
Removal of Acrylamide by Microorganisms

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1. Introduction

Acrylamide ($\text{CH}_2=\text{CHCONH}_2$) is a well-known bifunctional monomer, appearing as a white odorless flake-like crystal. It is soluble in water, methanol, ethanol, dimethyl ether, and acetone, but insoluble in benzene and heptane. Acrylamide is incompatible with acids, bases, oxidizing agents, irons and iron salts. It decomposes non-thermally to form ammonia while thermal decomposition produces carbon monoxide, carbon dioxide, and oxides of nitrogen [1].

As a commercial conjugated reactive molecule, acrylamide has been used worldwide for the synthesis of polyacrylamide and other polymers [2, 3]. It has also been used as a binding, thickening, or flocculating agent in grout, cement, sewage, wastewater treatment, pesticide formulation, cosmetics, sugar manufacturing, and to prevent soil erosion. Polymers of this compound have been used in ore processing, food packaging, plastic products, and in scientific and medical laboratories as solid support for the separation of proteins by electrophoresis [4]. Acrylamide monomer is also widely used as an alkylating agent for the selective modification of sulfhydryl proteins and in fluorescence studies of tryptophan residues in proteins. In 2002, there was an alarming report of the occurrence of acrylamide at high levels up to 3 mg/kg in plant-derived foods and thought to form during cooking allowing the formation of Maillard browning products [5]. Many reports have suggested that acrylamide seems to be found in foods that have been processed by heat-treatment methods other than boiling [6]. One possible pathway to the formation of acrylamide is via the Maillard reaction between amino acids, particularly asparagines, and reducing sugars at high temperatures [5, 6]. Some reports suggest acrylamide could form by acrolein (2-propenal, $\text{CH}=\text{CHCHO}$), a three-carbon aldehyde, by either the transformation of lipids or the degradation of amino acids, proteins and carbohydrates [7-12].

Acrylamide could be absorbed through unbroken skin, mucous membranes, lungs, and the gastrointestinal tract. Human exposure to acrylamide is primarily occupational from dermal

contact with the solid monomer and inhalation of dust and vapor. Although it is not toxic in polymer form, the monomer can cause peripheral neuropathy. Residual monomer in polymers is also of health concern [13]. Primary exposure occurs during the handling of monomers. Two acrylamide manufacturing factories showed breathing zone concentrations of 0.1 to 3.6 mg/m³ [1]. During normal operations, workers at another plant were exposed to not more than 0.3 mg/m³. Aside from occupational exposure, probable exposure to the general public is through consumption of certain foods [14]. Another source of acrylamide exposure to the general public could be through drinking water treated with polyacrylamide flocculants [13]. Acrylamide may not be completely removed in many water treatment processes with some remaining after flocculation with polyacrylamides probably due to its water solubility and is not absorbed by sediment [15].

Acrylamide is evidentially a neurogenic, teratogenic or carcinogenic toxicant in animals [16]. The neurotoxic properties of acrylamide have been studied for humans in relation to occupational exposures and, experimentally, in laboratory animals. Understanding of acrylamide-induced neuropathies is quite advanced, a consequence of more than 30 years of research on the possible mechanisms of action [17]. The mechanism underlying the neurotoxic effects of acrylamide as with other toxins are interference with the kinesin-related motor proteins in nerve cells or with fusion proteins in the formation of vesicles at the nerve terminus and eventual cell death [18]. Neurotoxicity and resulting behavioral changes in acrylamide-exposed laboratory animals can reduce reproductive fitness. Further, kinesin motor proteins are important in sperm motility, which could alter reproductive parameters. Effects on kinesin proteins could also explain some of the genotoxic effects on acrylamide. These proteins form the spindle fibers in the nucleus that function in the separation of chromosomes during cell division. This could explain the clastogenic effects of the chemical noted in a number of tests for genotoxicity and assays for germ cell damage [4].

2. Release of acrylamide in environment

Acrylamide is a synthetic monomer with a broad spectrum of industrial applications, mainly as a precursor in the production of several polymers, such as polyacrylamide [1, 19]. High molecular weight polymers can be modified to develop nonionic, anionic, or cationic properties for specific uses [1, 20]. Various grades of acrylamide are available with the industrial grade typically with a purity of 98 to 99%. Acrylamide for laboratory use ranges from routine to pure, the former for electrophoresis, the latter for molecular applications [21]. The largest demand for acrylamide polymers in industry is for flocculation of unwanted chemical substances in water arising from mining activities, pulp and paper processing, sewage treatment, and other industrial processes. Applications are based on the principles of colloidal suspensions and used to clean up liquids, particularly aqueous media, either for disposal or human consumption [20, 22-23]. Acrylamide is also used as a chemical intermediate in the production of *N*-methylol acrylamide and *N*-butoxy acrylamide and as a superabsorbent in disposable diapers, medical, and agricultural products [24]. Small amounts of acrylamide are also used in sugar beet juice clarification, adhesives, binders for seed coatings and foundry sand, printing ink

