



## Biological roles and applications of urease – A review

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### Abstract

Urease is a metalloenzyme that catalyzes the hydrolysis of urea to yield ammonia and carbon dioxide. Spontaneous degradation of urea occurs with a half-life of approximately 3.6 years, but in the presence of urease, the hydrolysis of urea is  $10^{14}$  times faster. The best genetic data concerning plant ureases are available for soybean. Separate genes encoding two urease isoenzymes, a tissue-ubiquitous and embryo-specific, as well as the unlinked genes encoding regulatory proteins, were identified in soybean and mutants are available. The embryo-specific urease is an abundant seed protein in many plant species, including soybean, jack bean and Arabidopsis, while the other type of urease (called ubiquitous) is found in lower amounts in vegetative tissues of most plants. Bacterial ureases have been shown to be important virulence determinants in the pathogenesis of many clinical conditions in human and animals. Urease is directly involved in the formation of infection stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma and urinary catheter encrustation. Urease is known to be the major cause of pathologies induced by *Helicobacter pylori*, which allows this pathogen to survive at the low pH of the stomach during colonization and therefore plays an important role in the pathogenesis of gastric and peptic ulcers, which in some cases may progress to cancer. In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. This further induces plant damage primarily by depriving plants from their essential nutrients and secondly through ammonia toxicity and carbon dioxide release that increases the pH of the soil. Most of our knowledge about the molecular mechanism of ureolytic catalysis by plant ureases is based on the 3-D structures of bacterial ureases.

**Key words:** urease, applications, human health, bacterial urease, plant urease

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### Introduction

Urease (urea amidohydrolase; EC 3.5.1.5) play diverse roles and mainly involved in the nitrogen metabolism. The bacterial urease has been extensively studied may be due to the simplicity of system and its significance in various pathological conditions and role in agriculture etc. Jack bean (*Canavalia ensiformis*) was the first enzyme to be crystallized (Sumner, 1926) and it played an important historical role as proof of the proteinaceous nature of enzymes. Also jack bean urease was the first nickel-containing enzyme to be described (Dixon *et al.*, 1980) and it is the only nickel-containing metalloenzyme identified so far in plants (Polacco and Holland, 1993). Its rapid catalysis for the hydrolysis of urea to ammonia and carbon dioxide plays an essential role in agriculture

and human health (Mulvaney and Bremner, 1981; Mobley *et al.*, 1995). Urease is a metalloenzyme that catalyzes the hydrolysis of urea to yield ammonia and carbon dioxide (Dixon *et al.*, 1980). Spontaneous degradation of urea occurs with a half-life of approximately 3.6 years, but in the presence of urease, the hydrolysis of urea is  $10^{14}$  times faster. Although urea is the major substrate of urease, this enzyme is capable of hydrolyzing (albeit poorly) other substrates such as acetamide, formamide, N-methylurea, semicarbazide, and hydroxyurea (Dixon *et al.*, 1980). In urea hydrolysis, plant and microbial ureases exhibit  $K_m$  values ranging from 0.1 to  $>100$  mM urea; jack bean urease has a  $K_m$  value of 2.9 mM (Mobley and Hausinger, 1989).

Urease is known to be a major cause of pathologies induced by *H. pylori*, which allows the

bacteria to survive at the low pH of the stomach during colonization and, therefore, plays an important role in the pathogenesis of gastric and peptic ulcers (including cancer) (Mobley *et al.*, 1995). In infections with *P. mirabilis* and *Y. enterocolitica*, urease has been implicated in urolithiasis (stone formation) and contributes to the development of acute pyelonephritis and infection-induced reactive arthritis, respectively. In agriculture, high urease activity (soil bacteria) causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. Accumulation of ammonium ions, loss of urea N as ammonia, nitrite as well as ammonia toxicity damages germinating seeds, seedlings, and young plants. These problems can be successfully tackled by undertaking the inhibition studies of urease of ureolytic bacteria (found in soil) and from any other source as model system. Several classes of urease inhibitors are known, and some have been examined for their pharmacological and agricultural value (Mulvaney and Bremner, 1981; Mobley and Hausinger, 1989). Hydroxamic acids, phosphorodiamidates, imidazoles, phosphazene and related compounds are some classes of urease inhibitors extensively studied. Earlier we have published few review articles on urease, where we have addressed the various issues on urease (Kumar, 2015; 2016)

