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Review

Ureases. II. Properties and their customizing by enzyme immobilizations: A review

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ABSTRACT

Ureases are enzymes highly desirable in immobilized form for a number of applications that exploit urea cleavage and an increase in pH, inherent to the reaction. Major among them are medical and analytical applications, but there have emerged new biotechnological and engineering areas, proving that there exists a growing demand for robust reliable immobilized urease preparations with defined properties. These can be assured by immobilizing the enzymes. By creating disturbance in the original state of enzymes, immobilizations inevitably change enzyme properties, enabling them to be customized for specific applications. In this context, this article offers a review of reports on immobilizations of ureases covering the last two decades. It surveys the immobilization techniques and support materials applied, in addition to the resulting properties of the enzymes. In this manner it attempts to provide useful guidance through the wealth of available immobilization data in the literature, but more importantly, to develop an integrated perspective on how to customize ureases for their applications, which may help establish rational immobilization procedures in place of tedious experimental optimization.

Contents

1. Introduction

Owing to their unparalleled catalytic properties, foremost among them being catalytic efficiency, specificity and mild conditions of operation, further to their biodegradability and derivation from renewable resources, enzymes have become desirable catalysts, whose utilization has emerged as one of chief strategies brought forward in the present-day drive towards more eco-friendly and energy- and material-saving chemical processes [1–3]. The broader use of enzymes, however, their advantages compared to conventional chemical catalysts notwithstanding, is constrained by a number of practical problems. In addition to the high cost of enzyme isolation and purification, the prime problem is their inherent fragility to environmental conditions other than their individual optimal ones. These include particularly temperature, pH and sensitivity to inhibitors, all of them capable of triggering enzyme dysfunction. This results in limited operational lifetime of enzymes, and besides, difficult or not at all feasible is their recovery in the active form after the process for reuse [1,4–6].

One way to overcome these constraints, arguably commonest and most successful, is immobilization of enzymes [1,4–6]. The immobilization consists of converting enzymes into insoluble form, most frequently by fixing them to or within solid supports, as a result of which heterogeneous enzyme systems are obtained,

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where structures of enzymes, hence their activities, are stabilized. Equally important in immobilization is that the heterogeneity of the systems allows them to be easily recovered separately from the product, and repeatedly reused. Possible are also continuous operation of enzymatic processes, their rapid termination and a greater variety of engineering designs.

Importantly, being an intrusion, immobilizations inevitably create disturbance of the original state of enzymes, thereby leading to alterations of their properties. Remarkably, this has opened the way for customizing enzyme properties for their specific applications.

Immobilizations of a great variety of enzymes have been studied for the application in diverse analytical, medical, industrial and biotechnological processes, and to date, several processes have been implemented on a larger scale, mainly in the food industry and in the manufacture of fine specialty chemicals and pharmaceuticals [1,5].

Among enzymes most extensively studied for immobilizations and practical applications are ureases. This is because of the significance of the processes in which ureases take part and of their possible exploitation in practical applications. The former, along with the kinetic and catalytic properties of the enzymes, were reviewed in a preceding article [7].

This article offers a review of reports on immobilizations of ureases covering the last two decades. It surveys the immobilization techniques and support materials applied, in addition to the resulting properties of the enzymes. In this manner it attempts to provide useful guidance through the wealth of available urease immobilization data in the literature, but more importantly, to develop an integrated perspective on how to customize ureases for their specific applications.

2. Immobilization of enzymes

Diverse enzyme immobilization techniques have been developed [1,4–6]. Their traditional classification is into chemical and physical ones, though very frequently their combinations or various follow-up treatments are applied [6]. To the chemical techniques belong: (i) covalent attachment to solid supports, and (ii) crosslinking with multifunctional, low molecular reagents, also sometimes performed with the addition of neutral compounds (co-crosslinking). By contrast, the physical techniques include: (i) adsorption on solid supports, (ii) gel entrapment, (iii) microencapsulation with solid or liquid membranes, (iv) containment in membrane reactors, (v) formation of Langmuir–Blodgett films, and (vi) layer-by-layer assembling. The choice of materials to be used in these techniques is practically unlimited, and include organic and inorganic, natural and synthetic materials, that may be configured as (micro-, nano-)beads, membranes, fibers, hollow fibers, (micro-)capsules, sponges to best suite a chosen biotransformation in a chosen bioreactor.

On the whole, to immobilize an enzyme is not a difficult task, but to obtain an enzyme with desired properties is a complex challenge. This is because, regrettably, there are no universal protocols how to immobilize enzymes and how to exactly predict their resulting properties. The following general guidelines, however, are worth taking into consideration [1,4,5,216]. First, each immobilization technique has its singular features. For instance adsorption is simple, cheap and effective, but very frequently reversible. Conversely, covalent attachment and crosslinking are effective and durable, but costly and easily lowering the enzyme activity. In membrane-confinement, entrapment and microencapsulation on the other hand, an important contribution to the overall enzyme performance is brought in by diffusional restrictions imposed on substrates and products of the reaction. Second, in choosing the support material the following material characteristics should be taken into account: high affinity to proteins, availability of reactive functional groups for direct reaction with enzymes or for chemical modifications, mechanical stability, regenerability and ease of preparation in different geometrical configurations that would provide the system with surface areas and permeabilities suitable for a chosen reaction. Third, understandably, the choice of support material should be correlated with the chosen application of the enzyme system. For instance, for food, pharmaceutical, medical and agricultural applications, nontoxicity and/or biocompatibility of the material are required. Besides, in response to the growing public health and environmental awareness, the material should be biodegradable, and to prove economical, inexpensive. Fourth, always, though to various degrees, the immobilization alters the properties of enzymes [1,5,8,105,106,217]. For the most cases the enzyme activity is lowered and its Michaelis constant increased. These alterations are a combined effect of a number of factors. One is brought about by structural changes in the enzyme occurring as a result of its binding, the other ones resulting from the heterogeneity of the system. These include creation of a microenvironment different from the bulk solution, strongly depending on the properties of the support (electric charge, hydrophobicity, etc.), the reaction itself (ions, pH change, etc.) and on the design of the reactor, in addition to inevitable mass transfer limitations. Effectively, it is both the enzyme and the support, and the interaction between the two that impart the system with specific physico-chemical and kinetic properties responsible for its operational performance.

