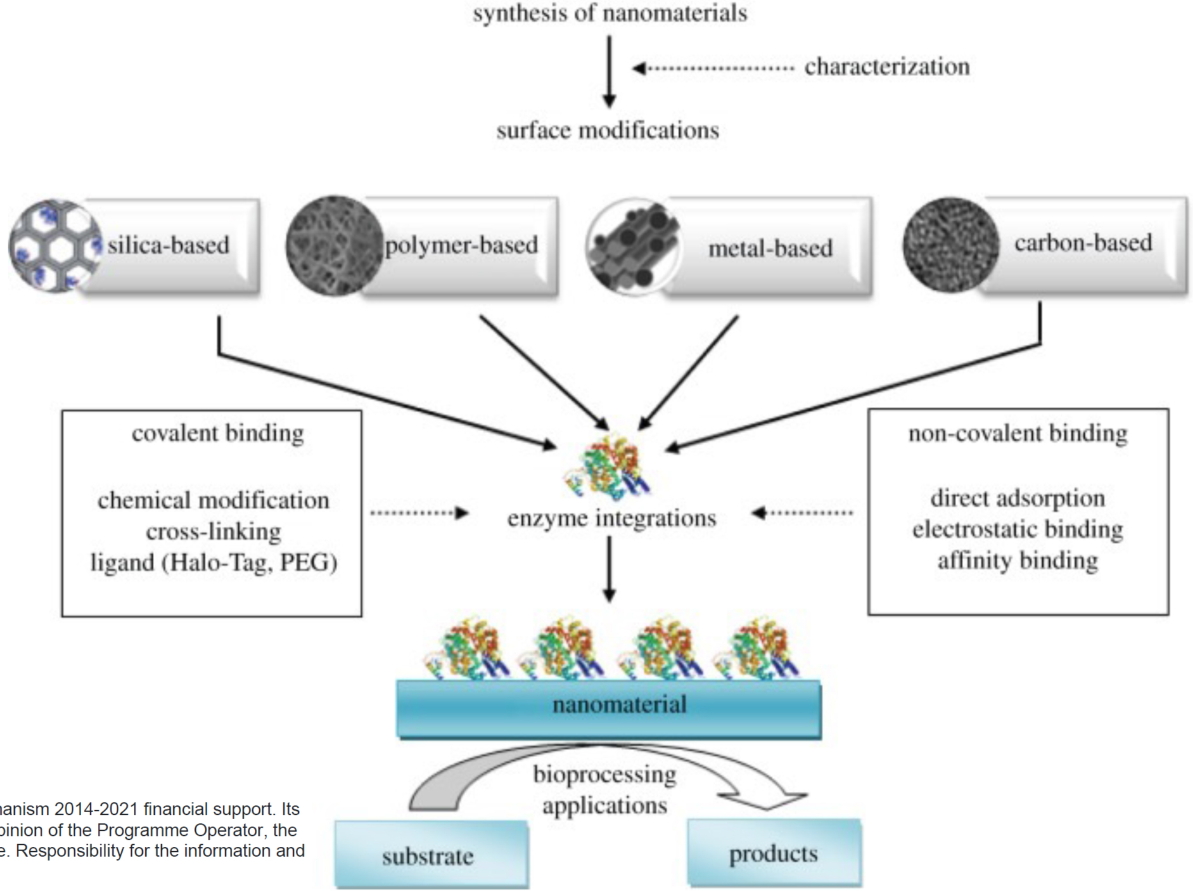
I. Applications from bio-nanocatalysis



synthesis of nanomaterials

characterization

surface modifications

silica-based

polymer-based

metal-based

carbon-based

covalent binding

chemical modification
cross-linking
ligand (Halo-Tag, PEG)

enzyme integrations

non-covalent binding

direct adsorption
electrostatic binding
affinity binding

nanomaterial

bioprocessing applications

substrate

products

Misson *et al*, 2015

Agenda

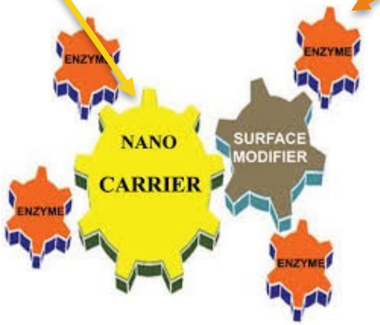
- Definitions
- Benefits of coupling enzymes to nanostructures:

(is it still a nanostructure and is it green chemistry??)

1. Increasing effectiveness?
2. Increasing stability?
3. Sustainability (re-useable)?

Fossil based?

Bio based?



- Cascade reactions with biocatalysis and nanocat.
- Production of biofuels by Biocat/Nanotech
- Production of agrochemicals by Biocat/Nanotech

Definition of biocatalysis

Biocatalysis is “the use of natural substances to speed up (catalyze) chemical reactions”.

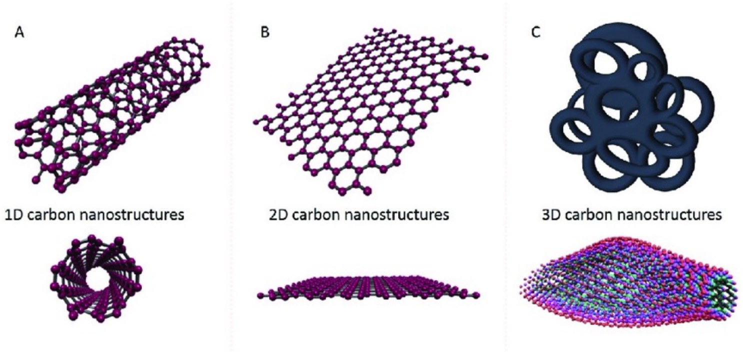
In most cases, a group of proteins called enzymes will be carrying out the catalysis, but a combination of enzymes as well as cells can be used.

These enzymes can be taken from the cell, either from the original cell or from a different cell that was modified to produce the enzyme.

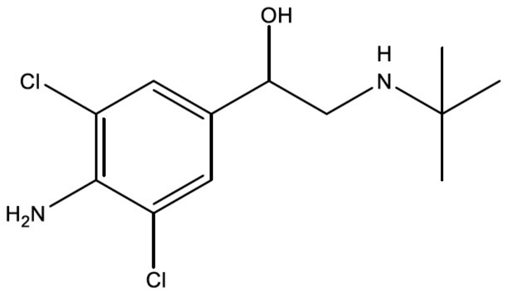
Definition of nanostructure

A nanostructure is a structure of intermediate size between microscopic and molecular structures.

Nanostructural detail is microstructure at nanoscale.



Carbon nanostructures-large polymers!



Clenbuterol- a molecule based on benzene- why not nanostructure?

In describing **nanostructures**, it is necessary to differentiate between the number of dimensions in the volume of an object which are on the nanoscale. (Wikipedia)

Definition of nanotechnology

Nanotechnology is the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications.

Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this small scale.

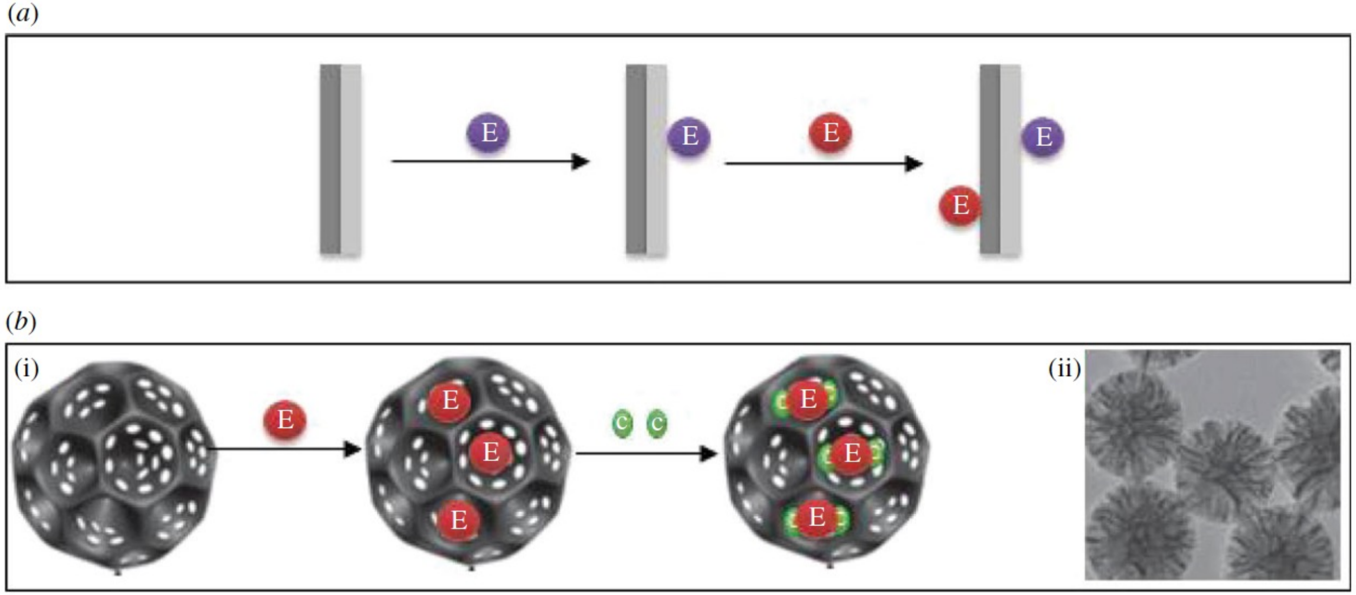
National Nanotechnology Initiative (NNI)

Nanofibers (NF´s=polymers) + enzymes

Immobilization onto solid supports may reduce enzyme activity, f. inst bovine serum albumin (BSA), penicillin acylase and β-galactosidase. This belief may not be true when enzymes are immobilized onto nanocarriers.