emulsion stabilizers, thickening agents for agricultural sprays, latex dispersions, textile printing paste, and water retention aids [25]. An aqueous 50% solution of acrylamide is used as acrylic copolymer dispersions in surface coatings and adhesives. In surface coatings, polymers are used as dispersants and binders to provide better pigment separation and flow. Surface coatings are used on home appliances and in the automotive trade [1, 26]. In addition, polyacrylamide has been used in both paper production process and treatment of mill wastewater [27]. Emulsions of polyacrylamide, calcium carbonate and clay are applied as a white coating in the manufacture of cardboard cartons [22]. These polymers are used as thickeners in soap and cosmetic preparations, and in skin care and hair grooming products, to impart a smooth after-feel and shine [22]. For oil drilling, liquid or powder partially-hydrolyzed polyacrylamide is used as additives to water based drilling mud to provide a lubricating film and reduce friction at the drill bit, impart stability to shale and clay and increase viscosity [1, 22, 26]. Moreover, specialized gels comprised in part of acrylamide polymers are manufactured for use as lubricants in the textile dyeing components to which fabric or finished garments are added. The gel lubricates the cloth preventing it from clumping together and aids pigment dispersion during the dyeing process to ensure an even color [22, 28]. In leather processing, acrylamide is used as polymers impart a gloss or specific feel and suppleness to leather. The hide is most commonly placed in a drum with the polymer and various other constituents such as dyes, formaldehyde and pigments, then rolled for about two hours. The polymer can also be applied by brush or spray. There is no set formulation for the components of the mix and the proportion of acrylamide polymer is at the discretion of the operator seeking to obtain the properties required in the tanned product [29-30]. Another major application of acrylamide is to reduce herbicide drift during spray applications. The polymer increases the viscosity of the herbicide solution, allowing for more uniform spray applications, and also increases plant contact time [31-33].

In worldwide usage, acrylamide is released into environment as waste during its production and in the manufacture of polyacrylamides and other polymers. Residual acrylamide concentrations in 32 polyacrylamide flocculants approved for water treatment plants ranged from 0.5 to 600 ppm [13]. Acrylamide may remain in water after treatment [15] and after flocculation with polyacrylamides due to its high solubility and is not readily adsorbed by sediment [34]. Other sources of release to water are from acrylamide-based sewer grouting and recycling of wastepaper. Another important source of contamination is from acrylonitrile-acrylamide production which releases approximately 1 g acrylamide in each liter of effluent [35]. Some reports have indicated that polyacrylamide, in the presence of sunlight and glyphosate, photolytically degrades to acrylamide monomer and this is a direct introduction of acrylamide into agricultural areas [36-38]. The half-life of acrylamide monomer in rivers ranges from weeks to months [22]. However, one report indicates that polyacrylamide does not degrade to acrylamide monomer in the presence of sunlight and glyphosate. Additionally, glyphosate appears to interact with either the acrylamide monomer or polymer, decreasing the rate of monomer degradation [39]. The most important environmental contamination results from acrylamide use in soil grouting [13]. Half-life of acrylamide in aerobic soil increases with decreasing temperature [40]. Under aerobic conditions, acrylamide was readily degraded in

fresh water by bacteria with a half-life of 55-70 h, after acclimatization for 33-50 h [41]. Acrylamide has been shown to remain slightly longer in estuarine or salt than fresh water [15].

Acrylamide releases to land and water from 1987 to 1993 totaled over 18.16 tons of which about 85 percent was to water, according to Toxic Chemical Release Inventory of the U.S. Environmental Protection Agency (EPA) [40]. These releases were primarily from plastic industries which use acrylamide as a monomer. In 1992, discharges of acrylamide, reported to the Toxic Chemical Release Inventory by certain US industries included 12.71 tons to the atmosphere, 4.54 tones to surface water, 1,906.8 tones to underground injection sites, and 0.44 tones to land [4]. In an EPA study of five industrial sites that produce acrylamide and polyacrylamide, acrylamide (1.5 ppm) was found in only one sample downstream from a polyacrylamide producer and no acrylamide was detected in soil or air samples [13]. Concentrations of 0.3 ppb to 5 ppm acrylamide have been detected in terrestrial and aquatic ecosystems near industrial areas that use acrylamide and/or polyacrylamides [42-43]. Cases of human poisoning have been documented from water contaminated with acrylamide from sewer grouting. The acrylamide monomer was found to remain stable for more than 2 months in tap water [22]. Atmospheric levels around six US plants were found on an average of $< 0.2 \mu\text{g}/\text{m}^3$ (0.007 ppb) in either vapor or particulate form [15]. The vapor phase chemical should react with photochemically produced hydroxyl radicals (half-life 6.6 h) and be washed out by rain [15].

