Supplementary information

Biocatalytic microgels (µ-Gel*zymes*): synthesis, concepts, and emerging applications

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Enzyme class	Enzyme	Section	Immobilisation technique	Microgel	Enzyme loading [mg/g microgel/ copolymer]	Enzyme loading efficiency [%]	Residual activity after loading* [%]	Performance of µ-Gel*zyme*	Ref.
Hydrolase	Cellubiase	1.1	Covalent immobilization (pre-synthesised microgels)	PAAm-AA-GMA	35.1	80	78	Reusability: 71% after 8 reaction cycles	1
	Cellulase	1.1	Covalent immobilization (pre-synthesised microgels)	PAAm-AA-GMA	37.9	100	90	Reusability: 72% after 10 reaction cycles	1
	Cellulase	2.2	Encapsulation during microgel synthesis (crosslinking of reactive polymer chains)	PVPS	-	-	24	-	2
	Cellulase	2.2	Encapsulation during microgel synthesis (crosslinking of reactive polymer chains)	PVPS	41.9	87	~10-40	Organic solvent stability: 70% against acetone (free enzyme 37.5%) Chaotropic agent stability: 51.5% against urea (free enzyme 37.9%)	3
	Cellulase	2.2	Encapsulation during microgel synthesis (crosslinking of reactive polymer chains)	PVGMA	-	-	39	Organic solvent stability: 87% against acetone (free enzyme 37%) Chaotropic agent stability: 81% against urea (free enzyme 38%)	4
	Cellulase	3.1	Encapsulation during microgel synthesis (crosslinking of reactive polymer chains)	PVP-PDS	15.5	77	4.5-8.8	Stimuli-responsive enzyme release: 55% activity of free enzyme reconstituted after release	5
	Galactosidase (β-galactosidase)	3.2	Encapsulation during microgel synthesis (APS/TEMED initiation)	PNIPAAm-AAm	-	-	-	Regulation of enzyme activity by temperature-induced collapse and swelling of microgels in packed bed reactor. Increased mass transfer rate due to collapse/swelling cycles "pumping process"	6
	Galactosidase (β-galactosidase)	3.2	Encapsulation during microgel synthesis (APS/TEMED initiation)	PNIPAAm-AAm	-	-	-	Regulation of enzyme activity by temperature-induced collapse and swelling of microgels in packed bed reactor. Increased mass transfer rate due to collapse/swelling cycles "pumping process"	7
	Galactosidase (β-galactosidase)	3.2	Encapsulation during microgel synthesis (APS/TEMED initiation)	PNIPAAm	-	-	-	Regulation of enzyme activity by pressure-induced collapse and swelling of microgels in packed bed reactor. Increased mass transfer rate due to collapse/swelling cycles "pumping process"	8

Hydrolase	Galactosidase (β-galactosidase)	3.1	Encapsulation during microgel synthesis (crosslinking of reactive polymer chains, enzymatic auto-oxidation of thiol groups)	Poly (glycidol) thiol-functionalised	21-41	40-44	83-85	Co-immobilisation with HRP	9
	Glucoamylase	3.1	Hydrophobic adsorption in the collapsed state of microgels followed by crosslinking with glutaraldehyde after adsorption	PNIPAAm-HEMA (aminated)	14.2	29	4.6	Storage stability: up to 100% activity after 30 days	10
	Glucosidase (β-D-glucosidase)	3.1	Adsorption (Hydrogenbond formation)	PNIPAAm with polystyrene core	620	-	325	-	11
	Lipase (CalB)	2.2	Encapsulation during microgel synthesis (crosslinking of reactive polymer chains)	Poly(glycidol)	0.4 mg/mL	75	25	Esterification activity: 87 % (free enzyme 13%) Molecular weight of polymer: 4900 M_n/Da (free enzyme 1140 M_n/Da)	12
	Lipase (CalB)	3.1	Immobilisation by solvent exchange from polar to unpolar media	PNIPAAm	3	2.5	-	Esterification acitivity: ~0.03 U in hexane (free enzyme~0.001 U); ~30-fold improvement	13
	Lipase (LCR)	1.1	Covalent immobilization (pre-synthesised microgels)	PAAm functionalized with amine groups	1.13 mg/m^{-2} of PVDF membrane	-	3.2	-	14
	Lysozyme	3.1	Adsorption (electrostatic interaction)	PNIPAAm-AA with polystyrene core	675	-	350	-	15
	Trypsin	1.1	Covalent immobilization (pre-synthesised microgels)	PNIPAAm-AA	493	-	155	Thermal stability: 70% after 3 h at 45 °C (free enzyme ~40%) Storage stability: 80% after 60 days (55% free enzyme)	16
	Trypsin	3.1	Hydrophobic adsorption in the collapsed state of microgels followed by crosslinking with glutaraldehyde after adsorption	PNIPAAm-HEMA (aminated)	0.3	44.2	18.6	Storage stability: up to 50% activity after 30 days	10
	Urease	4	Encapsulation during microgel synthesis (APS initiation)	PNIPAAm-VIm	16	-	-	Control of membrane permeability by urease induced microgel swelling collapse.	17

