Electronic Supplementary Material (ESI) for Green Chemistry

Electronic Supplementary Information

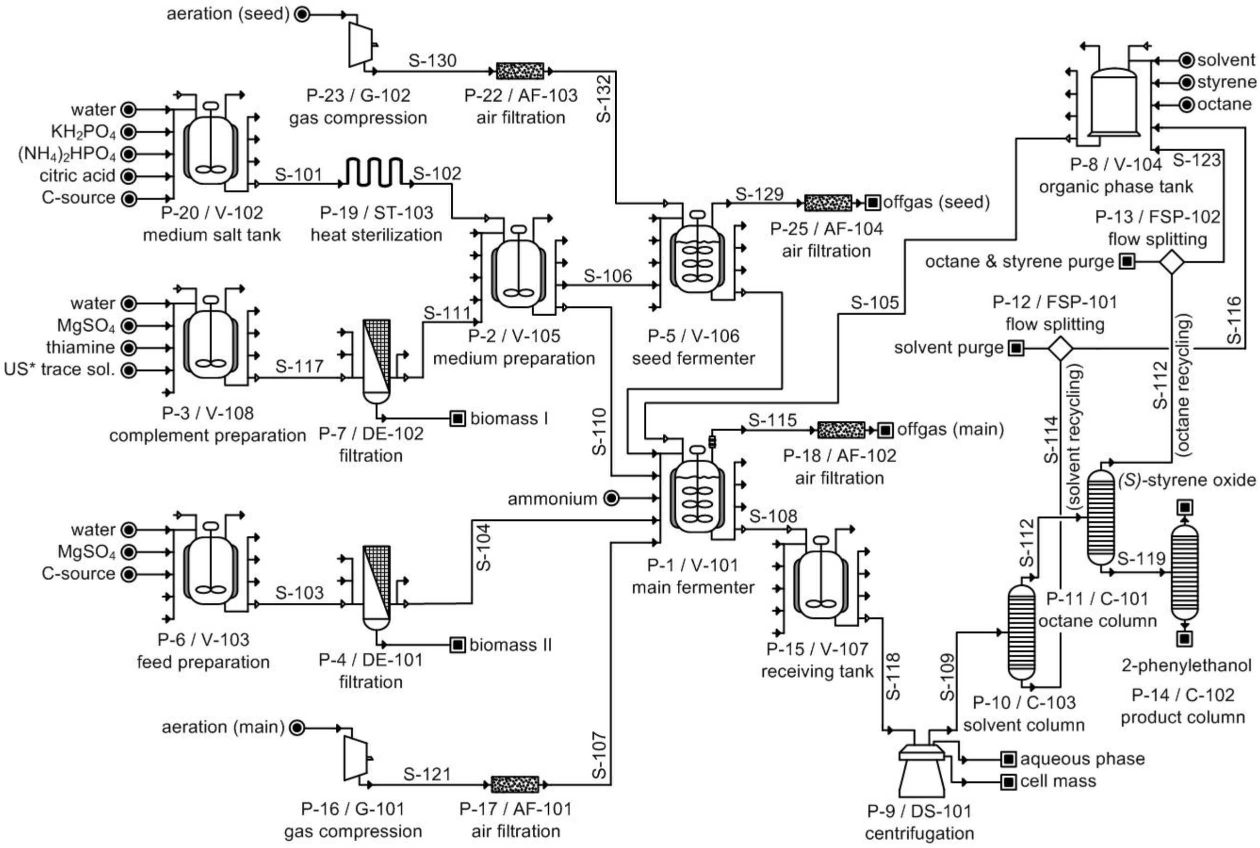

Fig. A: Flow-sheet for the biocatalytic (S)-styrene oxide production. The flow-sheet was generated in the SuperPro Designer software. For details see experimental part. Framed circles describe input mass streams and framed squares output or waste streams. C-source (glucose or glycerol), solvent (BEHP or EO).