NF´s offer a high surface-to-volume ratio to show a high adsorption capacity of enzyme loading:

enzymes penetrate the polymer and attach into inner region of polymer – giving optimal substrate diffusion



(a) Side-by-side hybrid nanofibers promote immobilization of two enzymes to perform simultaneous reactions.
(b) Schematic illustration of dendrimer-like nanoporous silica for the co-immobilization of enzyme with cofactors or other biomolecules (i), TEM image of dendrimer-like nanopores silica (ii)

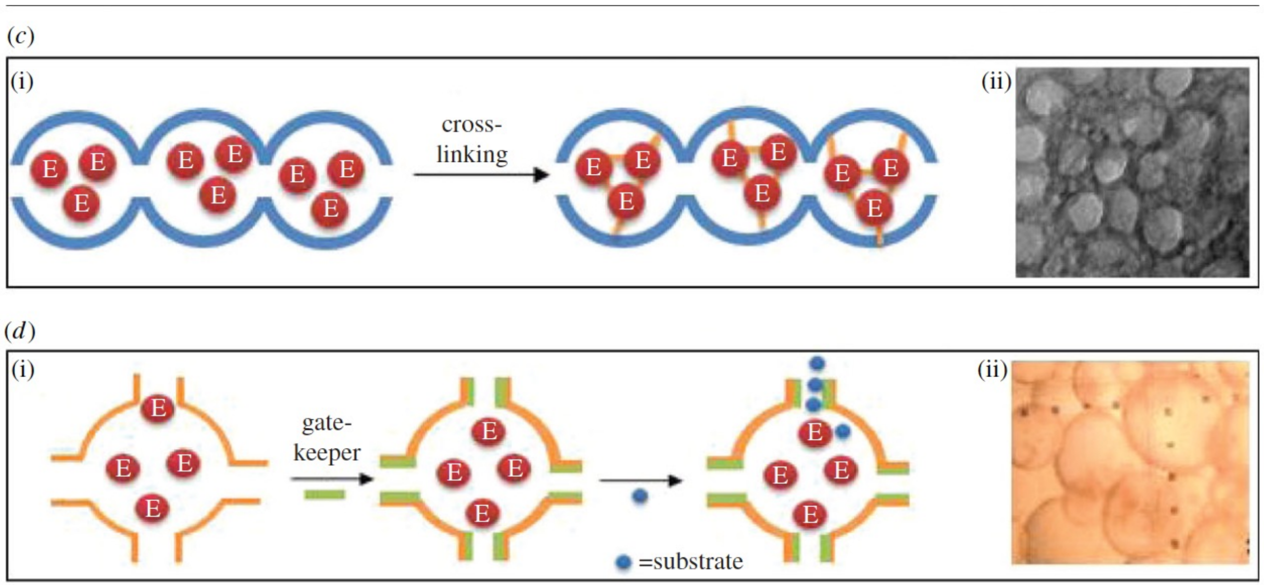
Du *et al* 2013

Nanocages (mesoporous silika) + enzymes

Enzymes can be attached to
- fibre surface or
- enclosed inside the pores.

However, enzyme leaching can be an issue if only the enzymes are attached by physical adsorption. Enzyme reactivity could be affected due to conformational change or is reduced by exposing to cross-linking reagents.

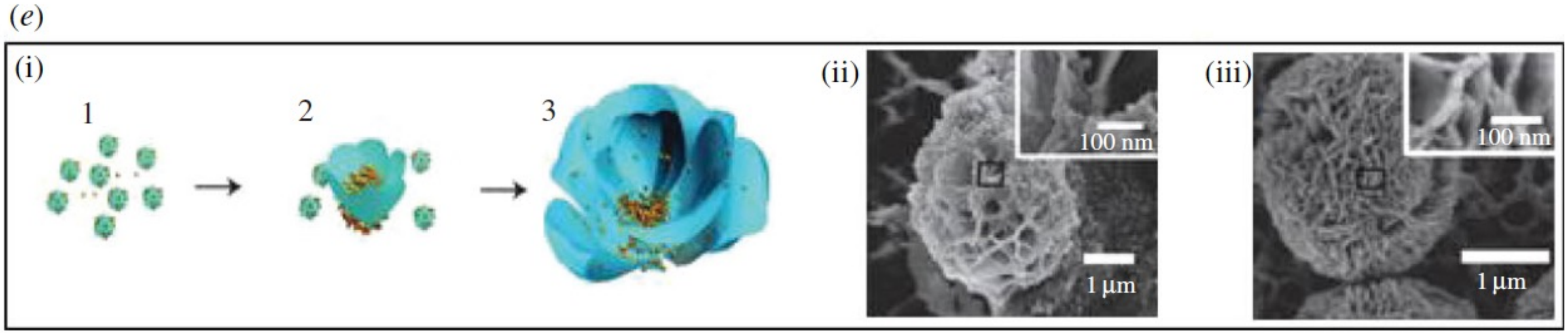
To encapsulate the enzyme molecules inside a nanoscale container is a promising approach to maintain enzyme activity
- substrates can freely diffuse into and out of the container



(c) Schematic illustration (i) and TEM image (ii) of ship-in-a-bottle pore structures to retain and stabilize enzymes inside the nanocages. (cross linking w glutaraldehyde) Pore size only large enough for diffusion of small molecules, not enzymes.
(d) Schematic illustration (i) and optical micrograph (ii) of nanocages with substrate-diffusion gatekeepers to prevent enzyme leaching.

Lee *et al.* 2005 , Liu *et al.* 2013

Nanoflowers + enzymes



(e) Schematic diagram (i) and SEM images of the formation of Bovine Serum Albumin (BSA)-incorporated $Cu_3(PO_4)_2.3H_2O$ nanoflowers (spheres in nanoflowers' core as protein molecules) at 12 h (ii) and 3 days (iii)

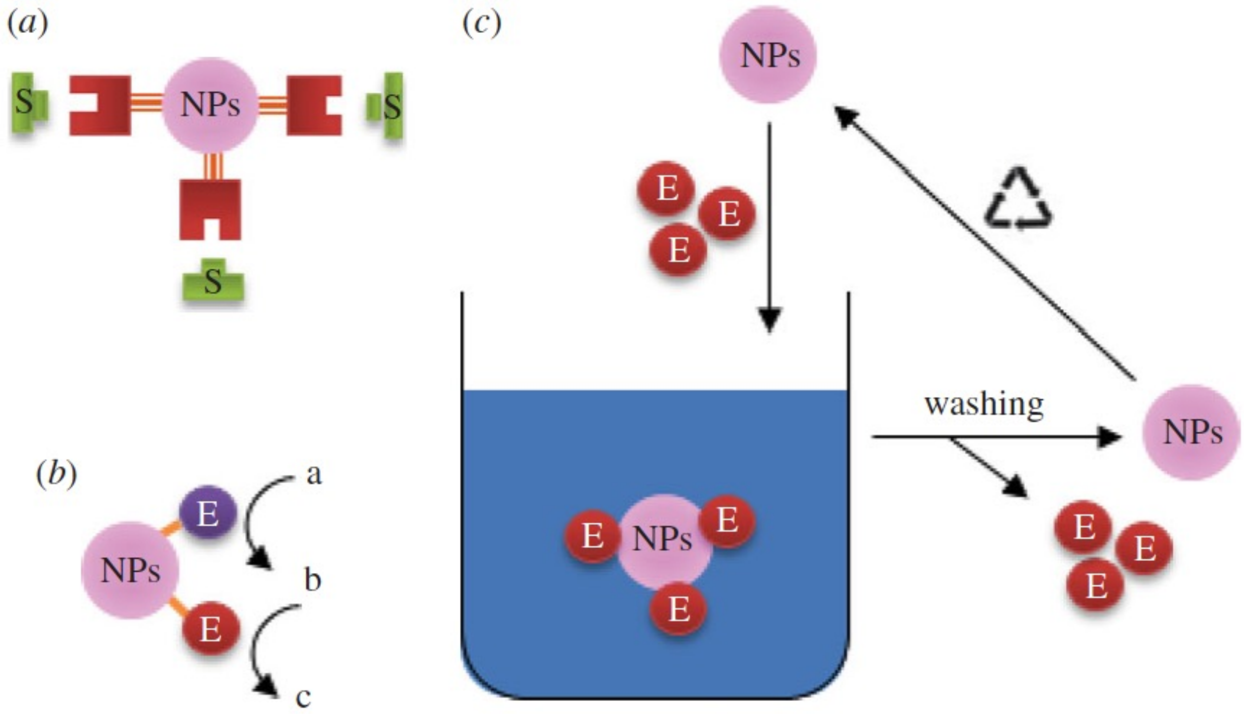
The hybrid nanoflower exhibit enhanced enzymatic activity and stability compared with free enzymes, which may be attributed to the confinement of the enzyme in the core of the nanoflower.

Enzymes : α-lactalbumin, laccase, carbonic anhydrase and lipase Ge *et al*, 2012

Engineering performance of NanoBioCatalysts in bioprocess applications.

(a) Enhancing enzyme activity by stabilizing the enzyme reactive sites towards the substrate.

(b) Accelerating biocatalysis through cascade reactions of the co-immobilized enzymes in one-pot medium.



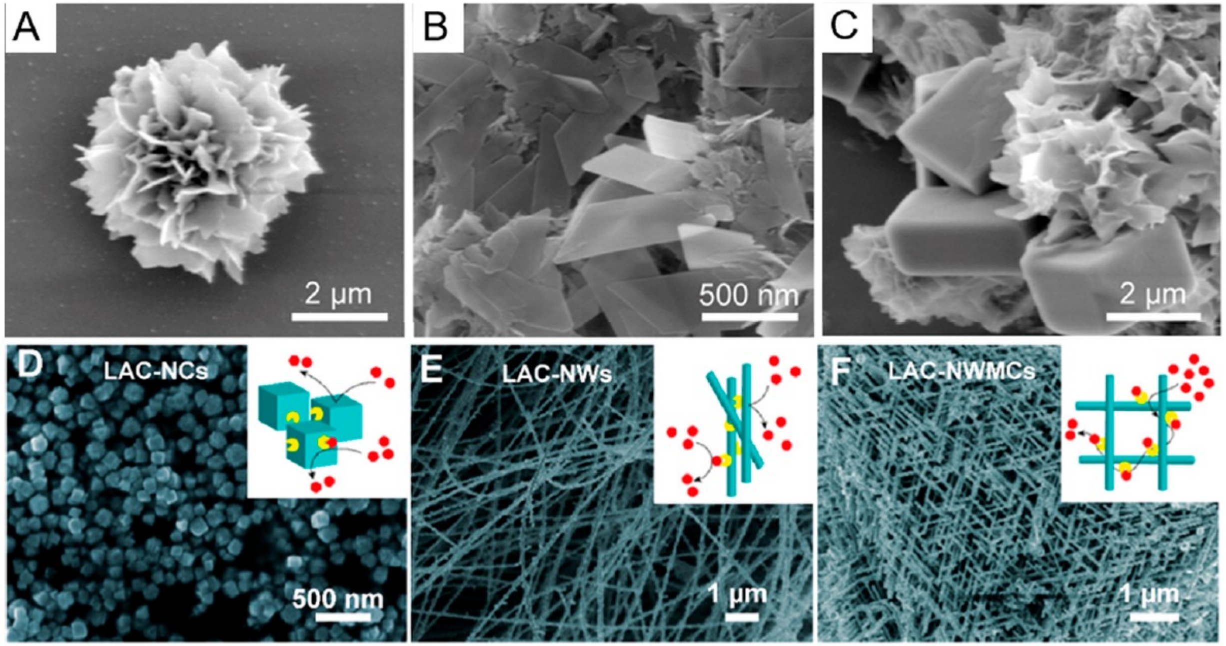
(c) Recycling the nanocarriers after the enzyme activity decays.