3. Microbial degradation of acrylamide

The interest in environmental problems is continuously growing and there are increasing demands to seek the sustainable and controllable process which do not burden the environment significantly. Biodegradation is one of the classic methods for removal of undesired organic compounds to concentrations that are undetectable or below limits established as acceptable by regulatory agencies.

Acrylamide is likely to partially biodegrade in water within approximately 8-12 days [13]. If released on land, acrylamide can be expected to leach readily into the ground and biodegrade within a few weeks. In five surface soils that were moistened to field capacity, 74-94% degradation occurred in 14 days in three soils and 79 to 80% in 6 days in the other two soils [44]. Acrylamide may not be completely degraded in domestic sewage and water treatment facilities if residence times are relatively short [13, 45]. Further degradation through bioremediation of acrylamide to less harmful substances would alleviate environmental concerns.

Since 1982, microbial degradation of acrylamide has been explored extensively with a diversity of isolates (Table 1), mainly *Bacillus*, *Pseudomonas* and *Rhodococcus* [3, 46-55]. Further, numerous other microorganisms including the representatives of *Arthrobacter*, *Xanthomonas*, *Rhodopseudomonas*, *Rastonia*, *Geobacillus*, and a newly family of Enterobacteriaceae [49, 56-62]. *Aspergillus oryzae*, a filamentous fungal has also been documented as an acrylamide degrader [63].

Several acrylamide degraders use a coupling reaction of nitrile hydratase (EC 4.2.1.84) and amidase (EC 3.5.1.4) for biotransformation of acrylonitrile to acrylic acid via acrylamide as an

intermediate [46, 56]. For example, *R. rhodochrous* J1 changed acrylonitrile to acrylamide and subsequently to acrylic acid [47] and *R. erythropolis* utilized either 2-arylpropionamides or acrylamide to form acrylic acid and ammonia [64]. In China, *Nocardia* sp. 163, a soil derived bacterium from Taishan Mountain harboring the highest nitrile hydratase activity on acrylonitrile was also used frequently for bioconversion of acrylamide [65]. Another prominent example is *Rhodococcus* sp. AJ270 which is a powerful and robust nitrile hydratase/amidase-containing microorganism isolated by Guo et al [66]. An aliphatic amidase (amidohydrolase) has been found to be the responsive enzyme for the deamidation of acrylamide to acrylic acid and ammonia [50, 59, 62, 64-67].

In 1990, Shanker and his colleagues isolated an acrylamide-degrading bacterium, *Pseudomonas* sp., from soil using an enrichment method. This bacterium degraded high concentration of acrylamide (4 g/l) to acrylic acid and ammonia. An amidase was also found to be the relevant enzyme for the hydrolysis of acrylamide and other short chain aliphatic amides like formamide and acetamide but not on acrylamide analogues, methacrylamide and *N, N*-methylene bisacrylamide [48].

Many aerobic microorganisms utilize acrylamide as their sole source of carbon and energy including *Pseudomonas* sp. and *Xanthomonas maltophilia*. Nawaz and his team found amidase in cell free extracts of these species and suggested it was involved in acrylamide degradation [49]. This is consistent with their earlier conclusion of acrylamide degradation by *Rhodococcus* sp. [50]. Later, the denitrifying bacteria, *Pseudomonas stutzeri* was found to use acrylamide as substrate in the acrylonitrile-butadiene-styrene resin wastewater treatment system. The strain could remove acrylamide at concentrations below 440 mg/l under aerobic conditions [52]. Acclimation of microorganisms is believed to enhance acrylamide biodegradation. Complete degradation of acrylamide at 10–20 ppm in river water occurred in about 12 days with non-acclimated microorganisms, but in only 2 days with acclimation [3]. In 2009, scientists in Malaysia reported two acrylamide-degrading bacteria, *Bacillus cereus* DRY135 and *Pseudomonas* sp. DRYJ7. Acrylic acid was also detected as a metabolite in the degradation [53-54]. *Aspergillus oryzae* KBN 1010 has been the only fungi documented as an acrylamide degrader [63].