Table A: Detailed mass-balances of resource consumption and waste generation

	BEHP glucose		BEHP glycerol		EO glucose		EO glycerol		Resting cells $10\ g_{CDW}\ L^{-1}$		Resting cells $30\ g_{CDW}\ L^{-1}$	
	Input /kg	Output /kg	Input /kg	Output /kg	Input /kg	Output /kg	Input /kg	Output /kg	Input /kg	Output /kg	Output /kg	Input /kg
2-phenylethanol	0.00	0.12	0.00	0.10	0.00	0.09	0.00	0.09	0.00	0.10	0.00	0.08
acetic acid	0.00	0.05	0.00	0.09	0.00	0.11	0.00	0.09	0.00	0.04	0.00	0.08
ammonium	0.08	0.01	0.09	0.01	0.09	0.01	0.11	0.01	0.03	0.00	0.08	0.00
biomass	0.00	0.54	0.00	0.59	0.00	0.61	0.00	0.75	0.00	0.25	0.00	0.60
carbon dioxide	0.00	1.43	0.00	1.43	0.00	2.09	0.00	2.63	0.00	1.28	0.00	2.52
carbon source [1]	1.69	0.00	1.83	0.00	2.28	0.00	2.87	0.00	1.21	0.00	2.52	0.00
carrier solvent [2]	0.88	0.88	1.06	1.06	0.82	0.82	0.89	0.89	1.64	1.64	1.28	1.28
complements [3]	0.04	0.04	0.05	0.05	0.06	0.06	0.08	0.08	0.03	0.03	0.06	0.06
octane	0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.08	0.10	0.10	0.08	0.08
oxygen	1.13	0.00	1.46	0.00	1.61	0.00	2.50	0.00	1.05	0.00	1.93	0.00
Riesenberg salts	0.27	0.27	0.32	0.32	0.35	0.35	0.38	0.38	0.49	0.49	0.38	0.38
styrene	1.00	0.03	1.07	0.12	1.11	0.17	1.11	0.17	1.10	0.15	1.05	0.11
styrene oxide	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
water	15.92	16.65	17.92	19.02	21.34	22.36	21.68	23.52	26.32	26.90	22.49	23.69
sum	**21.07**	**21.07**	**23.87**	**23.87**	**27.75**	**27.75**	**29.71**	**29.71**	**31.97**	**31.97**	**29.88**	**29.88**

[1] Carbon source was either glucose or glycerol

[2] Organic phase was either BEHP or EO

[3] Complements: magnesium sulfate, thiamine, trace element solution

Table B: Detailed environmental indices of the growing cell processes

	BEHP / glucose			BEHP / glycerol			EO / glucose			EO / glycerol		
	input	output	total	input	output	total	input	output	total	input	output	total
2-phenylethanol	0.0	0.5	0.3	0.0	0.4	0.3	0.0	0.4	0.2	0.0	0.4	0.2
acetic acid	0.0	0.9	0.5	0.0	1.6	1.0	0.0	2.1	1.2	0.0	1.7	1.0
ammonium	3.0	0.4	1.4	3.2	0.5	1.6	3.3	0.4	1.5	4.0	0.4	1.9
biomass	0.0	5.3	3.2	0.0	5.7	3.4	0.0	5.9	3.6	0.0	7.3	4.4
carbon dioxide	0.0	4.1	2.4	0.0	4.1	2.4	0.0	5.9	3.6	0.0	7.5	4.5
carbon source [1]	1.7	0.0	0.7	1.8	0.0	0.7	2.3	0.0	0.9	2.9	0.0	1.1
carrier solvent [2]	31.1	22.9	26.2	37.6	27.7	31.7	1.9	2.2	2.1	2.1	2.4	2.2
complements [3]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
octane	1.8	1.8	1.8	2.3	2.3	2.3	2.5	2.5	2.5	2.7	2.7	2.7
oxygen	2.8	0.0	1.1	3.6	0.0	1.4	4.0	0.0	1.6	6.2	0.0	2.5
Riesenberg salts	0.5	2.6	1.8	0.6	3.1	2.1	0.6	3.4	2.3	0.7	3.7	2.5
styrene	21.8	0.6	9.1	23.4	2.4	10.8	24.2	3.3	11.7	24.4	3.5	11.8
water	15.9	16.6	16.4	17.9	19.0	18.6	21.3	22.4	22.0	21.7	23.5	22.8
sum	**78.7**	**55.9**	**65.1**	**90.6**	**67.0**	**76.5**	**60.3**	**48.6**	**53.3**	**64.7**	**53.3**	**57.8**

[1] Carbon source was either glucose or glycerol

[2] Organic phase was either BEHP or EO

[3] Complements: magnesium sulfate, thiamine, trace element solution

Table C: Detailed environmental indices of the resting cell processes

	Resting cells 10 $g_{CDW}L^{-1}$			Resting cells 30 $g_{CDW}L^{-1}$		
	input	output	total	input	output	total
2-phenylethanol	0.0	0.4	0.2	0.0	0.3	0.2
acetic acid	0.0	0.7	0.4	0.0	1.4	0.8
ammonium	1.2	0.0	0.5	2.9	0.0	1.2
biomass	0.0	2.4	1.4	0.0	5.8	3.5
carbon dioxide	0.0	3.6	2.2	0.0	7.2	4.3
glucose	1.2	0.0	0.5	2.5	0.0	1.0
BEHP	58.1	42.8	48.9	45.4	33.4	38.2
complements [1]	0.1	0.1	0.1	0.1	0.1	0.1
octane	3.4	3.4	3.4	2.7	2.7	2.7
oxygen	2.6	0.0	1.0	4.7	0.0	1.9
Riesenberg salts	0.8	4.7	3.1	0.7	3.7	2.5
styrene	24.1	3.1	11.5	22.9	2.3	10.5
water	26.3	26.9	26.7	22.5	23.7	23.2
Sum	**117.9**	**88.2**	**100.1**	**104.5**	**80.6**	**90.2**