f. Inst Integration of magnetic technology with the enzyme immobilization on the nanocarriers can enhance recoverability and reusability of the NBC´s

Misson *et al*, 2015

Coupling of the α-amylase and laccase to nanostructures

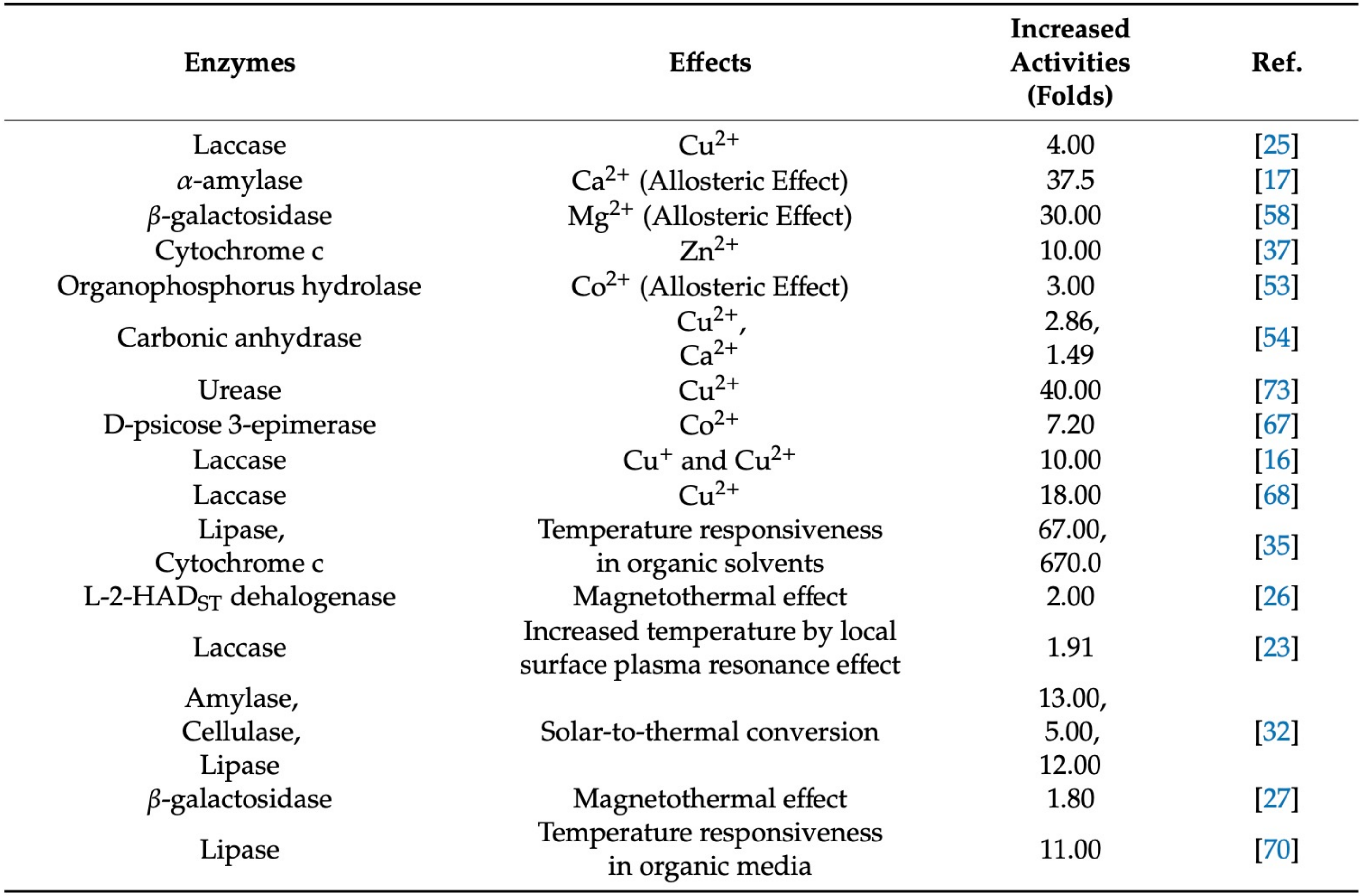
SEM images of $CaHPO_4$-α-amylase nanobiocatalysts, (A) nanoflowers, (B) nanoplates, and (C) parallel hexahedrons. (Wang *et al*, 2013, (ref [17] in An *et al* 2010))



SEM images of Cu_2O–laccase nanobiocatalysts, (D) nanocubes, (E) nanowires, and (F) nanowire mesocrystal, insets are the schematic illustrations of the plausible substrate diffusion pathways for these hybrid materials. (Li *et al*, 2018, ref [16] in An *et al* 2010)

An *et al* 2018 (review)

Effects of metal ion and temperature on enhanced activities of immobilized enzymes



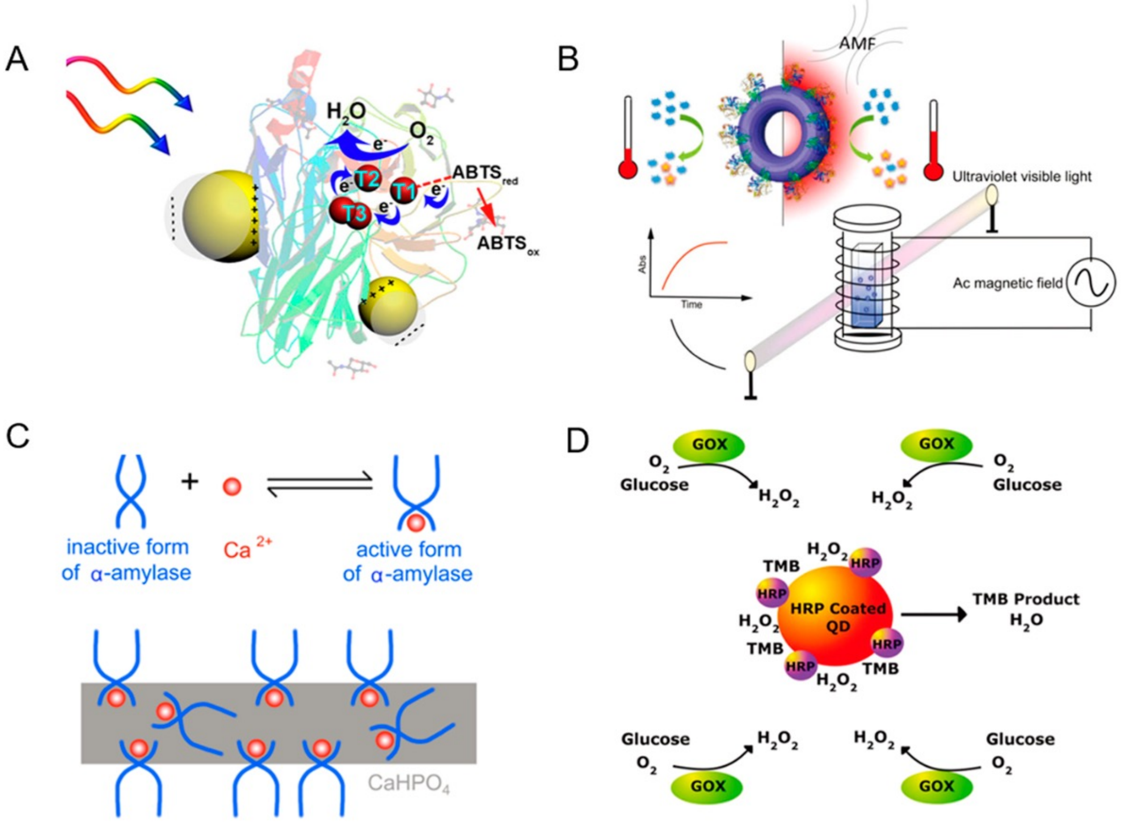
Enzymes	Effects	Increased Activities (Folds)	Ref.
Laccase	Cu^{2+}	4.00	[25]
α-amylase	Ca^{2+} (Allosteric Effect)	37.5	[17]
β-galactosidase	Mg^{2+} (Allosteric Effect)	30.00	[58]
Cytochrome c	Zn^{2+}	10.00	[37]
Organophosphorus hydrolase	Co^{2+} (Allosteric Effect)	3.00	[53]
Carbonic anhydrase	Cu^{2+}, Ca^{2+}	2.86, 1.49	[54]
Urease	Cu^{2+}	40.00	[73]
D-psicose 3-epimerase	Co^{2+}	7.20	[67]
Laccase	Cu^{+} and Cu^{2+}	10.00	[16]
Laccase	Cu^{2+}	18.00	[68]
Lipase, Cytochrome c	Temperature responsiveness in organic solvents	67.00, 670.0	[35]
L-2-HAD$_{ST}$ dehalogenase	Magnetothermal effect	2.00	[26]
Laccase	Increased temperature by local surface plasma resonance effect	1.91	[23]
Amylase, Cellulase, Lipase	Solar-to-thermal conversion	13.00, 5.00, 12.00	[32]
β-galactosidase	Magnetothermal effect	1.80	[27]
Lipase	Temperature responsiveness in organic media	11.00	[70]

An *et al* 2018 (review)

Experimental set ups

A. Au–laccase hybrids with enhanced electron transfer

B. Experimental set-up β-Gal hybrids



C. The α-amylase-$CaHPO_4$ nanoflower nanobiocatalyst. Ca^+ binds to allosteric sites in inactive α-amylase and generates active α-amylase

D. Diagram of the GOX/HRP–CdSe/ZnS QDs system with enhanced coupled enzymatic activity.

An *et al* 2018 (review)

Magnetic nanoparticles

Magnetic nanoparticles (MNP) suitable as supports to enzymes due to:

- low toxicity
- flexible surface modification by chemical reactions
- large enzyme capacity
- good reusability [30-in de Jesús Rostro-Alanis].

Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) widely used:

- low toxicity
- stability
- availability
- low environmental impact
- small size
- super-paramagnetic properties
- ease of separation from the reaction media [31–33-in de Jesús Rostro-Alanis.].

de Jesús Rostro-Alanis *et al*, 2016

Nanostructure characteristics enable design of robust biocatalysts

Main advantages of nanomaterials for immobilization of enzymes:

1. possibility of fine tuning the biological activity by designing specific materials

2. high surface area - allows for a high loading of enzyme.

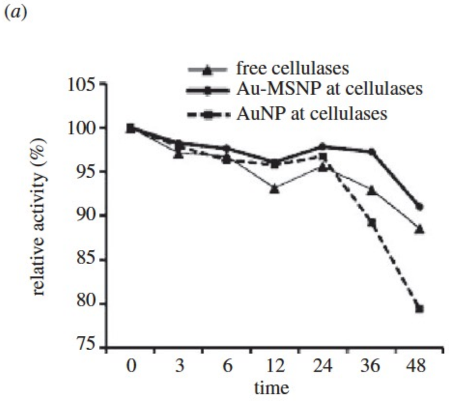
Desirable characteristics of Nanostructured materials for support for enzymes:

1. pore diameter on the scale of nanometers (5–100 nm)
2. hardness
3. defined geometry
4. hydrophobicity/hydrophilicity ratio
5. conductivity
6. magnetic properties

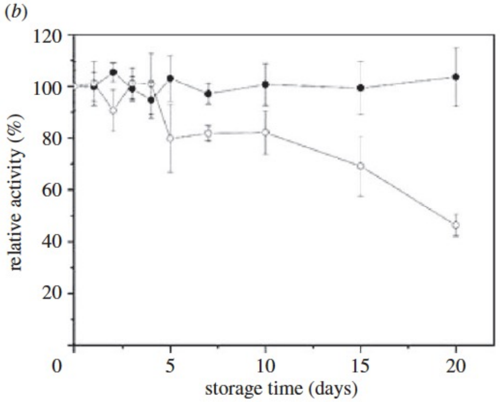
de Jesús Rostro-Alanis *et al*, 2016

Applications of NBC´s in bioprocesses.

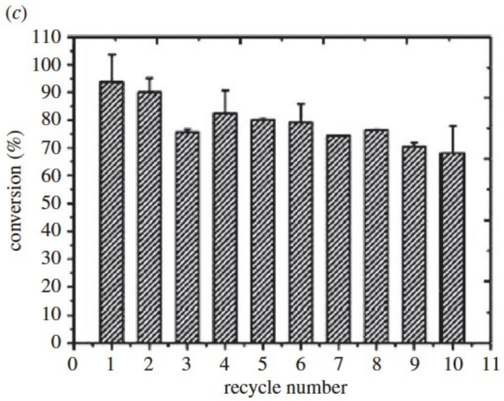
(a) Extension of enzyme activity from 24 to 36 h by immobilized cellulases on Au-magnetic silica NP´s [136] in Misson 2015.



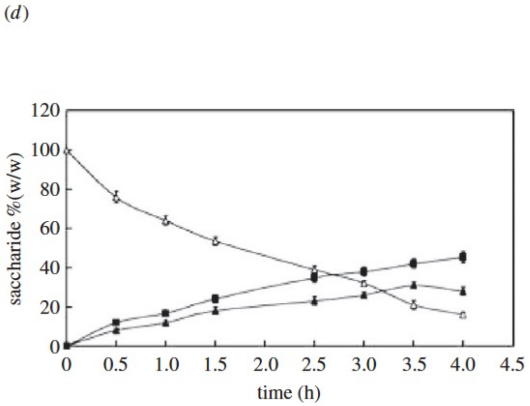
(b) Storage stability of free lipase (unfilled circles) and immobilized lipase (filled circles) on polyacrylonitrile nanofibrous membrane [145] in Misson 2015.



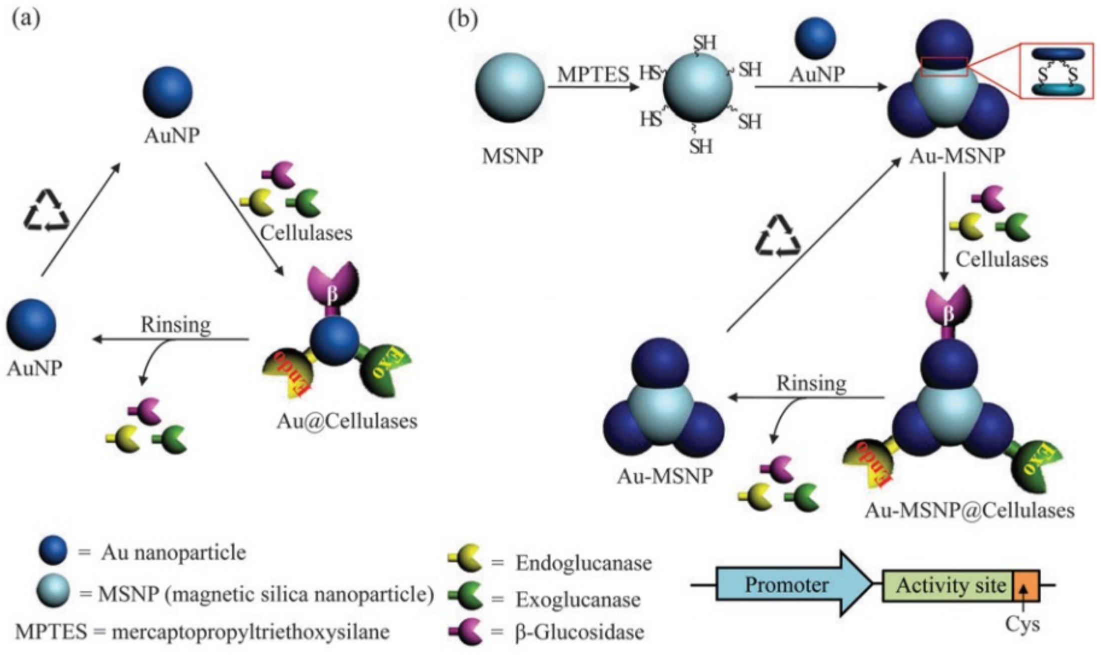
(c) Recyclability of lipase-nanoporous gold biocomposite for catalytic conversion of soybean oil to biodiesel [66] in Misson 2015.



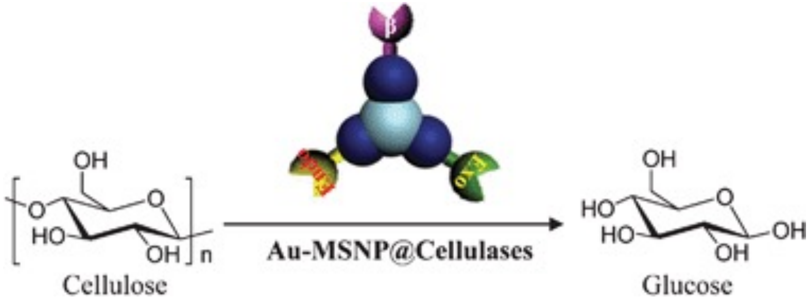
(d) Synthesis of GalactoOligoSaccharide (GOS) from lactose conversion by β-galactosidase-nanospheres (unfilled triangles, lactose; filled triangles, total GOS; filledsquares,monosaccharide) [146] in Misson 2015.



Co-immobilization of three cellulases on Au-doped magnetic silica nanoparticles for the degradation of cellulose



Scheme 1 Overall schemes for the synthesis of the cellulases immobilized on (a) **AuNP** and (b) **Au-MSNP**.



Cho *et al*, 2012

Large scale biocatalysis/nanocatalysis POTENSIAL

Immobilized enzymes used for large-scale industrial processes:

1. glucose isomerase for production of fructose corn syrup(HFCS) (107 tons per annum)
2. lipase for transesterification of food oils (105 tons per annum)
3. penicillin G acylase for antibiotic modification (104 tons per annum)

HOWEVER:

Studies on the development and application of nanocarrier-based NBC´s for bioprocesses still carried out in laboratory-scale bioreactor

A successful case using NBC´s in LARGE SCALE industrial bioprocesses has not been found in the literature so far.

Discussion: HOW CAN THIS UPSCALING BE POSSIBLE?

References nanobiocatalysis- from An *et al*, 2018

Vranish, J.N.; Ancona, M.G.;Walper, S.A.; Medintz, I.L. Pursuing the promise of enzymatic enhancement with nanoparticle assemblies. *Langmuir* **2017**, *34*, 2901-2925.

Zdarta, J.; Meyer, A.S.; Jesionowski, T.; Pinelo, M. A general overview of support materials for enzyme immobilization: Characteristics, properties, practical utility. *Catalysts* **2018**, *8*, 92.

Ge, J.; Lu, D.; Liu, Z.; Liu, Z. Recent advances in nanostructured biocatalysts. *Biochem. Eng. J.* **2009**, *44*, 53-59.

Lin, Y.; Chen, Z.; Liu, X.Y. Using inorganic nanomaterials to endow biocatalytic systems with unique features. *Trends Biotechnol.* **2016**, *34*, 303-315.

Kim, J.; Grate, J.W.; Wang, P. Nanobiocatalysis and its potential applications. *Trends Biotechnol.* **2008**, *26*, 639-646.

Cipolatti, E.P.; Valério, A.; Henriques, R.O.; Moritz, D.E.; Ninow, J.L.; Freire, D.M.G.; Manoel, E.A.; Lafuente, R.F.; Oliverira, D.D. Nanomaterials for biocatalyst immobilization-state of the art and future trends. *RSC Adv.* **2016**, *6*, 104675-104692.