### **Occurrence and role of urease**

Ureases are synthesized by numerous organisms, including plants, bacteria, algae, fungi and invertebrates, and also occur in soils as a soil enzyme. The substrate urea for the reaction is readily available. Its pervasive presence arises chiefly from urine excretion by animals and from the decomposition of N-compounds from dead organisms (Wang *et al.*, 2008), and also from its application as a fertilizer. Thus, owing to their occurrence, ureases play a prominent role in the overall nitrogen metabolism in nature. Their key function is to provide organisms with nitrogen in the form of ammonia for growth.

### **Urease in plants**

Biochemically, the best-characterized plant urease is that from jack bean (*Canavalia ensiformis*) (Hirai *et al.*, 1993; Karmali and Domingos, 1993; Takishima *et al.*, 1988). Urease from mulberry (*Morus alba*) leaves has also been purified and characterized (Hirayama *et al.*, 2000). The best genetic data concerning plant ureases are

available for soybean (*Glycine max*) (Polacco and Holland, 1993; 1994). Separate genes encoding two urease isoenzymes, a tissue-ubiquitous and embryo-specific, as well as the unlinked genes encoding regulatory proteins (see below), were identified in soybean (Meyer-Bothling and Polacco, 1987; Torisky *et al.*, 1994) and mutants are available. The embryo-specific urease is an abundant seed protein in many plant species, including soybean, jack bean (Polacco and Holland, 1994) and *Arabidopsis* (Zonia *et al.*, 1995), while the other type of urease (called ubiquitous) is found in lower amounts in vegetative tissues of most plants (Hogan *et al.*, 1983).

Urease, beside urea amidolase, is an essential urea-degrading enzyme in plants that catalyzes urea assimilation after uptake into plant cells (Wang *et al.*, 2008; Kojima *et al.*, 2006). Not entirely yet elucidated, higher plants were shown to possess various urea transport systems, passive and active, which allow them to optimize N-nutrition depending on the nitrogen form available from external environment or internally synthesized. From external environment, plants assimilate urea through roots as urea, but essentially as ammonia generated from urea hydrolysis, and this is possible due to the presence of ureases in soils, a fact exploited in urea fertilization practices. Importantly, high inputs of urea fertilizers applied may constitute a serious hazard both to plants and the environment. To enhance fertilization practices, urea is also applied through the foliage. Absorbed rapidly, foliar-applied urea, however, can be toxic in high concentrations (Wang *et al.*, 2008). Clearly, further knowledge on the mechanisms of urea-related plant nutrition is needed for the development of balanced strategies of urea-fertilization for best and sustainable agricultural crop production.

In plant cells on the other hand, urease participates in the metabolism of N-containing compounds (Wang *et al.*, 2008; Follmer *et al.*, 2008a; Kojima *et al.*, 2006). Therein, apart from being acquired from external environment, urea is an important intermediate resulting from two metabolic processes: arginase-catalyzed breakdown of arginine (Zonia *et al.*, 1995) and degradation of purines and ureides (Winkler *et al.*, 1988; Todd *et al.*, 2006). Metabolized rapidly, urea practically does not accumulate, however, constantly generated may serve as an N-source. It has also been hypothesized that due to the generation of ammonia, urease fulfils a defense function against plant pathogens (Polacco and Holland, 1993). In the

same context, recently, evidence has been provided that independent of their ureolytic activity, ureases also exhibit insecticidal (Follmer *et al.*, 2004b) and antifungal properties (Menegassi *et al.*, 2008), suggestive of their function in plant defense system. Present virtually in all plants, urease is especially abundant in leguminous seeds, those of soybean (*Glycine max*) containing 0.012% urease/dry mass and those of jack bean (*Canavalia ensiformis*) 0.07–0.14%, the latter thus being one of the commonest sources of the enzyme (Sumner, 1926; Weber *et al.*, 2008).

