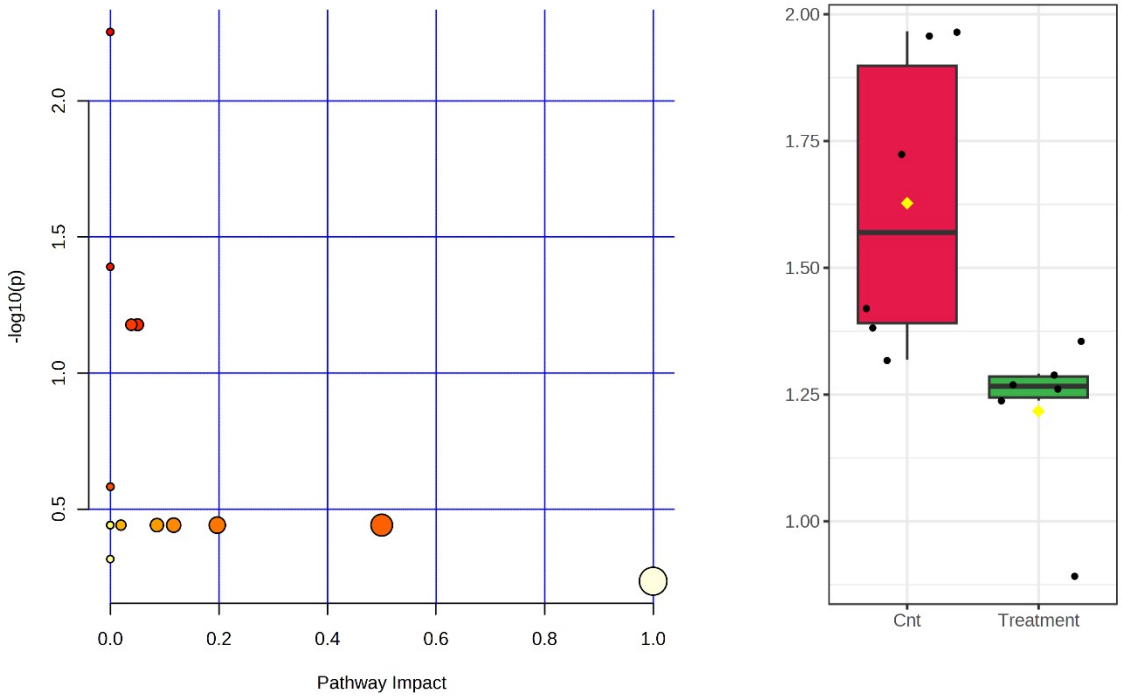
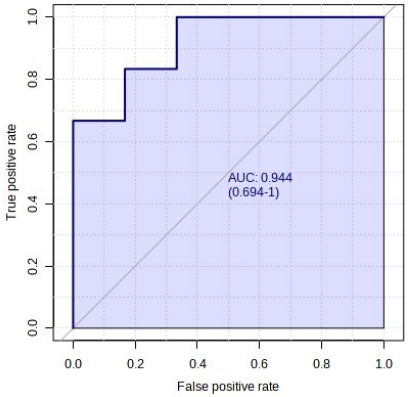
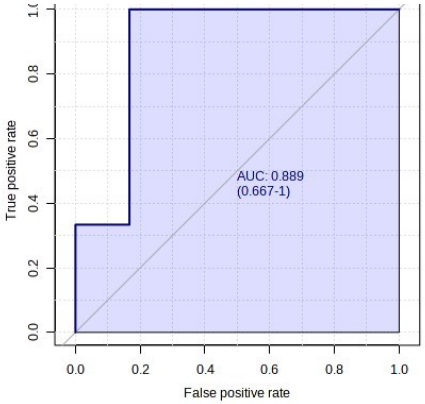


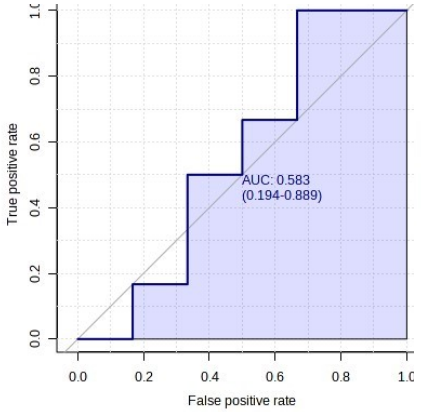
S4. Analysis of metabolic pathways for the 12 annotated metabolites.