Cascade enzyme catalysis-nanocatalysis

One pot combination

Benefits over traditional prosesses:

1. Avoiding purification and isolation of intermediates

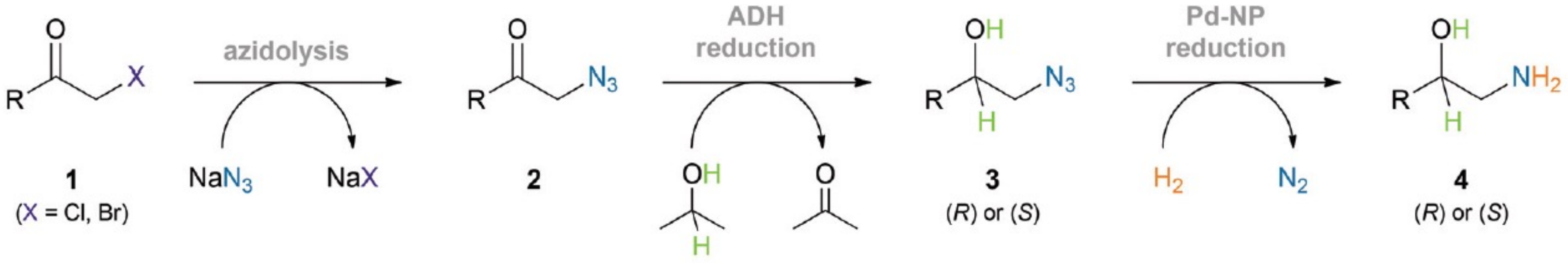
2. low ecological footprint, quantified by
    - the *E*-factor
    - solvent demand

The ideal *E*-factor is zero.
Kilograms of raw materials in, minus kilograms of desired product, divided by kilograms of product out.

Sheldon, 2017

Cascade enzyme catalysis-nanocatalysis

One pot combination

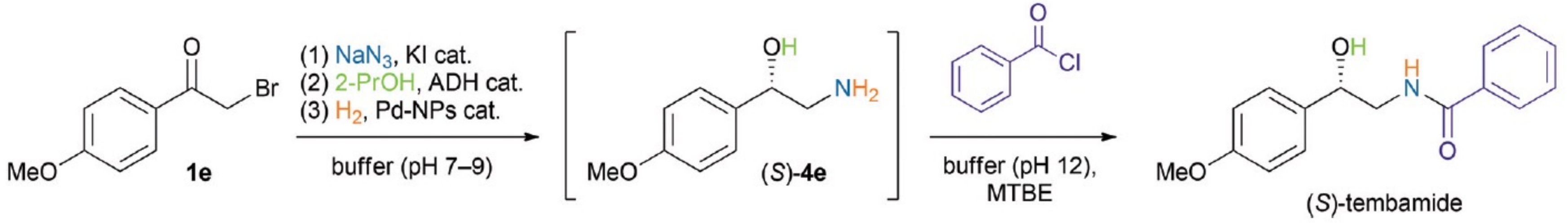


a: R = Ph, X = Cl; **b:** R = 4-Cl-C_6H_4, X = Br; **c:** R = 4-F-C_6H_4, X = Br;
d: R = 4-tolyl, X = Br; **e:** R = 4-MeO-C_6H_4, X = Br; **f:** R = 2-furyl, X = Br

Scheme: Chemo-enzymatic approach towards optically pure 1,2-amino alcohols via azidolysis, alcohol dehydrogenase (ADH) catalysed asymmetric reduction, and Pd nanoparticle (Pd-NP) catalysed azide hydrogenation

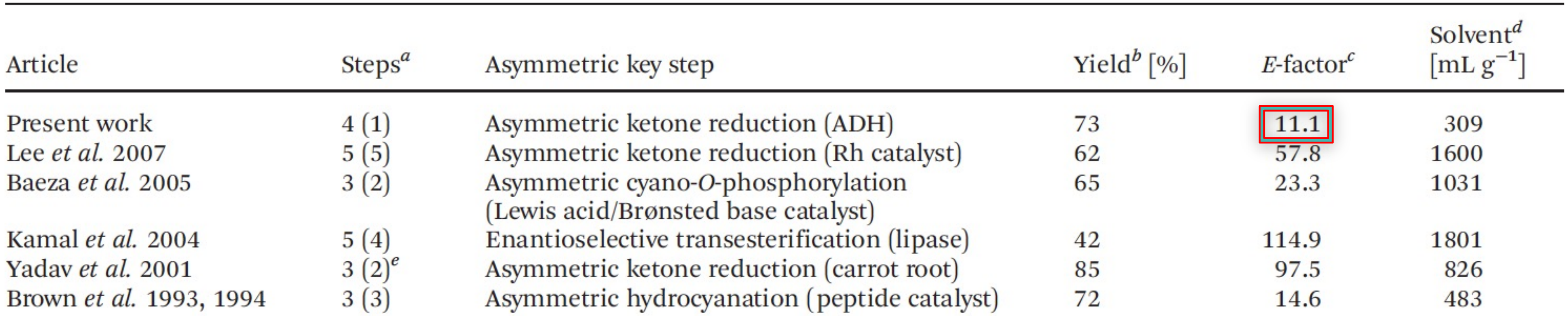
Schrittwieser *et. al,* 2013

Antiviral natural product (*S*)-tembamide (**1**), 73% yield, *ee* >99%



Scheme: Asymmetric synthesis of (*S*)-tembamide in a chemo-enzymatic four-step one-pot sequence.

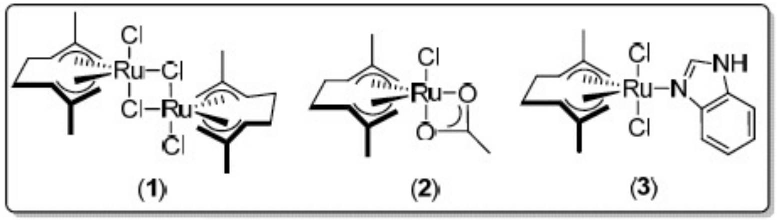
Table: Environmental impact comparison of catalytic asymmetric syntheses of tembamide



Article	Steps^a	Asymmetric key step	Yield^b [%]	*E*-factor^c	Solvent^d [mL g^{-1}]
Present work	4 (1)	Asymmetric ketone reduction (ADH)	73	11.1	309
Lee *et al.* 2007	5 (5)	Asymmetric ketone reduction (Rh catalyst)	62	57.8	1600
Baeza *et al.* 2005	3 (2)	Asymmetric cyano-*O*-phosphorylation (Lewis acid/Brønsted base catalyst)	65	23.3	1031
Kamal *et al.* 2004	5 (4)	Enantioselective transesterification (lipase)	42	114.9	1801
Yadav *et al.* 2001	3 (2)^e	Asymmetric ketone reduction (carrot root)	85	97.5	826
Brown *et al.* 1993, 1994	3 (3)	Asymmetric hydrocyanation (peptide catalyst)	72	14.6	483

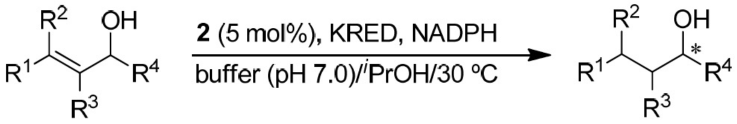
Schrittwieser *et. al*, 2013

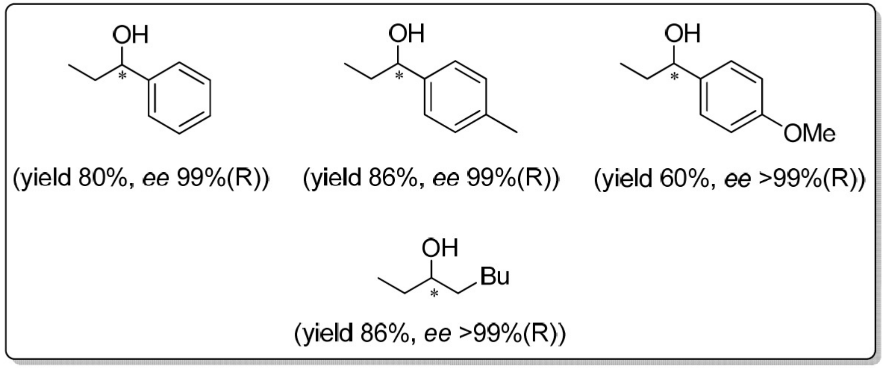
One-Pot Combination of Metal- and Bio-Catalysis in Water
Synthesis of enantiopure molecules



(**1**) (**2**) (**3**)

Fig. Highly-efficient and selective bis(allyl)-ruthenium(IV) catalysts (**1**–**3**) for the redox isomerization of allylic alcohols in water and under mild reaction conditions.