In domestic wastewater in Thailand, four novel acrylamide-degrading bacteria (*Enterobacter aerogenes*, *Kluyvera georgiana*, *Klebsiella pneumoniae*, and *Enterococcus faecalis*) were isolated. *E. aerogenes* and *K. georgiana* showed degradation potential of acrylamide up to 5000 ppm at the mesophilic temperatures and could degrade other aliphatic amides especially short to medium-chain length but not amide derivatives [60-61]. Removal of acrylamide and ammonium nitrogen from industrial wastewater by *E. aerogenes* was generally higher than that by mixed cultures of microorganisms [68].

Degradation of acrylamide under anaerobic conditions has been rarely described. Recently a new strain of *Rhodopseudomonas palustris* was found capable of using acrylamide under photoheterotrophic conditions but grew poorly under anaerobic dark or aerobic conditions. A study of acrylamide metabolism by nuclear magnetic resonance showed the rapid deamidation of acrylamide to acrylate and further to propionate [57]. More recently, the denitrifying

Microorganisms	Source	Conditions	Reference
Bacteria			
<i>Pseudomonas chlororaphis</i> B23	Soil	Aerobic (Enzymatic degradation)	[46]
<i>Arthrobacter</i> sp. J-1	Soil	Aerobic (Enzymatic degradation)	[56]
<i>Rhodococcus rhodochromis</i> J1	Soil	Aerobic (Free cells)	[47]
<i>Pseudomonas</i> sp.	Soil	Aerobic (Free cells)	[48]
<i>Pseudomonas</i> sp.	Soil	Aerobic (Immobilized cells)	[49]
<i>Xanthomonas maltophilia</i>			
<i>Rhodococcus</i> sp.	Soil	Aerobic (Enzymatic degradation)	[50]
<i>Rhodococcuserythropolis</i> MP50	Soil	Aerobic (Enzymatic degradation)	[64]
<i>Rhodococcus</i> sp.	Soil	Aerobic (Immobilized cells)	[51]
<i>Pseudomonas stutzeri</i>	Wastewater treatment system	Aerobic (Free cells)	[52]
<i>Rhodopseudomonas palustris</i>	Bovine slaughterhouse	Photoheterotrophic (Free cells)	[57]
<i>Pseudomonas aeruginosa</i>	Soil	Aerobic (Free and immobilized cells)	[3]
<i>Ralstonia eutropha</i> TDM-3	Wastewater treatment system	Anaerobic (Free cells)	[58]
<i>Bacillus cereus</i> DRY135	Soil	Aerobic (Free cells)	[53]
<i>Pseudomonas</i> sp. DRYJ7	Antarctic soil	Aerobic (Free cells)	[54]
Natural microbial populations	Rocky Ford Highline Canal, Colorado USA	Aerobic and anaerobic (Free cells)	[69]
<i>Ralstonia eutropha</i> AUM-01	Soil	Aerobic (Free cells)	[59]
<i>Enterobacter aerogenes</i>	Domestic wastewater	Aerobic (Free and immobilized cells)	[60]
<i>Kluyvera georgiana</i>	Domestic wastewater	Aerobic (Free cells)	[61]
<i>Klebsiella pneumoniae</i>			
<i>Enterococcus faecalis</i>			
<i>Geobacillus thermoglucosidasius</i> AUT-01	Soil	Aerobic (Free cells)	[62]
<i>Pseudomonas aeruginosa</i> DS-4	Soil	Aerobic (Free cells)	[55]
Fungi			
<i>Aspergillus oryzae</i> KBN 1010	Filamentous fungi used in food and beverage industries	Aerobic (Free cells)	[64]

Table 1. Acrylamide-degrading microorganisms.

bacterium, *Ralstonia eutropha* TDM-3 isolated from the wastewater treatment system associated with the manufacture of polyacrylonitrile fiber consumed acrylamide to concentration of 1446 mg/l, above which it was toxic [58]. This report is similar with the potential of soil bacteria, *Ralstonia eutropha* AUM-01 and *Geobacillus thermoglucosidasius* AUT-01 [59, 62]. One report, and perhaps most interesting, removal of acrylamide has been found potentially with the natural microbial populations in Rocky Ford Highline Canal, Colorado USA [69]. Degradation of acrylamide occurs under aerobic or anaerobic conditions, with nitrate serving as the most favorable anaerobic electron acceptor. Phylogenetic analysis of these cosmopolitan microorganisms suggest the potential for biodegradation in similar lotic systems such as *Pseudomonas*, *Rhodococcus*, and *Bacillus*. New proteobacterial genera (*Pectobacterium*, *Citrobacter*, *Delftia*, *Comomonas*, and *Methylobacterium*) were also found [69]. Microbial degradation of a lipid in conjunction with acrylamide was also report with *Pseudomonas aeruginosa* DS-4. Salad oil was believed to be an essential factor for acrylamide biodegradation by this bacterium. The degradation rate of acrylamide was affected by the incubation time of the acclimated strain DS-4. Longer incubation time with acrylamide resulted in more efficient degradation [55].