### Bacterial urease

Bacterial urease plays an important role in the pathogenesis of a number of bacterial species including *Proteus mirabilis*, *Staphylococcus saprophiticus*, *Yersinia enterocolitica*, *Ureaplasma urealiticum* and others (Mobley *et al.*, 1995). Due to urease activity, bacteria (e.g. *Klebsiella aerogenes*) are able to use urea as a sole nitrogen source (Mulrooney *et al.*, 1989). In the case of *Vibrio parahaemolyticus*, the ability to hydrolyze urea was proposed as a simple screening test to predict which strains are potentially pathogenic (Kaysner *et al.*, 1994). One of the most frequently mentioned examples in the recent literature is the urease from *Helicobacter pylori* because of its essential role in the pathogenesis of this microorganism and the high prevalence of this human pathogen (Eaton *et al.*, 1991). However, the best structural data are available for the urease from *K. aerogenes* (Jabri *et al.*, 1995).

Microbial ureases play an important role in the nitrogen metabolism of ruminants such as cattle, sheep, and other animals that contain a forestomach. Substantial amounts of animal-derived urea are recycled to the rumen, where ureolytic activity releases ammonia, the major source of nitrogen for most ruminal bacteria. The microbial biomass generated is then utilized as a nutrient by the ruminant. Urea hydrolysis also occurs in the intestinal tract of monogastric species such as humans, pigs, rats, cats, mice, and rabbits, but nitrogen cycling is quantitatively less important in these organisms compared with ruminants. In humans, approximately 20% of the urea produced by the liver is transferred by diffusion from the bloodstream to the intestinal tract and hydrolyzed by urease. As discussed above, several pathological states are associated with excessive intestinal urea hydrolysis. U.S. investigators have reported that the most numerous ureolytic bacteria in human faeces

was *Peptostreptococcus productus*, although ureolytic strains of *Ruminococcus albus*, *Clostridium innocuum*, *Clostridium beijerinckii*, *Fusobacterium prausnitzii*, *Coprococcus catus*, and *Streptococcus mitis* were also isolated. As in the case of the bovine rumen, most ureolytic bacteria in the human intestine are anaerobes.

### Urease from other organisms

Urease activity was found in several species of fungi, however, the nucleotide sequences of the genes encoding urease were reported for only a few of them, including a fungal respiratory pathogen of human *Coccidioides immitis* (Yu *et al.*, 1997) and *Schizosaccharomyces pombe* (Tange and Niwa, 1997). In the invertebrate *Aplysia californica*, urease, together with carbonic anhydrase, is required for the formation and homeostasis of statoconia, calcium carbonate inclusions in the lumen of the gravity-sensing organ, the statocyst (Pedrozo *et al.*, 1996a; 1996b). Urease is a cytosolic enzyme. In most of the studied cases the majority of its activity is associated with the soluble fractions of the cells (Mobley *et al.*, 1995).

### Soil urease and ammonia volatilization

The role of soil urease is in making urea available to plants through converting it into ammonia. Significant though it is, the hydrolysis may also have adverse effects. Namely, if too rapid, it may result in unproductive loss of nitrogen by ammonia volatilization, while ammonia toxicity and alkalinity along with accumulated nitrite may induce plant damage by affecting seed germination, seedling growth and early plant growth in soil, thereby causing severe environmental and economic problems (Sahrawat, 1980; Mulvaney and Bremner, 1981; Bremner and Krogmeier, 1989). Ammonia volatilization is also a problem faced in management of livestock wastes, these being presently produced in increasing amounts due to considerably intensified farming practices (McCrary and Hobbs, 2001). This volatilization entails a number of undesirable consequences. One is that livestock slurry, a valuable fertilizer for crop production, has its value considerably reduced by loss of nitrogen. Another one is that ammonia is a source of pollution, and besides, it contributes to odour that may have an adverse impact on people and animals. These problems emphasize the need for further research to find out effective methods to solve the problems encountered in the use of urea as fertilizer (Beaton, 1978; Engelstad and Hauck, 1974). One of the most favored approaches is the

inhibition of the urease of ureolytic bacteria found in soil, and thereby inhibiting the urea hydrolysis that means application of urease inhibitors to soils in conjunction with fertilizers. A number of studies have demonstrated that this approach can be very fruitful. In last several years, numerous compounds have been synthesized and patented as inhibitors of urea hydrolysis in soil (Hauck, 1984; McCarty, 1990).