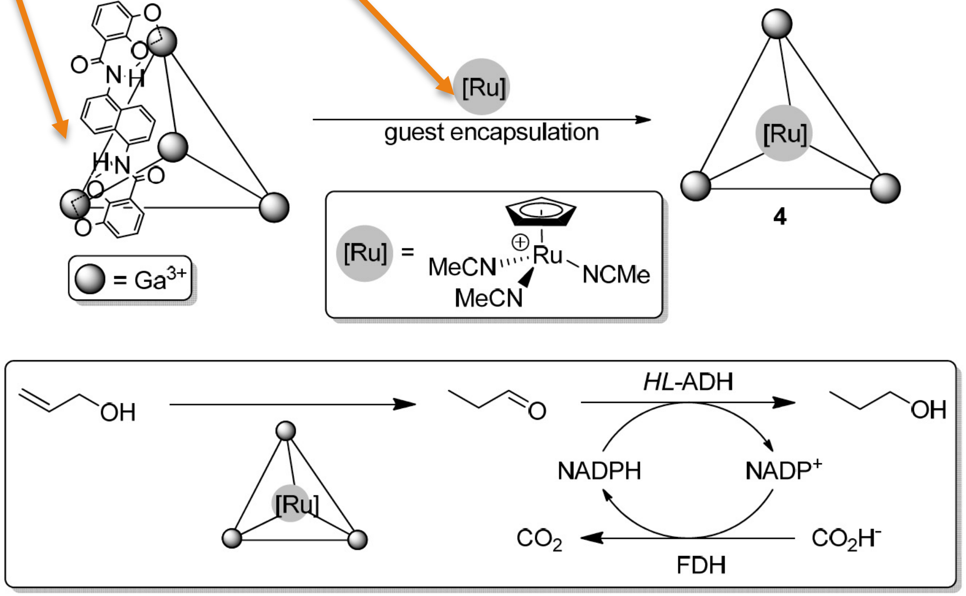


(yield 80%, *ee* 99%(R)) (yield 86%, *ee* 99%(R)) (yield 60%, *ee* >99%(R))

(yield 86%, *ee* >99%(R))

Ríos-Lombardía *et al*, 2018

Host –guest (nano) metal complexes + biocatalyst



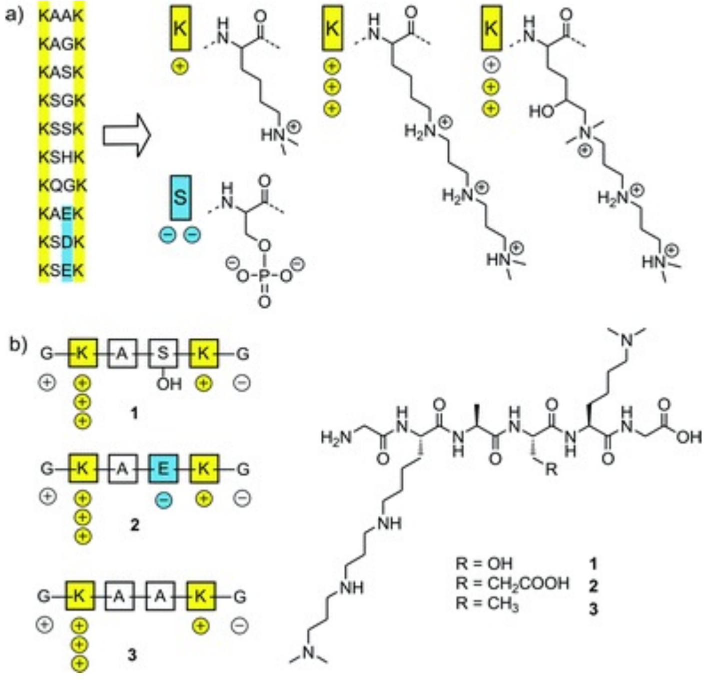
Synthesis of Ru(II)-host-guest Complex **4** and the design of a tandem isomerization/bioreduction of the allylic alcohol 2-propenol

Ríos-Lombardía *et al*, 2018

Another promising application of nanotechnology in the biofuel industry is enzyme (biocatalysts) immobilization during lipase-catalyzed biodiesel and cellulosic ethanol production processes (Kim *et al*., 2018).

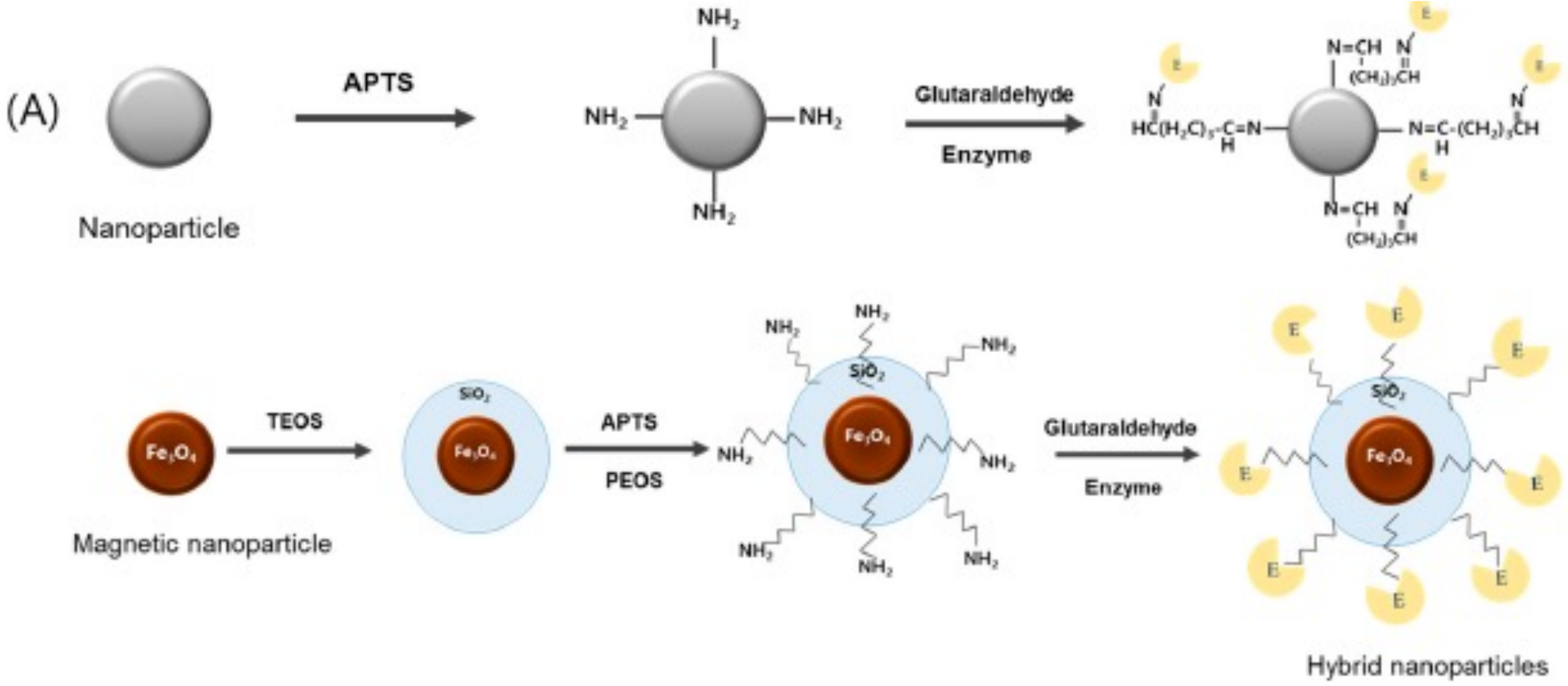
The benefits nanostructures offer in this domain include large surface area for high enzyme loading, higher enzymatic stability, and possibility of enzyme reusability, which could reduce the operational cost of large-scale biofuel production plants (Trindade, 2011).

Examples of the techniques developed for enzymes immobilization using nanotechnology are nano-encapsulation, self-entrapment with silaffin, and adsorption.



Silaffin polypeptide Sil-3 with increasing polarity from top to bottom

Enzyme immobilization techniques to nanoparticles
-For biofuel production

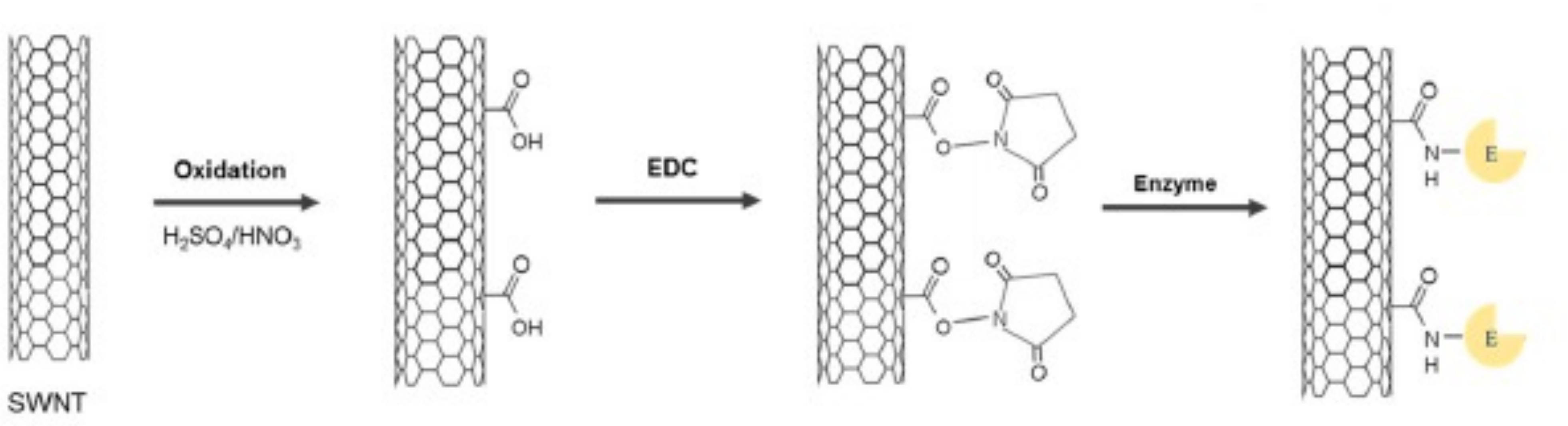


APTS: 3-aminopropyltriethoxysilane, TEOS: tetra-ethoxy silane, PEOS: poly-ethoxy silane

Kim *et al*, 2013, Netto et al, 2009

Nanotubes + enzymes *via* linker

(B)