In consequence, practically as a rule, immobilization procedures that would assure the desired properties to a chosen bio-system for a chosen application, are established through experimental optimization. For the efficacy of the immobilization procedure to be assessed, enzyme activity retention, alternatively protein immobilization yield, should be determined. By contrast, for the enzyme system to be characterized, the following properties are relevant: specific activity, the Michaelis constant K_M, the optimum pH and temperature, and the activation energy, the last four properties typically compared to the free enzyme. Obviously, a major emphasis in practical applications is on the stability of the enzyme. This includes thermal and storage stabilities, also compared to the free enzyme, in addition to operational stability and reusability.

3. Ureases and their applications

Ureases (urea amidohydrolases, EC 3.5.1.5) are a group of highly proficient enzymes, widely distributed in nature, whose catalytic function is to catalyze the hydrolysis of urea to carbonic acid and ammonia as final products:

$$H_2N\text{–}CO\text{–}NH_2 + 2H_2O \xrightarrow{\text{urease}} H_2CO_3 + 2NH_3$$

The products and the resulting increase in pH of the reaction environment that can reach pH up to 9.2, are consequential characteristics of the action of ureases [7]. Apart from its natural significance, ureases-catalyzed hydrolysis of urea is important in that it offers potential in practical applications. The most typical examples of such applications, where immobilized ureases are preferably used in place of free enzymes, are presented below.

3.1. Urea removal from aqueous solutions

The removal of urea from aqueous solutions is a problem faced in numerous areas, examples being urea-producing industry, agriculture and natural environment, food production and medicine. In the former, the weight of the problem derives from the fact that the production of urea has now reached the level of ca. 1×10^8 tons per year worldwide, more than 90% of which, for the use as a fertlizer. In the production, urea-containing (0.2–2%) wastes mainly result from the

urea purification and recovery process that follows the synthesis. Before discharging into the environment, the wastes need to have their urea content reduced to less than 0.006% [218]. In the environment, urea also comes from other industries that utilize urea, as well as from fertilized crop-planted soils as fertlizer wastewater effluents, also as effluents from households, but primarily from urine excretion by animals. The reported urea concentrations in the environment are in the micromolar range up to 70 μM for fertilized soils, these comparatively low values being a resultant of the rapid action of ureases [228]. Although urea has generally low ecotoxicity, the indirect long-term impact of its excessive levels in nature may be detrimental in causing eutrophication and groundwater pollution, in addition to the effects of ammonia resulting from urea hydrolysis, including toxicity, alkalinity and emissions to air [7,20], hence the importance of efficient urea removal modes.

Urea is a polar non-ionic compound, highly soluble and stable in water, showing little affinity to common sorbents [9], on the whole difficult to be removed from aqueous solutions. Industrially utilized are removal methods based on urea hydrolysis (nonenzymatic) and on biological conversion of urea nitrogen to dinitrogen. The methods, however, have drawbacks. The former requires elevated temperatures and pressures in addition to complex technological installations, and the latter suffers from instabilities of the microbial bed, both methods having high operation costs [218]. Medically by contrast, utilized is the removal method based on dialysis, exploited in the artificial kidney (see below). Other urea removal methods that include catalytic and electrochemical decompositions, oxidation with strong oxidants and adsorption, are presently only under laboratory investigations [218].

In this context, a removal mode based on the hydrolysis of urea catalyzed by urease is an attractive alternative. The mode has been examined for a number of applications, detoxification of blood being arguably a major one. The detoxification is a process done for clearing the blood of uraemic toxins, where blood urea concentration is typically reduced from 20–50 mM to less than 10 mM [28].

The underlying concept of this application derives from the search for blood detoxification techniques that could both simplify the artificial kidney machine and reduce its size, making it eventually portable/wearable [10]. Overwhelmingly used in the treatment of renal diseases and effective though they are, the conventional artificial kidneys based on haemodialysis are costly and inconvenient machines, difficult to handle and also largely limiting the mobility of the patient. In addition, they require as much as 100–300 l of dialysate solution per treatment, normally spent. Investigations into the application of urease as the basis for urea removal from the blood were initiated by Chang in 1964, with the invention of artificial cells [11] (updated review in Ref. [12]). In the cells urease was encapsulated within an ultra-thin, nontoxic, semipermeable membrane, which permitted the free diffusion of low molecular compounds (urea, ammonia) effectively retaining high molecular compounds. The cells were further developed to contain sorbents/ion exchangers to catch ammonium ions, and they were tried in extracorporeal haemoperfusion systems [13] and in oral therapies [14]. Though promising, their performance suffered from various physiological side effects, such as thromboembolism and platelet adhesion in the former, and indigestion, nausea and negative calcium balance in the latter. One alternative to circumvent these problems is the conventional haemodialysis associated with a dialysate regeneration system [15,16]. The system is a closed-loop unit through which the same small amount of dialysate is recirculated and cleared of the uraemic toxins. Urea is removed by hydrolyzing it with immobilized urease, the resulting ammonium and carbonate ions being caught by ion exchangers, whereas the other toxins are eliminated by adsorption on activated charcoal. The commercialized dialysate regeneration systems require 5 l of dialysate or less.

Another medical application of urease-hydrolysis of urea for its removal is in preparing urine for diagnosis of inborn metabolic errors [17]. The proposed procedure based on the simultaneous GC–MS analysis of amino acids, organic acids, sugars, sugar alcohols, sugar acids and nucleic acid bases in the pretreated urine was found capable of defining a large number of metabolic disorders, and these if found in newborns are effective for prevention or significant reduction of clinical conditions such as mental retardation.

Effectively, the hydrolysis of urea can be applied for removal of urea under any circumstances. One instance is the construction of a closed-loop environmental life-support system to be used for water reclamation aboard manned spacecraft, crucial especially for long duration flights or space stations [18]. Other instances include the removal of urea from industrial wastewaters, where the product ammonia can be recovered by air or stream stripping or by ion exchange [19], as well as the removal from fertilizer wastewater effluents.

In the food and beverage production area, a remarkable example of commercialized processes is the removal of urea from alcoholic beverages performed with use of acid ureases. These ureases, unlike the neutral ones, are known to have the optimum activity at acidic pHs [21–27]. Alcoholic beverages have comparatively low pHs, for example pH of sake is 4.4, that of wine is 3.2 [26]. This is why acid ureases meet the conditions of the process, whereas neutral ureases do not, which is on account of their too low activity at this pH range. This removal of urea is done to prevent the formation of ethyl carbamate, known to be carcinogenic, from the reaction of urea and ethanol taking place during alcohol manufacturing and preservation.