4. Metabolism of acrylamide

Until now, we can not deny possible routes for acrylamide other than deamination via amidase [50, 59, 62, 64, 67]. The subsequent fate of acrylate is not well understood but probably involves pathways and enzymes that have been characterized to various degrees for other acrylate utilizing bacteria (Figure 1). Acrylate metabolism is believed to proceed via hydroxylation to β -hydroxypropionate, then oxidized to CO_2 [48] or reduced to propionate [57]. Another plausible pathway for mineralization of acrylamide is via formation of acrylyl CoA which eliminates lactate as a final product [48].

A powerful tool that also enables unraveling acrylamide metabolic pathways is the sequential induction of catabolic enzymes and intermediary metabolites. Further, insight into degradative pathways is also provided from assaying the probable key proteins that are synthesized at sufficient levels when acrylamide is present. Using proteome analysis, fifteen proteins differentially expressed from *Enterobacter aerogenes* grown on acrylamide were identified. Six protein homologues with amidohydrolase, urease accessory protein, quaternary ammonium compound resistance proteins, dipeptide transport protein, Omp36 osmoporin and large conductance mechanosensitive channel proteins (MscL) are seemingly involved in acrylamide stress response and its degradation. Five proteins identified as GroEL-like chaperonin, ArsR-transcriptional regulator, Ts- and Tu-elongation factor and trigger factor and four proteins (phosphoglycerate kinase, ATP synthase β -subunit, malate dehydrogenase and succinyl-CoA synthetase α -subunit) are expected to be relevant to adaption of *E. aerogenes* in the presence of acrylamide [70]. Based on the results, Charoenpanich and Tani have proposed acrylamide may be assimilated using Omp36 osmoporin and dipeptide transport proteins. Acrylamide is toxic, indeed lethal, to most microorganisms, however some bacteria have adapted their metabolism to use this substance as an energy source. Important to this adaptation is the evolution of genes that encode amidohydrolase (amidase) and other synthesis proteins that deaminate acryla-

mide to acrylic acid and ammonium [48, 50-51, 60-61]. With this, acrylic acid can be changed to propionate and subsequently succinyl CoA [57, 71-72] to generate energy. Potentially harmful ammonium is detoxified and with MscL protein and released from the cell [70].

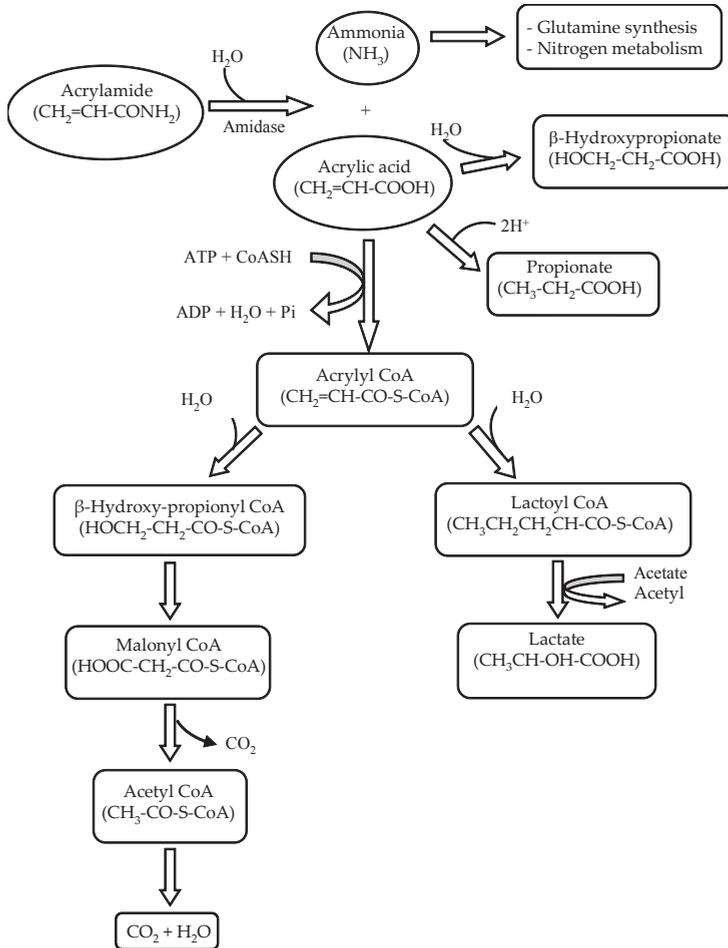


Figure 1. Possible biological fates of acrylate produced from acrylamide deamidation.