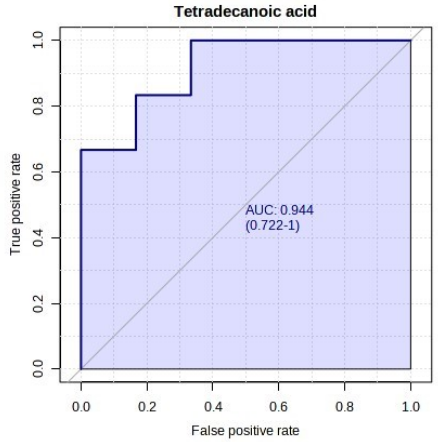


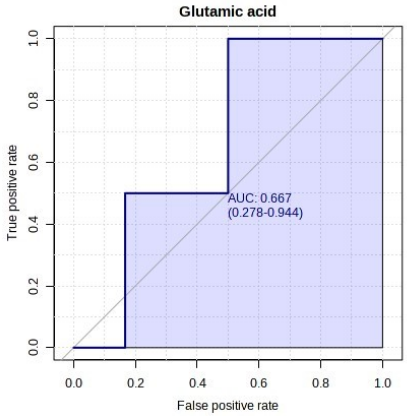
S5. Receiver operating characteristic curves.

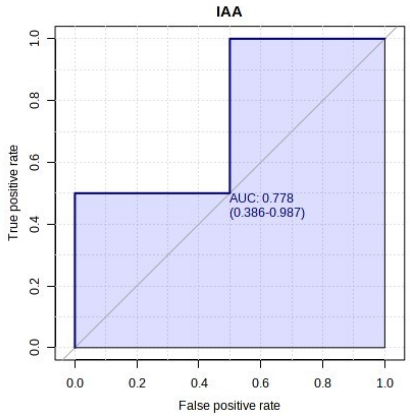


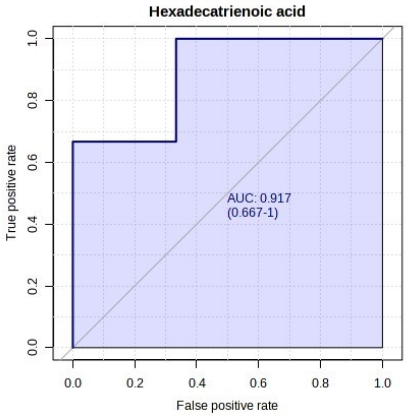


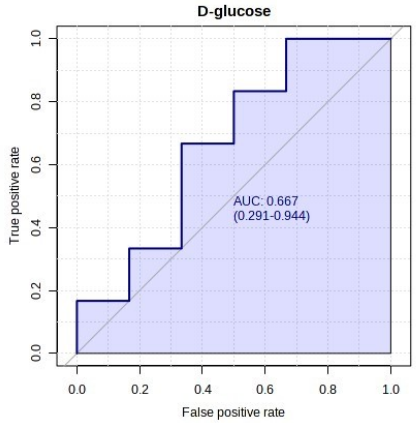


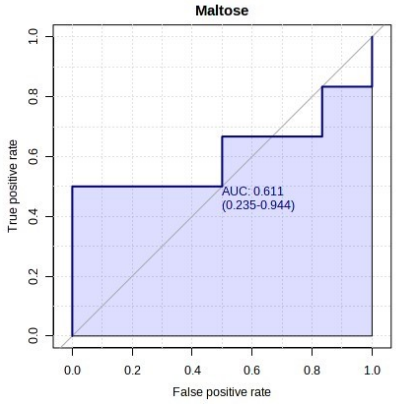


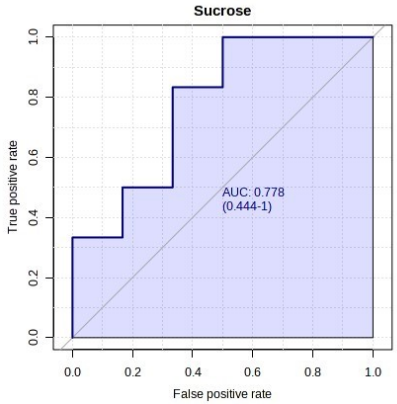












S6. Metabolic differences responsible for distinguishing *T. obliquus* exposed to nevirapine from those in the control grou

ChEBI ID	Metabolite	VIP score	*f-value*	*p*-value	FDR
CHEBI:28822	Eicosanoic acid	2.065475	17.185	7.5702E-4	0.002271
CHEBI:22340	Hexadecatrienoic acid	1.2806	17.855	6.6419E-4	0.002271
CHEBI:77524	Hexadecadienoic acid	1.19305	8.149	0.0081	0.013617
CHEBI:28875	Tetradecanoic acid	1.005595	5.2821	0.026	0.035527
CHEBI:16196	Oleic acid	1.22682875	8.0691	0.0083	0.013617
CHEBI:17306	Maltose	1.10190625	27.119	1.5233E-4	9.1399E-4
CHEBI:16411	Indole acetic acid	1.14572	61.15	7.3764E-6	8.8516E-5
CHEBI:17992	Sucrose	1.44262875	7.8506	0.009	0.013617
CHEBI:22599	DL-Arabinose	1.42318875	4.8522	0.032	0.039514
CHEBI:17351	Linoleic Acid	1.403075	11.489	0.0028	0.0068533
CHEBI:18237	Glutamic acid	1.35306625	15.3	0.001	0.002
CHEBI:17634	D-glucose	0.1618825	9.065	0.004	0.0062

ChEBI ID- Entities of Biological Interest; VIP- Variable Importance in Projection; f-value -ratio of two variances; probability value; FDR- false discovery rate.