3.2. Analytical applications of urease

The foremost analytical application of urease is for quantification of urea in aqueous solutions [20]. Even though the major interest has been on its medical application, there is a growing demand for sound, reliable, and fast urea analytical procedures in other areas, such as environmental, food and industrial.

In medical application, urea is mainly analyzed in blood and urine. Apart from being crucial as an indicator of liver and kidney function, the blood urea test is also used as a marker for quantification and monitoring of haemodialysis treatment. By contrast, in food analysis, urea is routinely quantified for instance in cow's milk and in alcoholic beverages [20]. In the former analysis, as the prime component of non-protein nitrogen in milk, the level of urea (typically 3–6 mM) is utilized as an indicator of protein-feeding efficiency. This, if improved, may help significantly enhance the economy of milk production and of animal husbandry [20,219]. The assay is also used for detecting urea adulteration in milk [220]. In the latter analysis on the other hand, control of urea level in alcoholic beverages is necessary to minimize the reaction of urea with ethanol, generating carcinogenic ethyl carbamate [20]. Furthermore, in environmental and industrial contexts, the necessity of urea quantification in waste- and natural waters is consequent on the production and wide use of urea-fertilizers, in addition to the use of urea in chemical industry. This includes the manufacture of resins, glues, solvents, medicines and cleaning products (liquid soaps, detergents). Urea has also been extensively used in the treatment of dry skin, both therapeutically and in cosmetics [20].

Compared to direct urea quantification procedures, such as diacetyl monoxime reaction, the indirect ones that make use of urease, are beneficial in that they eliminate the taxing application and disposal of noxious reagents [20]. In these procedures, urea is determined either by measuring the products of its hydrolysis or the effects brought about by the reaction, i.e. the increase in pH or in conductivity of the solution. Whereas ammonia can be determined colorimetrically by indophenol [29] or Nesslerization

Table 1
Immobilizations of ureases.

Covalent attachment
Urease from jack bean (*Canavalia ensiformis*)

Support material (remarks on immobilization)	Act. reten. (%)[a]	Specific activity (U/mg protein)[b]	K_M (mM) free imm	pH_{opt} free imm	T_{opt} (°C) free imm	Ea (kcal/mol) free imm	Stabilities, $t_{1/2}$ Thermal (min) free imm	Stabilities, $t_{1/2}$ Storage (days) free imm	Stabilities, $t_{1/2}$ Oper. (days) (Reusability)	Remarks	Refs.
Silk cloth activated with 1-ethyl-3-(3dimethylamino propyl) carbodiimide hydrochloride										Urease-aided precipitation of hydroxyapatite	[75]
Nylon membrane grafted with glycidyl methacrylate		32.954									[76]
Polypropylene fabric (thick. 0.48 mm, surface area 0.395 m^2/g) grafted with acrylic acid, activated with:										Column operation	[77]
- *N*-(3-dimethylaminopropyl)- *N*'-ethylcarbodiimide hydrochloride		12.5	12.4 27.5	7.2 7.6	45 50	4.30 4.73		(4°C) 23 98	66		
- *N*-cyclo-hexyl-*N*'-(b-[*N*-methyl-morpholino]-ethyl) carbodiimide *p*-toluene-sulfonate		8.1	12.4 36.5	7.2 7.6	45 50	4.30 5.22					
- GA[c]		3.0	12.4 79.4	7.2 7.6	45 55	4.30 5.22					
Gelatine beads activated with GA	68			7.3 8.0	45 60		(70°C) 4 31	(4°C) 20 90			[78]
Nylon membrane grafted with cyclohexyl methacrylate (thick.150 µm, pore diam. 0.2 µm) modified with hexamethylenediamine and GA	23		18.1 25.8	6.0 5.7	75 81					Applied in a membrane biorector under nonisothermal conditions	[79]
Poly(hydroxyethyl methacrylate-*co*-glycidyl mathacrylate) gel film	44	2.18 U/cm^2	18 32	7.0 6.5	45 45						[80]
- modified with 1,6-diaminohexane (spacer) and GA (activator)	56	3.25 U/cm^2	18 24	7.0 6.5	45 45		(55°C) 79 261 (65°C) 69 115	(4°C) after 28 days RA[d] free 0%, after 56 days RA imm 63%	After 80 h RA 93%		
Poly(styrene-*co*-acrolein) microspheres, diam. 373 nm										10-fold reduction of activity on binding	[81]
Polyacrylonitrile hollow fiber (outer surface) hydrolyzed, amidated with 1,6-hexanediamine and activated with GA	98	0.38		7 5-8				(4°C) after 42 days RA free 2%, RA imm 90%	15 reuses RA 86 %	To improve urea removal in dialysis; 2-fold improvement observed	[82,83]

Table 1 (*Continued*)