5. Bioremediation of acrylamide and future prospects

Bioremediation is viewed as a sustainable process for wastewater treatment, which under appropriate conditions, can promote an efficient reduction of organic matter with minimal

energy requirements and, therefore, low costs. Major limitations are the bioavailability of the organic matter and the finding of efficient biodegraders. Physico-chemical environmental conditions also greatly influence the rate and extent of degradation. In general, degradation efficiency is dependent on three overall factors (i) microorganisms that can degrade the specific chemical structure (ii) environmental conditions that allow the microorganisms to grow and express their degradation enzymes and (iii) good physical contact between the organic substrate and the organism.

Rapid degradation of acrylamide coupled with growth requires not only amidase or microorganism producing amidase, but also a whole pathway, i.e. a set of enzymes that are differentially synthesized in the presence of acrylamide. Although a complete catabolic pathway for acrylamide does not exist, recombination and mutation processes and exchange of genetic information between microorganisms may lead to the development of organisms with improved catabolic activities. Alternatively, microorganisms can cooperate by combining their catabolic potential in mixed cultures and in this way may completely mineralize acrylamide. Wang and Lee elucidated the effectiveness of *Ralstonia eutropha* TDM-3 and mixed cultures of wastewater from the manufacture of polyacrylonitrile fiber in treating acrylamide in synthetic wastewater. They found that mixed culture and *R. eutropha* TDM-3 can jointly consume acrylamide up to concentrations of 1446 mg/l and completely remove acrylamide with a sufficient supply of nitrate as electron acceptors [58]. A similar result has been found in *E. aerogenes*. If grown with mixed cultures from a municipal wastewater treatment plant, they can completely and rapidly convert acrylamide to acrylic acid [68]. Acrylamide up to 100 mg/L can efficiently be removed from amended canal water and sediment slurries under aerobic conditions. Using natural nitrate-reducing microorganisms in a canal environment, potential fate of acrylamide (70.3-85%) was found after 60 days [69].

Microorganisms typically require sufficient water, inorganic nutrients, carbon sources, and trace elements for maintenance and growth. Besides growth substrates, other specific organic compounds such as vitamins or other growth factors are essential for some microorganisms. Monosaccharides like glucose and fructose have been reported as support elements for the growth and degradation potential of acrylamide-degrading bacteria [53-54]. However, in some cases supplementation of acrylamide containing growth medium with glucose or succinate as additional carbon source demonstrated a severe repression in degrading ability [48, 71-75]. Addition of glutamate or ammonium sulfate as an additional nitrogen source to the growth medium demonstrated an increase in degradation potential compared to the cells grown only on acrylamide [48]. One interesting study found that *Pseudomonas aeruginosa* DS-4 isolated from lipid wastewater required salad oil for growth and acrylamide degradation [55].

Toxic compounds (e.g. heavy metals) should not be present at high concentrations, since they can inactivate essential enzymes. As explained in [51] iron (<10 mM) enhanced the rates of acrylamide degradation of *Rhodococcus* sp. but copper, cobalt and nickel inhibited the degradation. Mercury and chromium inhibited acrylamide degradation by *Pseudomonas aeruginosa* while nickel at lower concentrations (200 and 400 ppm) improved the degrading ability [3].

Optimum conditions for acrylamide biodegradation are achieved if pH and temperature are in the range of pH 6-8 and mesophilic temperature (15-30°C), respectively [3, 45-48, 53-55].

Most microorganisms consume considerably less energy for the maintenance of basic functions under neutral conditions. This means that more energy is available for growth. It has been known that metabolic activity of tropical soils typically is high and fosters several processes such as carbohydrate fermentation and carbon dioxide production leading to the lowering of pH. Thus, for successful bioremediation of pollutants including acrylamide pH control may be essential. Addition of an inexpensive chemical such as calcium carbonate to neutralize soil pH during bioremediation can optimize remediation [76].