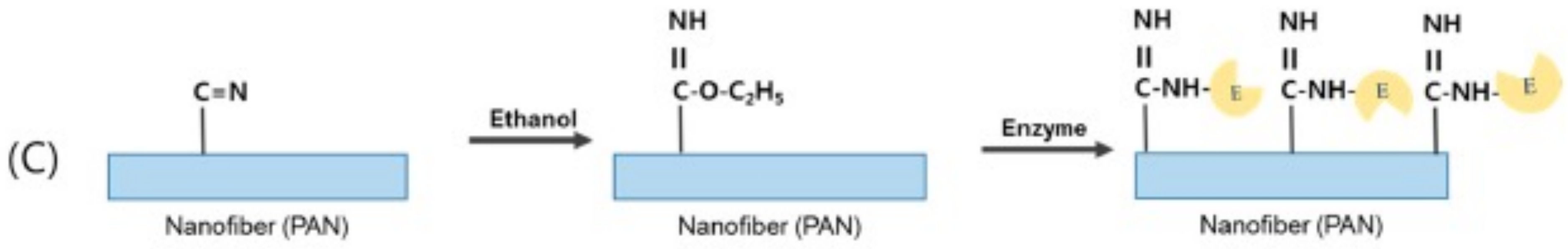


Single walled NT

EDC: N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride

Ji *et al.* 2010

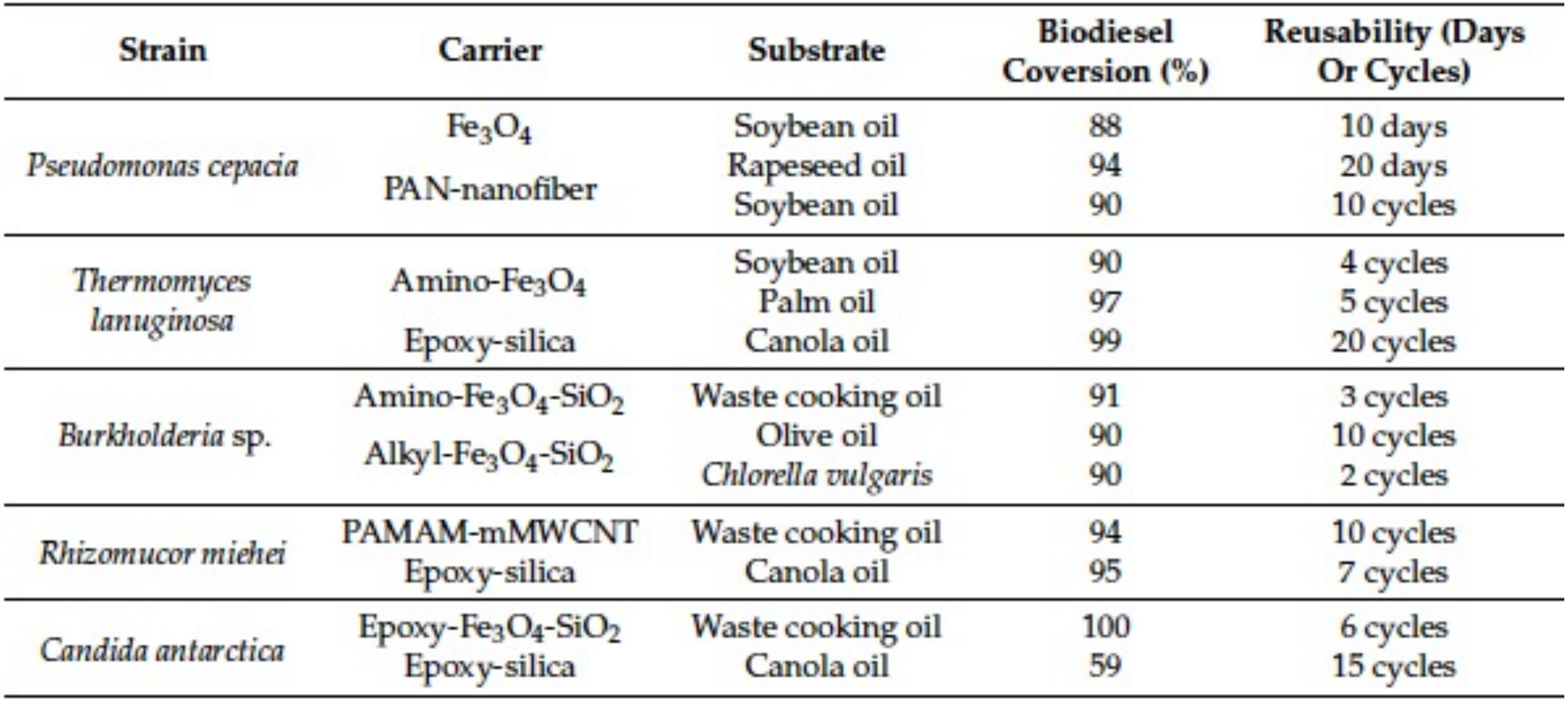
Nanofibers + enzyme *via* linker



PAN: polyacrylonitrile polymer

Li *et al*, 2007

Applications



Strain	Carrier	Substrate	Biodiesel Coversion (%)	Reusability (Days Or Cycles)
Pseudomonas cepacia	Fe_3O_4	Soybean oil	88	10 days
	PAN-nanofiber	Rapeseed oil	94	20 days
		Soybean oil	90	10 cycles
Thermomyces lanuginosa	Amino-Fe_3O_4	Soybean oil	90	4 cycles
		Palm oil	97	5 cycles
	Epoxy-silica	Canola oil	99	20 cycles
Burkholderia sp.	Amino-Fe_3O_4-SiO_2	Waste cooking oil	91	3 cycles
	Alkyl-Fe_3O_4-SiO_2	Olive oil	90	10 cycles
		Chlorella vulgaris	90	2 cycles
Rhizomucor miehei	PAMAM-mMWCNT	Waste cooking oil	94	10 cycles
	Epoxy-silica	Canola oil	95	7 cycles
Candida antarctica	Epoxy-Fe_3O_4-SiO_2	Waste cooking oil	100	6 cycles
	Epoxy-silica	Canola oil	59	15 cycles

Nano-immobilized lipase in packed-bed reactors

POTENTIAL for industrial biodiesel production

- high enzyme loading
- multiple reuses
- effective protection from enzyme denaturation

Goal:

- The integrated development of a high enzyme and nano-immobilization technique will play a key role in cost-effective biodiesel production

Further investigations necessary:

- scale-up of the biodiesel production process using nano-immobilized lipase necessary to implement these technologies on an industrial level.

Kim *et al*, 2018

Importance of engineered nanoparticles (ENP´s) for agrochemicals

- Engineered nanoparticles (ENPs) (polymers, carbon-based, inorganic, zero-valent metal NPs, etc.), have unique physicochemical properties:

  I. Novel approach to boost the efficiency of agrochemical remediation.
  II. Acting as biocides or as nanocarriers of particular conventional agrochemicals, ENP´s increase risk–benefit assessment of remediation.
  III. NPs used to assist alternative remediation processes such as phytoremediation and bioremediation.

- Use of highly efficient specific nanofertilizers and nanopesticides
- Engineering of nanodimensional devices for precise monitoring, so-called nanosensing of environmental parameters, and the right-time
- Efficient application of needed agro measures.
- The final goal is the creation of integrated agriculture supported by the development of nanotechnology and the evolution of efficient advanced agriculture and precision farming.

Boritzev *et al, chapter 19* 2020

Bioremediation: Degradation with NP and microorganisms

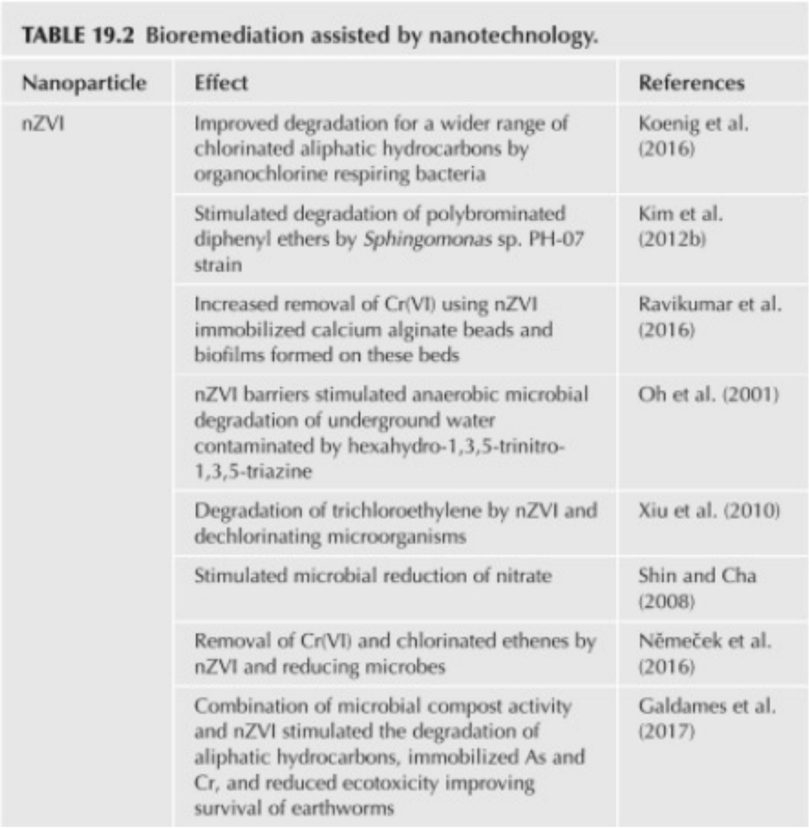
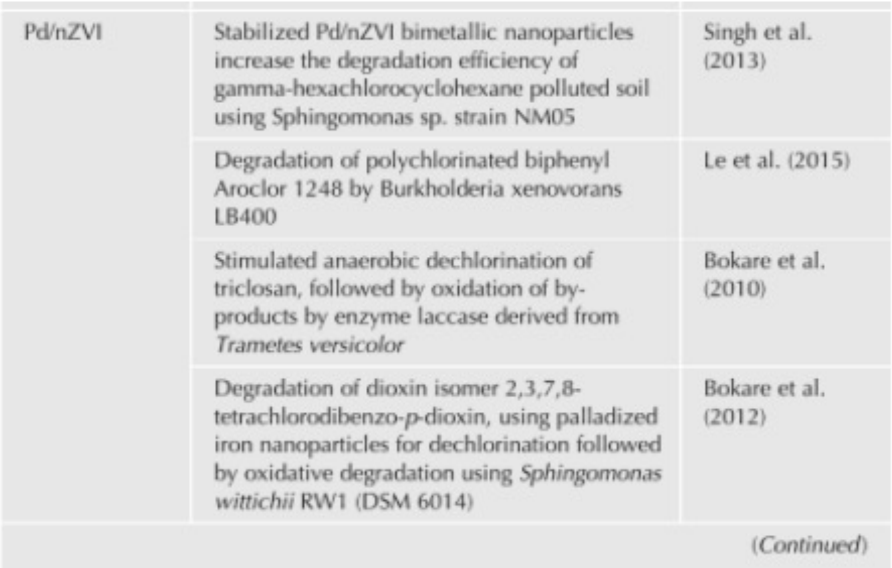


TABLE 19.2 Bioremediation assisted by nanotechnology.