Poly(ethylene glycol dimethacrylate/2-hydroxyethyl-methacrylate) microbeads, diam. ~115 μm, modified with periodate (oxidation), hexamethylene diamine (spacer) + GA (activator)		12.8 U/g beads	14.5 15.9					(4^oC) after 75 days RA imm 73%		V_{max} 10^4 times lower than of free enzyme; Blood coagulation and protein adsorption reduced. Blood urea removal	[84,85]
Polypyrrole microspheres, diam. 92 nm, embedded in conducting polypyrrole-polyvinylsulphonate films deposited electrochemically on indium-tin oxide glass plates				7.0 7.5						Design combines large immobilization surface area and film configuration.To be used in biosensors	[86]
Methoxypolyethyleneglycol 5000 activated with cyanuric acid, encapsulated in erythrocytes										Intravenously injectable system for removal of urea	[87,88]
Nylon membrane, thick. 150 μm, pore size 0.2 μm, grafted with butyl methacrylate and modified with hexamethylenediamine and GA			19.5 50.0	8.0 7.5	70 75	5.90 9.10					[89,90]
Poly(*N*-isopropylacrylamide-*co*-*N*-acrylosuccinimide-*co*-2-hydroxyethyl methacrylate) composite hydrogel membrane	55	5.71 U/cm^2	2.84 7.81	7.5 7.5	60 70	6.7 3.4	(70^oC) after 300 min RA free 5%, RA imm 67%			Thermal responsive gel; reactor/separator to remove urea with temperature swing	[91]
Poly(2-hydroxyethylmethacrylate) membranes, thick. 0.06 mm, activated with epichlorohydrin	27	16.2	18 34	7.2 7.2	45 50	1.47 1.83	(65^oC) 19 31 (55^oC) 53 73		After 40 h RA 87%	Column operation	[92]
Chitosan beads (Chitopearl BCW-3007), diam. 590-840 μm, pore diam. 0.15 μm, surface area 135 m^2/g, activated with GA			2.84 12.7	7.5 7.5	60 70	6.7 5.3	(70^oC) 70 175	(4^oC) after 150 days RA free 0%, RA imm 73%	10 reuses RA 100%	Column operation	[93]
Chitosan-poly(glycidylmethacrylate) copolymer (precipitate)	82		3.23 6.7	7.5 7.5	60 70			(4^oC) after 60 days RA 73%, (25^oC) RA 58%			[94]
Poly(vinyl alcohol) beads cross-linked with paraformaldehyde and activated with cyanuric chloride	70	0.0082	2.7 7.1	8.0 8.0	50 70	3.70 4.95	(70^oC) 74 210	(30^oC) 20 48	5 reuses RA 50%		[95]
Ethylene-vinyl alcohol membranes activated with cyanuric chloride	10	6.6 x 10^{-5} U/cm^2	29.9 12.0	7.0 7.0						Asymmetrical urease membrane	[96]
Nylon 6/6 tubes activated with GA	12			6.5 6.5	25 65			(4^oC) after 60 days RA 76%, (25^oC) RA 52%	5 reuses RA 78%		[97]

Vermiculite particles activated with GA	89		521 694	6.5 6.5	25 65			(4°C) after 60 days RA 81%, (25°C) RA 45%	4 reuses RA 61%		[98]
Acrylamide grafted poly(ethylene terphthalate) fibers activated with GA			2.82 4.50	7.0 7.0	52 60	2.81 3.38	(80°C) 102 225	(4°C) after 90 days RA free 46%, RA imm 92%, (25°C) RA free 21%, RA imm 63%	28 reuses RA 85%		[99]
Tris(hydroxymethyl)phosphine oxide-polyetheramine copolymer particles activated with GA		55 U/g dry carrier	5.0 15.0	7.0 6.4	60 70	3.5 5.0		(4°C) 31 66 (20°C) 20 41			[100,101]
Tris(hydroxymethyl)phosphine-polyetheramine copolymer films		5.5 U/cm^2								Spec. activity 1140 U/g dry support	[102]
Mica sheets plasma-treated and chlorobenzylated										AFM studies; Stable enzyme preparation	[103]
Chitosan gel membrane crosslinked/activated with GA, thickness 0.1 mm	94	31.8; 1.56 U/cm^2; 1100 U/g carrier	5.01 26.4	7.2 6.35	65 75	5.71 7.37	(70°C) 120 250	(4°C) after 32 days RA free 0%, after 64 days RA imm 90%, (25°C) after 12 days RA free 0%, after 64 days RA imm 70%	After 120 h RA 40%, 9 reuses RA 20%	Study of effects of support on enzyme kinetics; improved resistance to inhibition by heavy metal ions, F^- ions, boric and aceto-hydroxamic acid	[104-109]
Kaolinite modified with 3-amino-propyl-triethoxysilane and GA			29.4 60.8	7.0 7.0	60 60	4.6 6.2				Soil urease properties	[110]
Montmorillonite modified with 3-aminopropyl-triethoxysilane + GA			29.4 45.0	7.0 8.5	60 60	4.6 3.2				Soil urease properties	[110]
Aminated butylacrylate-ethylene-dimethacrylate copolymer beads, porosity 39%, pore volume 0.49 cm^3/g, surface area 30 m^2/g, activated with GA	56	26.6; 1192 U/g carrier	9.95 14.6	7 5-7		5.76 6.37	(70°C) 127 62	(25°C) 7 85 (4°C) after 40 days RA free 0%, after 80 days RA imm 100%	10 reuses RA 20%		[111]
Cation exchanger Amberlite XP-64 (20-40 mesh) activated with 1-cyclohexyl-3-2(morpholinoethyl)-carbodiimide metho-*p*-toluene-sulfonate				6.6 8.0						Fixed-bed reactor: theoretical model	[112]

Table 1 (*Continued*)

Alkylamine derivative of Ti(IV) chloride activated porous silica			31 92	7.4 8.0	65 75	8.55			Used several hours a day for 50 days RA 100 %		[223]
Collagen-glycidyl methacrylate graft copolymer	80	166 U/g carrier	3.3 10	7.5 7.5	60 70			after 60 days RA imm 82%	5 reuses RA 24%	pH stability studied	[113]
Nylon tubing (0.1 cm internal diam) activated with GA			3.5 3.5	7.0 7.0		9.2 9.6	(75°C) 30 105	(4°C) after 15 days RA free 40%, RA imm 80%			[114]
Copolymer of *p*-amino-DL-phenylalanine and L-leucine diazotized, reacted with urease reversibly inactivated with p-chloromercuribenzoate, further reactivated with cysteine								after 5 months RA imm 60%		Column operation; Analysis of urea in body fluids and urea removal	[115]
Urease from Brasilian jack bean (*Canavalia brasiliensis*)											
Vapour phase stain etched porous silicon wafers modified with polyethyleneimine and GA			93.3 53.0	6.5 6.5	37 37		(40°C) after 60 min RA free 85%, RA imm 100%				[116]
Urease from pigeon pea (*Cajanus cajan*)											
Alkylamine glass beads activated with GA	92		3.35 3.55	7.3 6.8	47 77		(77°C) 8.5 120	after 70 days RA free 10%, RA imm 85%	10 reuses RA 30%	Potentiometric biosensing of blood urea in patients	[117]
Arylamine glass beads activated by diazotation	90		3.35 3.43	7.3 7.0	47 77		(77°C) 8.5 60	after 70 days RA free 10%, RA imm 83%	10 reuses RA 18%	Potentiometric biosensing of blood urea in patients	[117]
Chitosan beads activated with GA	64		3.0 8.3	7.3 8.5	47 77		(95°C) 6	(4°C) 31 110		Column operation for analysis of urea	[118]
Urease from watermelon (*Citrullus vulgaris*) seeds											
Cyanuric chloride DEAE-cellulose ether	72	636	3.3 12.5	7.5 7.5	55 65	7.1 7.7		(4°C) 49 106 (wet) ~188 (dry) (25°C) 24 33 (wet) 53 (dry)	20 reuses RA 83%	Resistance to heavy metal ion inhibition	[119]