Hydrolase	α-Chymotrypsin	2.1	Encapsulation during microgel synthesis (APS/TEMED initiation)	PNIPAAm with Calcium-alginate as the polymerisation mould	558-2991	75-80	~10	Reusability: 75% after 30 reaction cycles (1 month)	18
Ligase	Acetly-CoA snythetase	1.2	Covalent immobilization (pre-synthesised microgels)	PNIPAAm-AEMA	20	68	61	Storage stability: 80% after 9 days (63 % free enzyme) Thermal stability: 75% after 15 min at 60 °C (60 % free enzyme) Reusability: 50% after 4 cycles	19
	Acetly-CoA snythetase	4	Adsorption (electrostatic interaction)	PNIPAAm-PEI	278	97	120	Reusability: 70 % after 7 cycles	20
Lyase	DERA	2.1	Encapsulation during microgel synthesis (enzyme also as crosslinker; covalent bonds to microgel network)	PNIPAAm-TlaAm	-	44	Strongly impaired	Storage stability: 100 % activity after lyophilisation	21
Oxidoreductase	Alcohol dehydrogenase	4	Encapsulation during microgel synthesis	PNIPAA-MAA PNIPAAm-PNIPMAAm	-	-	-	Stabilisation and heat-induced breaking of emulsions for catalysis	22
	Glucose oxidase	2.1	Encapsulation during microgel synthesis (APS/TEMED initiation)	PAAm-AA	-	-	-	Storage stability: 100% after 8 months	23
	Glucose oxidase	2.1	Encapsulation during microgel synthesis (enzyme initiated polymerisation)	PVCL	-	-	40	-	24
	Glucose oxidase	2.1	Encapsulation during microgel synthesis (enzyme initiated polymerisation, enzyme also as crosslinker; covalent bonds to microgel network)	PNIPAAm-TlaAm	-	31	4	-	21
	HRP	1.2	Covalent immobilization (pre-synthesised microgels)	PHEAA	22	-	87-96	Thermal stability: 76 % activity after 5.5 h at 50° C (free enzyme 20%)	25
	HRP	1.2	Covalent immobilization (pre-synthesised microgels)	PEGMA	44	-	33	Thermal stability 50 °C: 75% after 5 h (free enzyme 40%)	26
	HRP	2.1	Encapsulation during microgel synthesis (enzyme initiated polymerisation)	PDMAA	-	-	102	Storage stability: 98 % activity after 3 months Thermal stability: 33% after 30 min at 70°C (14% free enzyme)	27

Oxidoreductase	HRP	2.2	Encapsulation during microgel synthesis (crosslinking of reactive polymer chains)	dPG	1.6	40	-	Thermal stability: 70% after 42 h at 50°C (free enzyme 10%) Reuseability: 50% after 6 cycles	28
	HRP	3.1	Non-covalent immobilisation Immobilisation by solvent exchange from polar to unpolar media	PNIPAAm	12	-	-	Organic solvent stability: 0.016 μmol min^{-1} μg^{-1} in isopropanol(free enzyme $6.15*10^{-5}$ μmol min^{-1} μg^{-1}) ; ~260-fold improvement	29
	HRP	3.1	Encapsulation during microgel synthesis (crosslinking of reactive polymer chains, enzymatic auto-oxidation of thiol groups)	Poly(glycidol) thiol-functionalised	-	-	90	-	9
	Laccase (CueO)	1.2	Covalent immobilization (pre-synthesised microgels)	PVCL-GMA	64.2	-	126	Organic solvent stability: up to 3.8-fold improved activity in DMSO compared with free enzyme Decolorisation of indigo carmine : 79% decolorisation efficiency after 8 cycles Reusability: 55% after 11 cycles	30
	Laccase (Trametes vesicolor)	2.1	Encapsulation during microgel synthesis (APS/TEMED initiation)	PNIPAAm-VIm	-	35	-	-	31
	Laccase (Trametes vesicolor)	2.1	Encapsulation during microgel synthesis (APS/TEMED initiation)	PNIPAAm-AAm	-	96.4	100	Storage stability: 91% activity after 56 days (free enzyme 42%) Reusability: 78 % after 10 cycles	32
	Lactic acid dehydrogenase	1.1	Covalent immobilization (pre-synthesised microgels)	PNIPAAm-PEI	-	37	78	Thermal stability	33
	Cytochrome P450 BM3	1.2	Covalent immobilization (pre-synthesised microgels)	PVCL-GMA	89.6	-	22	Organic solvent stability: up to 4-fold improved activity in DMSO compared with free enzyme	30
Transferase	Pyruvate kinase	1.1	Covalent immobilisation (pre-synthesised microgels)	PNIPAm-PEI	-	20	223	Thermal stability Storage stability: ~70% after 14 days (free enzyme ~40 %)	33

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