Nanoparticle	Effect	References
nZVI	Improved degradation for a wider range of chlorinated aliphatic hydrocarbons by organochlorine respiring bacteria	Koenig et al. (2016)
	Stimulated degradation of polybrominated diphenyl ethers by *Sphingomonas* sp. PH-07 strain	Kim et al. (2012b)
	Increased removal of Cr(VI) using nZVI immobilized calcium alginate beads and biofilms formed on these beds	Ravikumar et al. (2016)
	nZVI barriers stimulated anaerobic microbial degradation of underground water contaminated by hexahydro-1,3,5-trinitro-1,3,5-triazine	Oh et al. (2001)
	Degradation of trichloroethylene by nZVI and dechlorinating microorganisms	Xiu et al. (2010)
	Stimulated microbial reduction of nitrate	Shin and Cha (2008)
	Removal of Cr(VI) and chlorinated ethenes by nZVI and reducing microbes	Němeček et al. (2016)
	Combination of microbial compost activity and nZVI stimulated the degradation of aliphatic hydrocarbons, immobilized As and Cr, and reduced ecotoxicity improving survival of earthworms	Galdames et al. (2017)

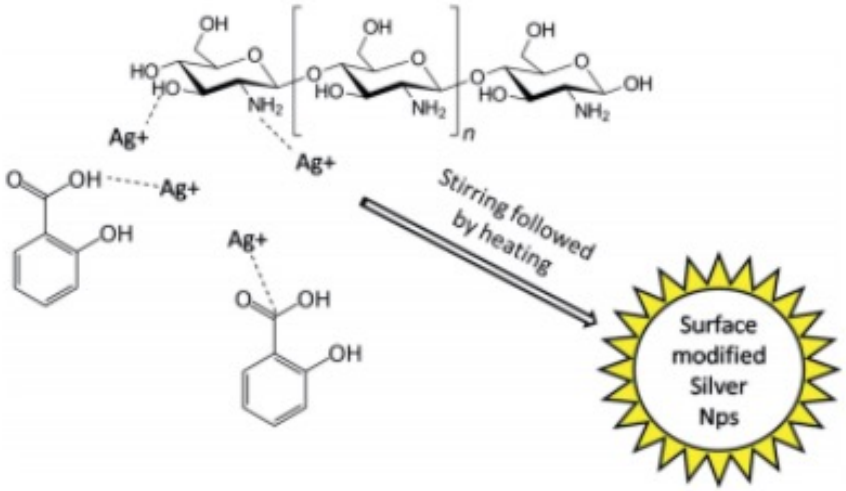


Pd/nZVI	Stabilized Pd/nZVI bimetallic nanoparticles increase the degradation efficiency of gamma-hexachlorocyclohexane polluted soil using Sphingomonas sp. strain NM05	Singh et al. (2013)
	Degradation of polychlorinated biphenyl Aroclor 1248 by Burkholderia xenovorans LB400	Le et al. (2015)
	Stimulated anaerobic dechlorination of triclosan, followed by oxidation of by-products by enzyme laccase derived from *Trametes versicolor*	Bokare et al. (2010)
	Degradation of dioxin isomer 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, using palladized iron nanoparticles for dechlorination followed by oxidative degradation using *Sphingomonas wittichii* RW1 (DSM 6014)	Bokare et al. (2012)

(Continued)

Borisev *et al*, 2020

Agrochemical degradation with nanoparticles



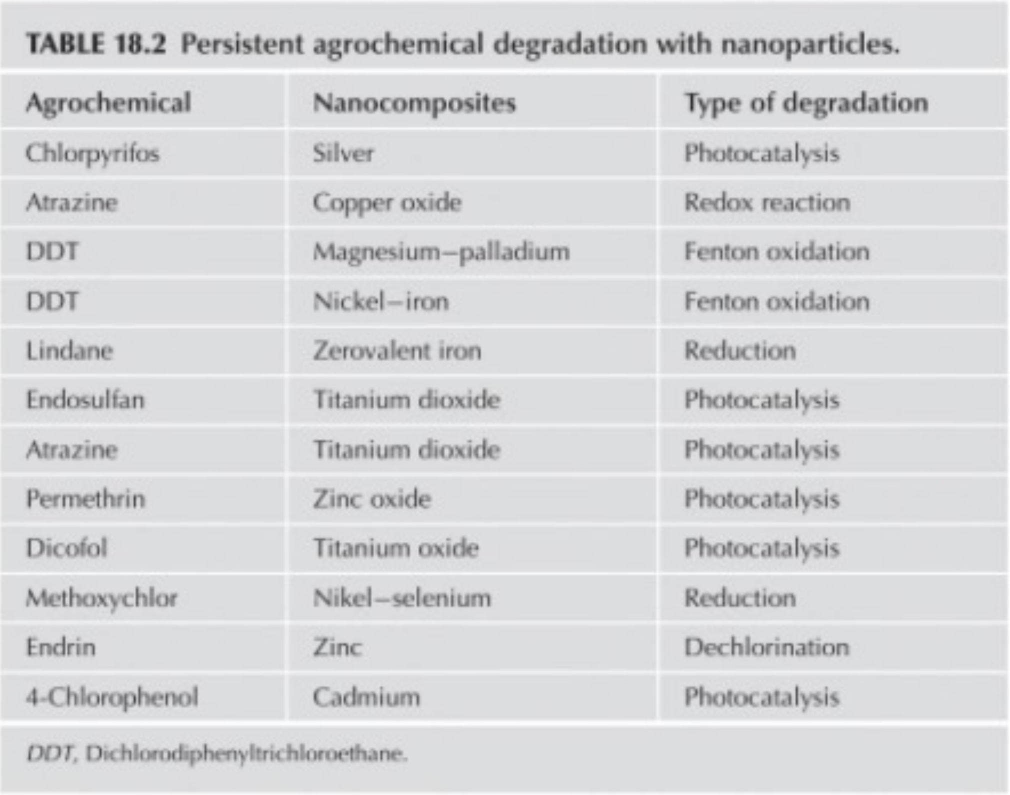


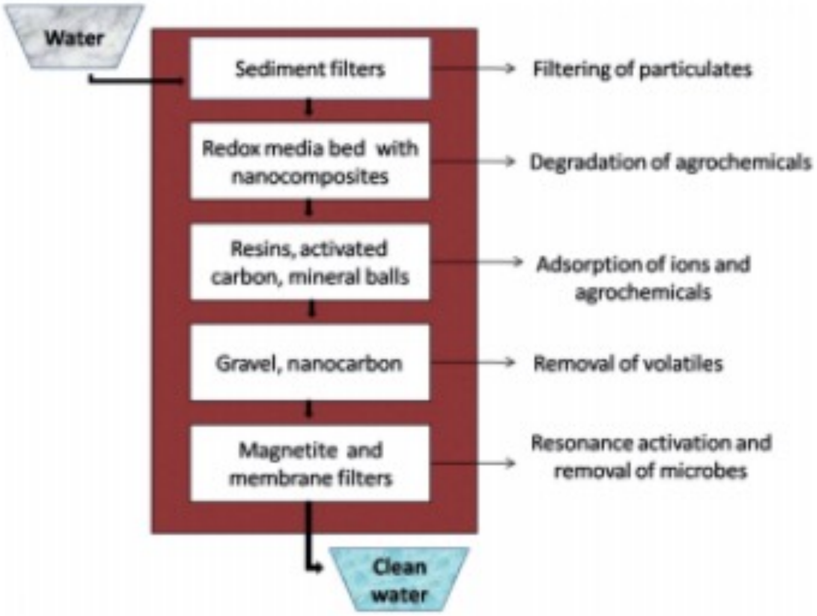
TABLE 18.2 Persistent agrochemical degradation with nanoparticles.

Agrochemical	Nanocomposites	Type of degradation
Chlorpyrifos	Silver	Photocatalysis
Atrazine	Copper oxide	Redox reaction
DDT	Magnesium–palladium	Fenton oxidation
DDT	Nickel–iron	Fenton oxidation
Lindane	Zerovalent iron	Reduction
Endosulfan	Titanium dioxide	Photocatalysis
Atrazine	Titanium dioxide	Photocatalysis
Permethrin	Zinc oxide	Photocatalysis
Dicofol	Titanium oxide	Photocatalysis
Methoxychlor	Nikel–selenium	Reduction
Endrin	Zinc	Dechlorination
4-Chlorophenol	Cadmium	Photocatalysis

DDT, Dichlorodiphenyltrichloroethane.

Sebastian *et al*, 2020

Mulitistep removal of agrochemicals from water



Chlordane, dieldrin, endrine, toxaphene: persistent in agriculture residues, soil particles and in water present in irrigation channels.

Can migrate into deeper layers of soil resulting in ground water pollution.

Nanoparticles degrade persistent agrochemicals: photocatalytic reactions creating electron-hole pairs, results in the formation of free radicals (such as hydroxyl radicals)
* then the free radicals direct secondary reactions that end up in degrading the chemicals

Sebastian *et al*, 2020

Conclusions

Process-related traits of NanoBioCatalysts (NBC´s) are not fully understood.

Exploitation of NBC technologies still in the infant stage in the bioprocessing industry.

Success of NBC technology in the large-scale manufacturing processes relies on:

1. specific activity under the process conditions;
2. stability of the NBC´s when exposed to pH/temperature variations, organic solvents, high shear stress and other harsh environments;
3. reusability of biocatalysts
4. high throughput for large-scale processes.

It is economically and technically crucial that the NBC´s are able either to maintain stable activities in the long term in a continuous process, or to be recycled for re-use in a batch operation process for many runs in which the NBCs are separated from the reaction media after the reaction is completed.

For a continuous operation, stability of enzyme activity as well as reduction of enzyme leakage are the main targets for immobilization, while for batch operations, recyclability and constant enzyme activity of NBC´s are the key challenges.

Future improvements of NBC´s

To further promote the applications of nanobiocatalysts, the following urgent challenges need to be addressed:

1. an evolutional nanobiocatalyst that can permit the simple recycle and reuse of enzymes
2. minimizing the “dead areas” of nanobiocatalysts in catalytic reactions to make the hybrid systems more economically friendly
3. improving the biocompatibility and stability of nanobiocatalysts for in vivo and in vitro biomedical applications
4. smart nanobiocatalysts that can respond efficiently to remote stimuli for modulating the activities of nanobiocatalysts on demand.

An *et al* 2018 (review)

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