Urease from sources unspecified in the reports cited

Poly(acrylonitrile)-chitosan composite membrane activated with GA	94	15.6 U/cm^2		5.8 5.8	28 30		(70°C) 127 173	(4°C) after 25 days RA free 0%, after 60 days RA imm 78%			[120]
Membrane, copolymerization of vinylized urease, acrylamide, 2-hydroxyethylmethacrylate and *N,N′*-methylenebisacrylamide			0.92	7.5	45					Membrane reactors	[121]
Expanded PTFE films grafted with 2-hydroxyethylmethacrylate				7.5 7.5	30 50						[122]
Polyethylene films (thick. 40 μm) grafted with acrylic acid activated with *N,N'*-dicyclohexylcarbodiimide (cation exchange membranes)	80			5.8 6.0	28 30		(40°C) after 5 h RA free 0%, RA imm 40%	(4°C) 11 56			[123]
Chitosan-tripolyphosphate complex beads, activated with GA	48		12.5 9.1	7.0 6.5	60 65			(4°C) 35	10 reuses RA 40%		[124]
Silica gel (60-100 mesh) aminated and phospholipid-coated							(100°C) after 1 h RA 100%	(25°C) after 42 days RA 100%		Very good stabilities	[125,126]
Polyamide ultrafiltration membrane (binding to membrane porous sub-structure by acyl-azid coupling)	10		4.87 59.9	6.8 7.2						Urease conversion in enzyme ultrafiltration experiments	[127]

Adsorption

Urease from jack bean (*Canavalia ensiformis*)

Pore expanded silica (surface area 920 m^2/g, pore size 10.4 nm); silica (surface area 1078 m^2/g, pore size 3.8 nm); silica gel adsorbent (surface area 443 m^2/g, pore size 6.5 nm)										Urease activity: silica > silica gel >> pore expanded silica; Control of soil urease activity	[128]
Poly(2-hydroxyethyl methacrylate-*co*-*N*-methacryloly-L-histidine-methylester) beads, surface area 13.5 m^2/g		0.0086 U/cm^2	16 26	7.0 6.5	45 50		(60°C) 64.5 193.5 (70°C) 11.0 77.9				[129]
Poly(2-hydroxyethyl methacrylate-*co*-*N*-methacryloly-L-histidine-methylester-Ni(II) beads (23.8 μmol Ni/g polymer)		0.015 U/cm^2	16 21	7.0 6.5	45 50		(60°C) 64.5 272.7 (70°C) 11.0 95.2			Column operation	[129]
Procion Brown MX-5BR-Ni(II) attached polyamide hollow fibers	37		18 22	7.0 6.0	45 55	1.47 2.73	(60°C) 47 115		After 60 h RA 92%		[130]

Table 1 (*Continued*)

Palmityl-substituted Sepharose 4B (hydrophobic support); prepared by mixing urease with support in water, denaturating enzyme with acidic pH followed by renaturation	105		2.85 1.11	7.5 7.5			(65°C) after 120 min RA free 70%, RA imm 100%		After 50 h RA 100%	Column operation	[131]
Polyaniline membrane		0.022 U/cm^2	8.7 11.7					(4°C) 20 14 (25°C) 6 6	7 reuses RA 10%		[132]
Hydroxyapatite (adsorption prevented by humic acid)		304	7.45 6.89	7.0 8.0				(25°C) 3.84 7.60		Resistance to proteolysis. Properties of soil urease	[133,134]
Vermiculite particles	82		521 793	6.5 6.5	25 65			(4°C) after 60 days RA 69%, (25°C) RA 30%	4 reuses RA 75%		[135]
Petroleum-based activated charcoal	80							7-9		Hexamethyldisiloxane coating to improve biocompatibility	[136]
Poly(ethyleneterephthalate) fibers grafted with methacrylic acid-acrylamide			2.82 3.71	7.0 7.0	52 60	2.81 4.12	(85°C) 81 205	(4°C) after 120 days RA free 35%, RA imm 100%, (25°C) after 120 days RA free 8%, RA imm 84%	40 reuses RA 100%		[137]
Aminated polysulphone membrane			5.0 22.1	7 5-6.5	62 70	1.36 1.86	(70°C) 120 50	(4°C) after 30 days RA free 0%, RA imm 75% (25°C) 2 15	After 140 h RA 40%, 17 reuses RA 60%		[138]
Diatomaceous earth activated with TiO_2, ethylenediamine-crosslinked		1								Water reclamation in spacecraft	[18]
Montmorillonite	71	180	11.7 3.6	7.1 7.1	60 60		(60°C) after 1 h, RA free 70%, RA imm 55%			Proteolysis easier than of free enzyme. Properties of soil urease	[139]
Non-crystalline $Al(OH)_3$	15	51	11.7 8.9	7.1 7.1	60 60		(60°C) after 1 h, RA free 70%, RA imm 55%			Proteolysis easier than of free enzyme. Properties of soil urease	[139]

$Al(OH)_3$-montmorillonite	64	67	11.7 6.6	7.1 7.1	60 60		(60°C) after 1 h, RA free 70 %, RA imm 55%			Proteolysis easier than of free enzyme. Properties of soil urease	[139]
Alumina particles (80-100 mesh), surface area 231 m^2/g, porosity 0.507 cm^3/g	141	25.17 U/g carrier						(4°C) after 1 month RA 57%			[140]
Polyvinylidene difluoride hydrophobic membrane (Immobilon)								1.36 year		Determinations of urea in serum	[141]
Kaolinite			12 30	6.65 6.65						Two-fold activation	[142]

Urease from pigeon pea (*Cajanus cajan*)

DEAE cellulose paper	51		3.0 4.75	7.3 6.8	47 67			(4°C) 21 150 (27°C) 10 120		Low-tech dipstick method of urea estimation	[143]
Gelatine beads activated with GA	75		3.0 8.3	7.3 6.5	47 65		(74°C) 30	(4°C) 31 240		Blood urea estimations	[144]
Cotton cloth activated with polyethyleneimine and crosslinked with dimethyl suberimidate	56							(4°C) 21 70	7 reuses RA 75%		[145]