Studies on acrylamide biodegradation are mainly concerned with the isolation and identification of suitable microbial strains. Most studies use either free or immobilized cells for acrylamide removal. Of these, immobilized cells are advantageous because the immobilized cells are less likely than free cells to be adversely affected by predators, toxin, or parasites [77-78]. Additionally, they can be reused, saving resources and time. However, the implementation of immobilized cells may be sensitive to pH, temperature and acrylamide concentration. Moreover, large accumulations of the metabolic intermediate, acrylic acid, may affect some microbial activity [3, 51, 60]. Hence, the attempt to biotransform acrylamide with amidase or nitrile-converting enzymes via hydrolysis.

Microbial degradation of nitriles proceeds through two enzymatic pathways. Nitrilase (EC 3.5.5.1) catalyzes the direct cleavage of nitriles to the corresponding acids and ammonia, and nitrile hydratase (NHase) catalyzes the hydration of nitriles to amides. Both nitrile-converting enzymes have increasingly attracted attention as catalysts for processing many organic chemicals [79-81]. Nitrile hydratase is commonly used as the catalyst in the production of acrylamide and is known as one of the most important industrial enzymes [82-83]. Generally, the gene operon of nitrile hydratase consists of the genes for the alpha and beta subunits of NHase, the NHase activator and amidase. The amides produced by NHase are degraded to their corresponding free carboxylic acids and ammonia by the action of amidases [84]. Thus, nitrile-converting enzymes are of broad use as alternatives for acrylamide biotransformation.

Acrylic acid, the intermediate product in acrylamide catabolism, is a commodity chemical with an estimated annual production capacity of 4.2 million metric tons [85]. Acrylic acid and its esters can be used in paints, coatings, polymeric flocculants, paper and so on. It is conventionally produced from petrochemicals. Currently, most commercial acrylic acid is produced by partial oxidation of propene which produces undesirable by-products and large amount of inorganic wastes [86]. Currently, there is an innovative manufacturing method using nitrile-amide converting enzymes. For acrylamide degraders, it is initially degraded to ammonia and acrylic acid (acrylate), a process catalyzed by amidase. Then acrylate is reduced to generate energy for growth. Until now, the acrylate-utilizing enzyme has not been well characterized but believed to be acrylate reductase [48, 57]. The identification of the gene encoding this enzyme remains a challenge. Moreover, from an economic aspect, the acrylate reductase-deficient strains created by a gene-disruption method, lead to acrylic acid accumulation in wastewater and are recommended for acrylamide bioremediation in the future.

Sequence similarities have been identified using computer methods for database searches and multiple alignment, between several nitrilases, cyanide hydratase, β -alanine synthase and the first type of aliphatic amidases which hydrolyze only short-chain aliphatic amides [87]. All

these enzymes involving the reduction of organic nitrogen compounds and ammonia production exhibited several conserved motifs. One of which contains an invariant cysteine that is part of the catalytic site in nitrilases. Another highly conserved motif includes an invariant glutamic acid that might also be involved in catalysis. Sequence conservation over the entire length of these enzymes, as well as the similarity in the reactions constitutes a definite family which points to a common catalytic mechanism [88]. Chemical mutagenesis and X-ray crystallography have been analyzed for three-dimensional structures of amidases. Only a few crystal structures of nitrilase-related amidases have been reported with *Pseudomonas aeruginosa* amidase the first [89-90]. The three dimensional-structures showed a conserved α - β - β - α sandwich fold resembling the conserved structural fold of the nitrilase superfamily structures. Analysis of the three dimension-structures identified E59, K134, and C166 as a catalytic triad [89]. Similar catalytic triad residues were also reported in the three dimensional structural models of amidase from *Rhodococcus erythropolis*, *Helicobacter pylori*, and *Bacillus stearothermophilus* [89] and also in the amidase of novel acrylamide-degrading *Enterobacter aerogenes* [91]. The crystal structure of *Xanthomonas campestris* XC1258 amidase showed a monomeric structure of globular α/β protein comprising mainly six α helices and two six-stranded β -sheet (Figure 2). This is the typical nitrilase-superfamily α - β - β - α fold. The hexamer preserving the eight-layered α - β - β - α - β - α structure in holoenzyme across an interface has also been reported [92]. The analysis of small asymmetric catalytic site of the *Geobacillus pallidus* RAPc8 amidase suggested that access of a water molecule to the catalytic triad (C, E, K) side chains would be impeded by the formation of the acyl intermediate. The conserved E142 in the catalytic site acts as a general base to catalyze the hydrolysis of this intermediate [93]. This confirmed the conservation of the E, K, C catalytic triad across the nitrilase superfamily members and also supported the classification of the amidases in the nitrilase superfamily.