S7. List of nevirapine degradation products and the biotransformation rule.

Compound	Chemical name	Rule	Transformation
1	11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e] [1,4] diazepin-6-one	-	-
2	2-((3-amino-4-methylpyridin-2-yl) (cyclopropyl)amino) nicotinate	bt0067	secondary Amide -> Carboxylate + primary Amine Lactam -> Aminecarboxylate
3	2-((3-amino-5,6-dihydroxy-4-methylpyridin-2-yl) (cyclopropyl)amino) nicotinate	bt0005	*vic*-unsubstituted Aromatic -> *vic*-Dihydroxyaromatic
4	(E)-2-(N',3-dicarboxylato-N-cyclopropyl-2-iminobutanimidamido) nicotinate	bt0254	*vic*-unsubstituted Aromatic -> *vic*-Dihydroxyaromatic
5	4-(cyclopropyl(methyl)amino)-3-imino-2-methyl-4-oxobutanoate	bt0350	Formamidine -> Amide + Methyl or Amine derivative
6	4-(carboxylatoamino)-3-imino-2-methyl-4-oxobutanoate	bt0350	Formamidine -> Amide + Methyl or Amine derivative
7	2-(cyclopropylamino)nicotinate	bt0350	Formamidine -> Amide + Methyl or Amine derivative
8	4-(cyclopropyl(methyl)amino)-2-methyl-3,4-dioxobutanoate	bt0391	primary Imine -> Aldehyde cyclic Imine -> Ketone + Amine
9	methanimine, (R)-2-(5-carboxylato-N-cyclopropyl-4-hydroxy-4-methylpentanamido) nicotinate	bt0241	tertiary Aliphatic -> tertiary Alcohol
10	2-(3-carboxylato-2-iminobutanamido) nicotinate	bt0243	N-substituted Amide -> Amide + Aldehyde or Ketone N, N-disubstituted Amide -> N-substituted Amide + Aldehyde or Ketone N-substituted Urea derivative -> Urea derivative + Aldehyde or Ketone N, N-disubstituted Urea derivative -> N-substituted Urea derivative + Aldehyde or Ketone
11	cyclopropanone	bt0243	N-substituted Amide -> Amide + Aldehyde or Ketone N, N-disubstituted Amide -> N-substituted Amide + Aldehyde or Ketone N-substituted Urea derivative -> Urea derivative + Aldehyde or Ketone N, N-disubstituted Urea derivative -> N-substituted Urea derivative + Aldehyde or Ketone
12	4-amino-3-imino-2-methyl-4-oxobutanoate	bt0318	Carbamyl -> Amine + Carbonate Carbamate -> Amine
13	4-(carboxylatoamino)-2-methyl-3,4-dioxobutanoate	bt0391	primary Imine -> Aldehyde cyclic Imine -> Ketone + Amine
14	carboxylatoalaninate	bt0067	secondary Amide -> Carboxylate + primary Amine Lactam -> Aminecarboxylate
15	4-(carboxylatoamino)-2-hydroxy-3-imino-2-methyl-4-oxobutanoate	bt0241	tertiary Aliphatic -> tertiary Alcohol
16	4-(carboxylatoamino)-2-(hydroxymethyl)-3-imino-4-oxobutanoate	bt0333	aliphatic Methyl [H1] -> primary Alcohol
17	2-(cyclopropylamino)-5,6-dihydroxynicotinate	bt0005	*vic*-unsubstituted Aromatic -> *vic*-Dihydroxyaromatic

References

1. B. Gao, F. Wang, L. Huang, H. Liu, Y. Zhong and C. Zhang, Biomass, lipid accumulation kinetics, and the transcriptome of heterotrophic oleaginous microalga Tetradesmus bernardii under different carbon and nitrogen sources, *Biotechnology for Biofuels*, 2021, **14**, 4.
2. J. Wang, X. Zhang, Y. Chen, M. Sommerfeld and Q. Hu, Toxicity assessment of manufactured nanomaterials using the unicellular green alga Chlamydomonas reinhardtii, *Chemosphere*, 2008, **73**, 1121-1128.