Urease from watermelon (*Citrullus vulgaris*) seeds

2-hydroxyethyl methacrylate and itaconic acid copolymer gel; γ-irradiation polymerization	41	216.8	3.3 6.25	7.5 7.5					7 reuses RA 20 %	Improved resistance to inhibition by heavy metal ions	[224]
Macroporous styrene-divinylbenzene copolymer granules		11 U/g carrier								Inhibition by Ag, Pb, Cd for their sensing	[146]

Urease from soybean (*Glycine max*)

Poly(3-mercaptopropyl)siloxane precipitate	102-125							after 300 days RA 90 %			[225]

Urease from horse gram (*Dolichos biflorus*) seeds

Porous silicon								26	7 reuses RA 77%		[147]

Urease from soil bacterium *Bacillus pasteurii*

Ca-polygalacturonate gel	100		235 315	8.0 8.0				(30°C) 3.3 13.7		Soil urease properties No proteolysis.	[148]

Table 1 (*Continued*)

Urease from sources unspecified in the reports cited

Porous polyethylene hollow fibers modified with diethylamine (anion exchange membrane), inner diam 2.0 mm; urease adsorption followed by crosslinking with transglutaminase										For decomposition of concentrated urea by permeation through membrane; 4 M urea decomposed in 3 min	[149]
Membranes made of acrylonitrile modified with: -2-dimethylaminoethyl methacrylate -diacrylamido-2-methyl- propane-sulfonic acid	93 75			6.0 6.0 7.0 7.5	30 30 30 30					Test-strips for blood urea analysis	[150]

Encapsulation

Urease from jack bean (*Canavalia ensiformis*)

Alginate beads			3.13 5.56								[151]
Chitosan-alginate polyelectrolyte complex beads, diam. 2 mm			4.5 3.03	7.5 8.0	55 60		(75^0C) 22 27	(4^oC) 7 70	20 reuses RA 55%		[152]
Polyelectrolyte capsules by LbL of poly(L-lysine) and poly(L-glutamic acid) on mesoporous silica spheres with adsorbed urease (diam. 2-4 μm, surface area 630 m^2/g, pore diam. 2-40 nm); the core dissolved with HF; urease loading 25 mg/mL capsule	49									Capsules used as biomimetic reactors for $CaCO_3$ (calcite and vaterite) precipitation exclusively inside the capsules	[153]
Polyelectrolyte capsules by LbL of poly(allylamine) hydrochloride and sodium poly(styrene-sulfonate) on melamine formaldehyde particles diam. 5 μm, the core decomposed at pH 1; wall thickness 16 nm; Urease loading from water/ethanol solution	13							(7^oC) after 5 days RA free 55%, RA imm 100%		Enzymatic nanoreactors	[154]
Chitosan-coated alginate capsules (coating prevents proteolysis)										Therapeutic enzyme intestinal delivery	[155]
Alginate capsules coated with poly(methylene co-guanidine), diam. 1.2 mm	31	41.7									[156]
Carboxymethylcellulose/alginate microspheres coated with chitosan, diam. 400-700 μm			2.85 3.94	7.5 7.0	50 65	2.83 4.52	(80^oC) 79 142		20 reuses RA 80%		[157]

Xanthan-alginate spheres activated with GA, diam. 1.8 mm				7.5 7.5	50 60				20 reuses RA 75%		[158]
Magnetic alginate beads(magnetized by a urease-dependent reaction)		43 U/g carrier								Magnetic drug delivery systems	[159]
Nylon microcapsules containing urease and haemoglobin; interfacial polymerization, diam. 205 μm	84		7.6 8.4	6.0 6.0							[160]
Nylon microcapsules; interfacial polymerization, diam. 266 μm; 94 % urease in capsules, 6 % bound	92	129.5								Concentration of urease in micro-capsules 62.3 mg/mL	[161]
Cellulose acetate butyrate micro-capsules containing urease and zeolite; phase separation										Oral therapy for urea removal in uraemia	[162]
Egg lecithin liposomes, diam. 0.2-1.3 μm			68 167	6.9 6.9		7.5	(70°C) 121 170	5 20		T<30°C, E_a = 17.5 kcal/mol; T<30°C, E_a = 8.9 kcal/mol	[163]
Nylon capsules, diam. 10 μm	90		2.72 3.2	6.65 5.0							[164]
Urease from watermelon (*Citrullus vulgaris*) seeds											
Alginate capsules		~2800	11.4 13.3	8.0 7.0	65				7 reuses RA 50-60%	Resistance to inhibit-tion by urea; Stability in organic solvents	[165,166]
Urease from recombinant *Helicobacter pylori*											
Hydrophobically (C12 alkyl chains) modified alginate capsules, diam. 10 μm										Nasal, subcutaneous and oral immuniza-tion of mice against *H.pylori*	[167]
Urease from bacterium *Lactobacillus fermentum*											
Alginate spheres (precipitated with Al^{3+}), diam. 2 mm										Urease-aided preci-pitation of porous alumina particles	[168]
Urease from sources unspecified in the reports cited											
κ-carrageenan capsules			65.6 96.4	7.0 7.0	37 37			(4°C) 3 4			[169]
Chitosan/poly(vinyl alcohol) capsules, diam.0.9 mm; coacervation				6.0 5.4							[170]
Tetraethoxysilane sol-gel films	12									Biosensors	[171]

Table 1 (*Continued*)

Gel entrapment

Urease from jack bean (*Canavalia ensiformis*)