[1] Complements: magnesium sulfate, thiamine, trace element solution

Table D: Criteria for ABC Classification [a]

Impact category	Class A	Class B	Class C
Land use	≥ 100 m^2 kg^{-1}	≥ 10 m^2 kg^{-1} and < 100 m^2 kg^{-1}	< 10 m^2 kg^{-1}
Raw material availability	Non-renewable resources, predicted exhaustion within 30 years	Non-renewable resources, predicted exhaustion in 30-100 years	Renewable resources, guaranteed long term supply (> 100 years)
Complexity of the synthesis	> 10 stages	3-10 stages	< 3 stages
Thermal risk [b, c]	R1-9, 11, 12, 14-19, 30, 44 F^+, E	R 10 F, O	No or negligible thermal risks
Acute toxicity [b,c,d]	T^+, T R23-29, 31, 32, 35, 39, 42, 43, 50 CH-poison class 1,2	X_n, X_i, C R20-22, 34-38, 41, 63, 65-67 CH-poison class 3,4	CH-poison class 5
Chronic toxicity [b,c,e]	MAK < 1 mg m^{-3} R33, 40, 45-49, 60, 61, 64 T, T^+	MAK 1-10 mg m^{-3} R 53, 58, 62, 63 X_n, X_i	MAK > 10 mg m^{-3}
Biological risk [f]	Biosafety level 3, 4	Biosafety level 2	Biosafety level 1
Ecotoxicity [b,g]	R 50, WGK 3	R 51, 52, 54-57 WGK 2	WGK 1
Global warming potential (GWP) [h]	GWP > 20	GWP < 20	No global warming potential
Ozone depletion potential (ODP) [h]	ODP > 0.5	ODP < 0.5	No ozone depletion potential
Acidification potential (AP) [h]	AP > 0.5	AP < 0.5	No acidification potential
Photochemical ozone creation potential (POCP) [h]	POCP > 30 or NO_x	30 > POCP > 2	POCP < 2 or no effect known
Odor	Odor threshold < 10 mg m^{-3}	Odor threshold < 500 mg m^{-3}	Odor threshold > 500 mg m^{-3} or odourless
Eutrophication potential	N-content > 0.2 or P-content > 0.05	N-content < 0.2 or P-content < 0.05 or C-content > 0.2	Nitrogen and phosphate-free compounds

[a] Criteria are based on [44]

[b] Standard Risk phrases (R phrases) as defined by and commonly used in the countries of European Union. Definition of R-phrases as published by the EU directive on dangerous substances.

[c] Official abbreviations for the classification of chemicals according to EU directive on dangerous substances: F^+= extremely flammable; E = explosive; F = flammable; O = oxidizing; T^+= very toxic; T = toxic; X_n = harmful; X_i = irritant; C = corrosive.

[d] Swiss poison class (CH-poison class) rating appears on European Material Safety Data Sheets (MSDS). The rating reflects the acute oral lethal dosis (usually in rats): Class 1: 0-5 mg kg^{-1}; Class 2: 5-50 mg kg^{-1}; Class 3: 50-500 mg kg^{-1}; Class 4: 500-2000 mg kg^{-1}; Class 5: >2000 mg kg^{-1}.

[e] MAK (maximale Arbeitsplatzkonzentration) = German workplace threshold value

[f] Biosafety level defined by Centers for Disease Control and Prevention (United States) was adapted in the EU. Biosafety level 1: not causing any disease; Biosafety level 2: moderate potential hazard to personnel and environment; Biosafety level 3: serious or potentially lethal disease as a result of exposure by inhalation; Biosafety level 4: aerosol-transmitted severe to fatal disease (no vaccines or treatment available).

[g] WGK (Wassergefährdungsklasse) = German water hazard classification. WGK 1: low hazard to waters; WGK 2: hazard to waters; WGK: severe hazard to waters.

[h] Global warming potential, ozone depletion potential, acidification potential, and Photochemical ozone creation potential are summarized in the database of the University of Leiden, The Netherlands. Scientific background information and guidelines is supplied by H. Wenzel and M. Hauschild, *Environmental assessment of products: Volume 1: Methodology, tools and case studies in product development*, Kluwer Academic Publisher, Dordrecht, NL, 1997 and by J. B. Guinée, *Handbook on life cycle assessment: Operational guide to the ISO standards*, Kluwer Academic Publisher, Dordrecht, NL, 2001.