### Human and animal health

Bacterial ureases have been shown to be important virulence determinants in the pathogenesis of many clinical conditions in human and animals. Urease is directly involved in the formation of infection stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma and urinary catheter encrustation. Urea is known to be the major cause of pathologies induced by *Helicobacter pylori* (HP), which allows *H. pylori* to survive at the low pH of the stomach during colonization and therefore plays an important role in the pathogenesis of gastric and peptic ulcers which in some cases may progress to cancer (Moblely and Hausinger, 1989; Mobley *et al.*, 1995; Smoot *et al.*, 1990).

Furthermore, it has also been suggested that the urease may have a role in the inactivation of “complement” (a component of the host defense mechanism). Therefore, strategies based on urease inhibition are now seriously considered as the first line of treatment for infections caused by urease-producing bacteria to reduce environmental pollution and enhance efficiency of urea nitrogen uptake by plants. The discovery of urease inhibitors has to date mainly relied upon random screening of tens of thousands of chemical compounds (Hamilton-Miller and Gargan, 1979). However, with the determination of high-resolution X-ray structures of native and inhibited ureases from BP (Benini *et al.*, 1999) and KA (Jabri *et al.*, 1995) it is now possible to rationally search for these inhibitors. These structural studies have revealed the intimate details of the molecular geometry of the enzyme as well as mechanism of urea hydrolysis, which has paved the way for structure-based design of potent inhibitors. In all the instances that require the control of urease activity (medical, agricultural, environmental), to counteract its deleterious effects, the use of enzyme inhibitors is proposed.

### Urease inhibitors

The inhibitions of urease were extensively studied because of their potential uses like: (i) therapy against bacterial urease (eg. *Helicobacter pylori*) that induced human pathogenic states, such as, urinary stone formation, peptic ulcer, pyelonephritis and hepatic coma, (ii) to protect soil from pH elavation and loss of nitrogen after use of urea fertilizer by controlling hydrolysis of urea in soil, and (iii) as an analytical technique for determining substances acting as enzyme inhibitor. Phosphate buffer, very common in the kinetic studies of urease, and long known to be inhibitory at neutral pH, had its inhibitory strength shown to be pH-dependent. This strength decreases with an increase in pH to cease at pH 7.0–7.5 (Krajewska and Zaborska, 1999; Todd and Hausinger, 1989). The inhibitory action of the buffer was ascribed to  $H_2PO_4^-$  ion (Krajewska and Zaborska, 1999; Todd and Hausinger, 1989; Dixon *et al.*, 1980), a point verified by the crystal structure of urease–phosphate complex at pH 6.3 (Benini *et al.*, 2001).

Several urea analogs have been examined as urease inhibitors, including alkylated ureas, various thioureas, hydroxyurea, and numerous hydroxamic acids. Substrate urea, product ammonium ions, and substrate analogues are weak inhibitors of urease. As revealed by pH-dependent kinetic study, thiols inhibit urease competitively in their thiolate anion form (Todd and Hausinger, 1989). Acetohydroxamic acid, a representative of numerous acylhydroxamic acids R-NHOH studied as inhibitors of plant (Blakeley *et al.*, 1969; Dixon *et al.*, 1980; Krajewska *et al.*, 2001), bacterial (Moblely *et al.*, 1988), fungal and soil ureases, was shown to be a slow-binding inhibitor with moderate strength. Owing to its low toxicity, it is one of the most intensively studied inhibitors for medical therapies to be used in ureolytic bacteria-induced pathological conditions (Rosenstein and Hamilton-Miller, 1984; Griffith *et al.*, 1978; Andersen, 1975).