Poly(acrylamide-*co*-acrylic acid)/κ-carrageenan inter-penetrating polymer network gel			4.5 3.30	7.5 8.0	55 55		(75°C) 22 40	(4°C) 7 ~130	20 reuses RA 80%		[152]
Sol-gel sodium silicate			1.3 1.9							Coimmobilization with fluorescein-dextran; reagentless kinetic assays	[172]
pH-responsive gel particles; redox polymerisation of *N*-isopropyl-acrylamide (thermosensitive monomer) and *N*-vinylimidazole (pH sensitive monomer)										Biochemo-mechanical system with enzyme reaction-regulated properties of the gel	[173,174]
Conducting poly(methylmethacryl-ate)/pyrrole matrix on Pt electrode			2.82 13.9								[175]
Poly-*N*-isopropylacrylamide gel, particle size 20-100 μm			2.4 3.7	6.2 5.2		7.6 7.6				Thermo-sensitive material;Temperature dependent anomalies of K_M, v_{max} and *Ea*	[176,226]
Polysiloxane polymer composed of 3-aminopropyltriethoxysilane and tetraethylorthosilicate (1:3)			225 170	6.5 6.0	50 >70			(4°C), after 18 weeks RA 65%			[177]
Poly[di(methoxyethoxyethoxy) phosphazene] hydrogel crosslinked by γ-radiation	80										[178]
Copolymer of 2-hydroxyethyl methacrylate and *N*-vinyl-pyrrolidone crosslinked with ethylene glycol dimethyl acrylate; γ-radiation polymerization		5.62 U/cm^2								Study of urea diffusion across gel	[179]
Polyacrylamide gel crosslinked with *N,N'*-methylenebisacrylamide			25 24.5	6 7	30 40	9.15 6.09		(5°C) after 30 days RA 100%, (15°C) after 45 days RA 50%	15 reuses RA 94%	Batch and column reactor	[180]
Cellulose-TiO_2 composite gel fibers, diam. 0.12-0.5 mm, surface area < 1 m^2/g; 8 wt% enzyme and 18 wt% TiO_2 in dry fibers			0.94 800	7.0 7.0	60 70				24, 20 reuses RA ~100%	Resistance to inhibition by urea; Urea determinations with NH_3 electrode	[181,182]

Urease from pigeon pea (*Cajanus cajan*)

Agar gel	52		3.23 5.07	7.3 7.5	30 60			(4°C) 21 53		Assays of blood urea	[183]
Polyacrylamide gel crosslinked with *N*,*N*′methylenebisacrylamide	50							(4°C) 21 200	9 reuses RA 90%	Gel strips for urea analysis in blood	[184]

Urease from watermelon (*Citrullus vulgaris*) seeds

Agarose gel membrane	88	2660	11.4 9.3	8.0 8.0	65 65		(52°C) 16.7			Resistance to inhibition by urea	[185]

Urease from leaves of lambsquarters (*Chenopodium album*)

Gelatine film activated with GA		230			30 40		(70°C) 8 65			Stability against detergents and inhibition by DTNB, and Hg compounds	[186]

Urease from soil bacterium *Bacillus pasteurii*

Polyurethane foam			17.3 23.0					(30°C) after 7 days RA free 10%, RA imm 90%		Resistance to proteolysis. Enzyme-aided calcite precipitation	[187]

Urease from sources unspecified in the reports cited

Polyion complex membrane composed of quaternized chitosan and Na carboxymethyl-cellulose			1.27	7.5	37					Membrane reactors	[121]
Cellulose acetate-Zr gel fibers	19		95 158						After 50 days RA 40%		[188]
Polypyrrole-polyvinyl-sulphonate films deposited on indium-tin oxide glass plates			2.9 2.77	7.5 8.0						Films to be applied in biosensors	[189]
Gel of 2-hydroxyethylmethacrylate and *N*-vinyl pyrrolidone; γ-irradiation polymerization	56								10 reuses RA 72%		[190]
pH-sensitive gel composed of *N*-isopropylacrylamide (hydrophobic monomer) and acrylic acid (ionic monomer) crosslinked with *N*,*N*′-methylenebisacrylamide										Biochemo-mechanical system with enzyme reaction-regulated properties of the gel	[191]

Table 1 (*Continued*)

Gelatin (also with poly(acrylamide) and CMC) crosslinked with Cr(III)				8.0 8.0	52 52				24 reuses RA 76%	T_{opt} 65°C for CMC modified gelatine	[192,194]
Dimethylamino nylon gels quaternized with cationic oligomers			4.8 20.0	7.5 6.0						Effects of support electrostatic potential and of urea diffusion - partition in gels on enzyme kinetics	[195,196]
Crosslinking											
Urease from jack bean (*Canavalia ensiformis*)											
BSA films crosslinked with GA, thick. 15 μm deposited on polyurethane foam	50	20.4	19.2 19.8	7.2 7.0		7.8 8.7		(10°C) after 120 days RA 100%		Resistance to urea inhibition; Batch squeezer, flow reactor, electrodialyer	[197-199]
Other methods											
Urease from jack bean (*Canavalia ensiformis*)											
Langmuir-Blodgett films on quartz crystal/plate and on ITO, of: (i) urease (ii) urease with dipalmitoyl phosphatidyl glycerol	36 300							(10°C) after 7 days RA free 82%, RA imm (i) ~82%, RA imm (ii) 93%			[200]
LbL assemblies of poly(diallyl-dimethyl-ammonium chloride) and urease at pH 4.0, and of Na poly-(styrene-sulfonate) and urease at pH 8.0, on polystyrene colloid particles, diam. 180 nm	24							(25°C) after 4 days RA free 18%, RA imm 80%		Stable assemblies with urease at pH 8.0; Addition of salts enhances activity; Nanobioreactors	[201]
LbL assemblies of polyethyleimine, and poly(styrene-sulfonate) + urease at pH 8.5, on silicon microchannels									5		[202]
Nonwoven cellulose membrane with attached avidin; urease biotinylated; carrier-enzyme complex formation based on biotin-avidin recognition		1617								Urea removal via enzymatic hydrolysis and electrodialysis	[203,227]
Micelles of dioctyl Na sulpho-succinate and polyoxyethylene isooctyl phenol in xylene; entrapment in reverse micelles			250 110	7.0 7.0	55 55			(20°C) after 15 days RA free 0%, RA imm 35%			[204]

LbL assemblies of poly-(dimethyl-diallyl ammonium chloride) and Na poly(styrene-sulfonate) + urease at pH 8.5 and arginase on NH_3 electrode										Biosensor for L-arginine detection in multistep enzyme reaction L-arginine → urea → NH_3	[205]
LbL assemblies of poly(dimethyl-diallyl ammonium chloride) and urease at pH 8 on polystyrene spheres, diam. 470 nm	25									Bionanoreactors	[206]
Containment of urease between isoelectric membranes in a reactor operating under electric field										Removal of urea	[207]
Urease immobilized between cation- and anion-exchange membranes (biopolar membrane)										Transport properties of biopolar membrane	[208]
Filter paper (electron irradiation polymerization in the presence of A-14G monomer)											[209]
Containment in the shell side of a hollow fiber module			58.4 52.5						After 2 months RA 100%		[210]
Complex formation with - tannic acid - Fe^{3+}-tannic acid - OH-Al-tannic acid	 21 46 62		 23.6 25.1 23.6 25.1 23.6 24.3	 7.0 7.5 7.0 7.0 7.0 7.0	 60 70 60 70 60 70	 5.21 6.78 5.21 5.77 5.21 5.13				No resistance to proteolysis; Study for properties of soil urease	[211]
Urease conjugated to polyclonal antiurease antibody covalently bound with GA to nylon-coated stirrers, nylon tubing or discs			4.5 7.5	7.0 7.0				(4°C), after 30 days RA 85%		Biorector connected to differential pH-meter for urea determination	[212,213]
Urease from soybean (*Glycine max*)											
Solution of urease and Na alginate contained in a membrane reactor				7.0 7.5						Diffusion-reaction and electrostatic potential effects	[214]
Urease from sources unspecified in the reports cited											
Metallochelate immobilization on silica carrier: vulcasil, grain size 20 nm, activated with $TiCl_4$		565.14	18 27.6	5.8 5.8		5.08 5.05	(50°C) 70 105				[215]