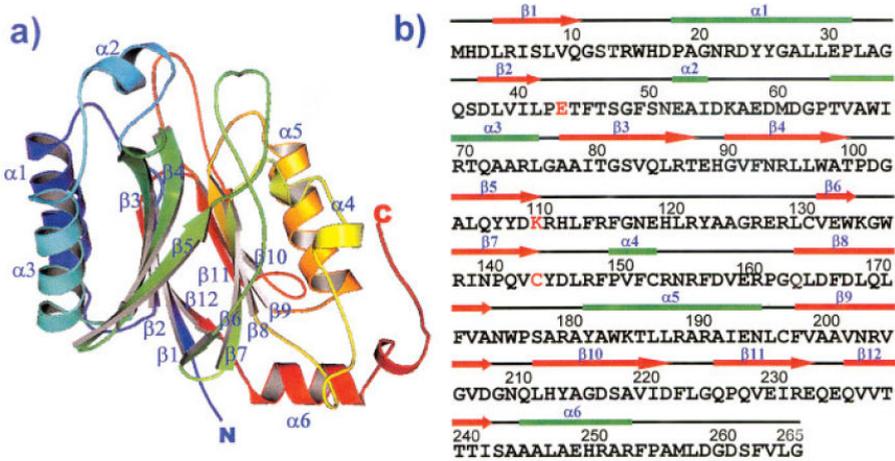


Figure 2. (a) The monomeric tertiary structure of amidase from *Xanthomonas campestris* XC1258, color-coded from blue (N-terminal) to red (C-terminal), and (b) the primary sequence of XC1258 amidase. Reprinted from Ref. [92].

Acrylamide amidases have similar sequences with nitrilases and seem to have descended from a common ancestry along with members of the sulfhydryl enzyme family. In these amidases an invariant cysteine residue was reported to act as the nucleophile in the catalytic mechanism and is confirmed by the three dimensional structural model of the amidase of *Pseudomonas aeruginosa*. This was built by comparative modeling using the crystal structure of the worm nitrilase fusion protein, NitFhit as the template. The putative catalytic triad C-E-K is conserved in all members of the nitrilase superfamily [89]. The signature amidases possesses two real active site residues D191 and S195 among the various conserved residues within the signature sequence common to all enantioselective amidases. D191N and S195A substitutions in *Rhodococcus* amidase has been shown to completely suppress amidase activity [94-95]. These sequences are also present within the active site sequences of aspartic proteinases. Thus, amide bond cleaving enantioselective amidases that are coupled with nitrile hydratases are evolutionary related to aspartic proteinases. Further structural characterization of the amidase produced by acrylamide-degrading bacteria should reveal what other differences are present. It may be possible to use this information to aid protein engineering of the enzymes in order to improve their efficiency and specificity.

Development of thermostable amidase is also important. Based on the three-dimensional structure of amidase, additional disulfide bridges can be engineered by site-directed mutagenesis for enzyme stabilization. Novel amidases that show broad substrate specificity may be developed to biodegrade the toxic environmental pollutants, acrylamide and amides. Random approaches such as directed evolution, reverse engineering and site-directed mutagenesis could be applied to achieve such ends.

Our understanding of the biochemistry and molecular biology of amidase is advancing rapidly and already providing information that is of use today. Moreover, recent developments in amidase studies have broadened the scope of potential applications of the enzyme in acrylamide bioremediation as well as that of acrylic acid production. I predict that these developments combined with progress in genetic engineering and enzyme crystallography will have a major effect on the practical applications of acrylamide bioremediation.

6. Concluding remarks

A huge demand for acrylamide as an ubiquitous monomer for industry led to its environmental presence, however the International Agency for Research on Cancer has classified this compound as a probable human carcinogen. Bioremediation seems to be the only efficient and environmentally friendly process to decompose this monomer. The first step in developing acrylamide bioremediation is to choose high potent microorganisms. Choice of microorganisms is challenging owing to the large scale degradation of acrylamide and elucidation of the intermediate in catabolic pathways is the first important step. Nevertheless, the main problem is the rapid conversion of intermediate acrylic acid to other metabolites. Research on the relationship between degradation mechanisms and membrane structure of acrylamide-utilizing bacteria awaits further characterization. It is noteworthy that successful remediation

of acrylamide depends on the ability of microbes to adapt to new environmental conditions and the availability of active and stable chemical degrading bacteria. Indigenous predators, parasites and toxicants are known to severely restrict biodegradation and should be a concern.

Nomenclature

Amino acids

E: Glutamic acid

K: Lysine

C: Cysteine

D: Aspartic acid

N: Asparagine

S: Serine

A: Alanine

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