Boric and boronic acids are rapidly binding urease inhibitors, comparatively weak. For boric acid, the maximum inhibitory activity was observed at pH between 6 and 9 (Breitenbach and Hausinger, 1988; Krajewska and Ciurli, 2005), suggestive of its action in the molecular form  $B(OH)_3$ . In contrast to the above inhibitors, the data on fluoride inhibition are less consistent. Namely, in a comprehensive study of *Klebsiella aerogenes* urease (Todd and Hausinger, 2000), fluoride was found to be an uncompetitive slow-binding inhibitor, however, for

jack bean urease, by virtue of F- binding to an active-site nickel ion, this inhibition was defined as competitive, while its time-dependent character also suggested that it be uncompetitive (Dixon *et al.*, 1980), and in (Krajewska *et al.*, 2001) it was interpreted as competitive slow-binding.

Heavy metal ions inhibit both plant and bacterial ureases at the following approximate order of effectiveness:  $\text{Hg}^{2+} \approx \text{Ag}^+ > \text{Cu}^{2+} \gg \text{Ni}^{2+} > \text{Cd}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Fe}^{3+} > \text{Pb}^{2+} > \text{Mn}^{2+}$  (Krajewska, 1991), with  $\text{Hg}^{2+}$ ,  $\text{Ag}^+$  and  $\text{Cu}^{2+}$  ions nearly always listed as the strongest inhibitors. Classified on the basis of the initial reaction rates measurements as noncompetitive, in the reaction progress curve studies this inhibition was best described as slow binding. This inhibition has been habitually ascribed to the reaction of the ions with the thiol groups of the enzyme, resulting in the formation of mercaptides. In practice, this inhibition is important for two reasons. One is that in view of heavy metal ion pollution, appropriate levels of urease activity in agricultural soils may be endangered. The other one is that this inhibition may be exploited in constructing urease inhibition-based sensing systems for in situ and real time determination of trace levels of the ions, e.g. in environmental monitoring, food control and biomedical analysis. Likewise, the involvement of urease thiol groups was found in the inhibition of the enzyme by bismuth compounds (Zhang *et al.*, 2006). The data on this inhibition (Zhang *et al.*, 2006) are of medical importance, because bismuth compounds are widely used as bactericidal agents in the treatment of peptic ulcers and *Helicobacter pylori* infections.

Amides and esters of phosphoric acid are also slow binding inhibitors of urease, classified as the strongest inhibitors. The kinetic analysis of their inhibition implied that irrespective of the compound, the inhibition is always brought about by the same diamidophosphate (DAP), a product of their hydrolysis (Andrews *et al.*, 1986). An interesting group of compounds within this class of inhibitors are derivatives of thiophosphoric acid, chiefly amides, which were shown to effectively be only precursors that become inhibitors upon their conversion into oxygen analogues. Due to their efficacy, a variety of derivatives of both phosphoric and thiophosphoric acids have been intensively studied for retarding urease hydrolysis in soils and against ureolytic bacteria infections.

The inhibition of ureases by quinones on the other hand has been mainly tested for their

potential application with urea fertilizers (Mulvaney and Bremner, 1981). The inhibition was reported non-competitive, but in other reports also slow-binding. A variety of other compounds were tested for their inhibitory potential towards ureases. Among those are ketones ( $\alpha,\beta$ - unsaturated,  $\alpha$ -hydroxyketones and cyclic  $\beta$ -triketones), Schiff base metal (Cu, Ni, Co, Cd, Mn) complexes, and notable for medicinal usage, compounds of natural origin, garlic- and herbs-derived. A group of novel inhibitors, P-methyl phosphinic and thiophosphinic acids, were designed, synthesized and studied. They proved to be competitive inhibitors, simple and slow binding, respectively, with  $K_i$  constants varying between  $1.7 \times 10^{-4}$  and 0.34mM for *Bacillus pasteurii* urease. P-methyl thiophosphinic acids appeared to be stronger inhibitors than their oxygen analogues.

In addition to their potential value in medicine and agriculture, the study of urease inhibitors can provide insight into selected aspects of the enzyme mechanism and active-site structure. The discovery of urease inhibitors has to-date mainly relied upon random screening of tens of thousands of chemical compounds. However, with the determination of high-resolution X-ray structures of native and inhibited ureases from *K. aerogenes* (Jabri *et al.*, 1995) and *B. pasteurii* (Benini *et al.*, 1999), it is now possible to rationally search for these inhibitors. These structural studies have revealed the intimate details of the molecular geometry of the enzyme as well as mechanism of urea hydrolysis, which has paved the way for structure-based design of potent inhibitors.