[a] Activity retention.
[b] Specific activity is given in U/mg protein, where U stands for μmol NH_3/min, unless otherwise stated.
[c] Glutaraldehyde.
[d] Remaining activity.

method, potentiometrically with use of ammonium ion-selective electrodes [30], enzymatically with use of glutamate dehydrogenase or horseradish peroxidase, in addition to simple titration, carbon dioxide can be determined with use of ^{13}C or ^{14}C labeled urea [31] or with carbon dioxide gas-selective electrodes. Measurements of pH [32] and of conductivity [33] are also applied. These biosensing systems commonly operating with soluble urease, become overwhelmingly simplified if changed into biosensors, where the enzyme is integrated with a transducer [34–36]. The integration is achieved by immobilizing the enzyme directly on transducer's working tip or in/on a membrane tightly wrapping it up. Since the first urea biosensor prepared by Guilbault et al. in 1969 [37,38], a great number of urease-based biosensors have been constructed and tested [220]. They employ techniques, such as spectrometry [39,40], potentiometry with the application of pH-sensitive electrodes, ammonium ion selective electrods and ammmonium ion-sensitive field effect transistors [41–46], conductometry [28,47,48], amperometry [49,50], as well as acoustic [51] and thermal [52] methods, to name the few. Practical, cost-effective and portable analytical devices, especially useful for in situ and real-time measurements, the biosensors are predicted to become widely accepted for use, once their storage and operational stabilities are improved.

The same promising features have urease-based biosensors and biosensing systems for the analysis of substances that act as inhibitors of the enzyme [53,54]. The measurements are based on the amount of inhibition provoked by the inhibitors, and they exploit enzyme sensitivity to sometimes infinitesimal concentrations of some inhibitors. Such biosensors offer enormous potential for measurements of trace levels of pollutants in environmental screening and monitoring, food control and in biomedical analysis. Due to its pronounced sensitivity, urease is especially disposed for the determination of heavy metal ions [55–57], Hg ions in particular [58]. Yet, in addition to the stability problems, the inhibition-based biosensors also suffer from the lack of selectivity in real samples [59]. This, however, has been proposed to be solved by developing hybrid systems of enzymes showing different sensitivities to different inhibitors [60,61].

3.3. Urease-aided mineralization processes

Comparatively new, urease-aided mineralization processes take advantage of the supply of dissolved inorganic carbon derived from urea hydrolysis and of an increase in pH generated by the reaction [62]. The latter, in the presence of calcium(II) ions in the reaction medium, induces the precipitation of calcium carbonate. The processes mimic calcium carbonate formation occurring in nature, where beside photosynthesis and sulphate reduction, bacterial urease-catalyzed hydrolysis of urea is believed to play a vital role [62]. Compared to the typical techniques of preparative solid-state chemistry, the biomineralization processes usually occur at room temperature and under mild conditions. Their application derives from the increasing demand for the preparation of advanced carbonate materials in an environmentally benign manner. Interestingly, the formation of different amounts and different polymorphic phases of calcium carbonate (calcite, aragonite, vaterite) have been reported depending on the type of urease and reaction conditions used [63,64]. In addition to preparing advanced carbonate materials, bio-induced precipitation of $CaCO_3$ has been proposed for a number of novel biotechnological applications. One is a solid-phase capture of excess soluble Ca^{2+}, radionuclide and trace element contaminants, utilized in cleaning waste- and groundwaters [65–67]. Another exciting application is as microbial sealants for plugging surface cracks and fissures in buildings [68,69], notably in restoration of historic monuments [70] for remediation of their surfaces and structures. The remediation consists of in situ carbonate precipitation upon filling the site to be plugged, with a reaction mixture containing urea, urease and Ca(II) ions. A similar carbonate plugging is also applied in oil reservoirs. There, its function is to prevent sand transportation during oil production from unconsolidated reservoir formations as well as to reduce permeability of porous areas of the reservoirs done to improve secondary oil recovery [221,222]. Apart from calcium carbonate, in a similar urease-aided biomimetic manner also other inorganic materials have been prepared, including aluminium hydroxide [71], aluminium basic sulfate [72], hydrotalcite [73], hydroxyapatite precursors [74] and hydroxyapatite-like phases, these to be used for bone regeneration [229].

3.4. Other applications of immobilized ureases

In addition to the presented applications of ureases, the enzymes are also immobilized for other purposes. For instance certain urease-entrapped gels are studied as smart materials having enzyme reaction-regulated properties. Owing to the controlled hydrolysis of urea the gels are capable of converting biochemical energy into mechanical work through swelling and shrinking. Ureases are also immobilized on selected soil materials in order to gain insights into behaviour and properties of soil urease. In the same agricultural context adsorption of urease on selected materials is tested as a possible means of reducing the activity of soil urease. Also, various multi-enzyme immobilizations are performed mainly for analytical purposes. The immobilizations are included in the compilation in Table 1.

4. Immobilizations of ureases

Table 1 compiles reports on immobilizations of ureases covering the last two decades, with few earlier reports found important. The compilation was prepared with the intention to survey the range of techniques and support materials applied for urease immobilizations, but first and foremost to collect the properties of the enzymes resulting from the chosen immobilization procedures. This was done in the hope that it may provide useful guidance through the wealth of data available in the literature, but more importantly to develop an integrated perspective on how to customize the enzymes for their specific applications. The number of reports collected implies that there is an ongoing vivid search for such customized ureases, and they can play a decisive role in advancing their applications.

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