### Ureases and their applications:

The catalytic function of ureases is to catalyze the hydrolysis of urea to carbonic acid and ammonia as final products. 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 (Blakeley *et al.*, 1969). The most typical examples of such applications, where immobilized ureases are preferably used in place of free enzymes, are presented below.

### Urea removal from aqueous solutions

The removal of urea from aqueous solutions is a problem faced in numerous areas, e.g., in urea-producing industry, agriculture and natural environment, food production and medicine. In the environment, urea also comes from other industries that utilize urea, as well as from fertilized crop-

planted soils as fertilizer wastewater effluents, also as effluents from households, but primarily from urine excretion by animals. Although urea has generally low eco toxicity, 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 (Blakeley *et al.*, 1969; Mobley and Hausinger, 1989), 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, 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. Medically by contrast, utilized is the removal method based on dialysis, exploited in the artificial kidney.

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 20–50mM to less than 10mM. 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. 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 100–300l 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. One approach is the conventional haemodialysis associated with a dialysate regeneration system. 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. 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, 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.

### **Analytical applications of urease**

The foremost analytical application of urease is for quantification of urea in aqueous solutions. 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. In the former analysis, as the prime component of non-protein nitrogen in milk, the level of urea (typically 3–6mM) 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. The assay is also used for detecting urea adulteration in milk. 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.

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. 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. 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 or Nesslerization method, potentiometrically with use of ammonium ion-selective electrodes (Follmer *et al.*, 2008a), enzymatically with use of glutamate dehydrogenase or horseradish peroxidase, in addition to simple titration, carbon dioxide can be determined with use of <sup>13</sup>C or <sup>14</sup>C labeled urea (Zonia *et al.*, 1995) or with carbon dioxide gas-selective electrodes. Measurements of pH (Winkler *et al.*, 1988) and of conductivity (Polacco and Holland, 1993) 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 (Follmer *et al.*, 2004a; Follmer *et al.*, 2004a; Menegassi *et al.*, 2008). 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 in 1969, a great number of urease-based biosensors have been constructed and tested. They employ techniques, such as spectrometry (Weber *et al.*, 2008), potentiometry with the application of pH-sensitive electrodes, ammonium ion selective electrodes and ammonium ion-sensitive field effect transistors, conductometry, amperometry, as well as acoustic and thermal 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 (McCarty, 1990). 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 Hg ions in particular. Yet, in addition to the stability problems, the inhibition-based biosensors also suffer from the lack of selectivity in real samples. This, however, has been proposed to be

solved by developing hybrid systems of enzymes showing different sensitivities to different inhibitors.

### 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. 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. 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. In addition to preparing advanced carbonate materials, bio-induced precipitation of CaCO<sub>3</sub> has been proposed for a number of novel biotechnological applications. One is a solid-phase capture of excess soluble Ca<sup>2+</sup>, radionuclide and trace element contaminants, utilized in cleaning waste- and groundwaters. Another exciting application is as microbial sealants for plugging surface cracks and fissures in buildings, notably in restoration of historic monuments 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. Apart from calcium carbonate, in a similar urease-aided biomimetic manner also other inorganic materials have been prepared, including aluminium hydroxide, aluminium basic sulfate, hydroxalite, hydroxyapatite precursors and hydroxyapatite-like phases, these to be used for bone regeneration.

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.

### Conclusion

Urease play a vital role in human health, environment and agriculture. In plants it plays important role in metabolism and defense system. Bacterial urease is important virulence factor in causing several diseases in human such as urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, peptic ulcer and cancer. So far a lot of work has been done on bacterial and plant urease to understand its catalytic mechanism and structural details. Irrespective of source urease shows similarity in catalytic mechanism and structure of active site. In agriculture there is huge economical losses due to hydrolysis of urea to volatile ammonia which escape in the atmosphere and therefore responsible for air pollution and soil pollution. Such unwanted ammonia formation in the soil can be minimized by the application of urease inhibitors in conjunction with urea based fertilizers. In industry the urease can be used to remove urea from aqueous solutions and also it has major applications in agriculture, food production and medicine.

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