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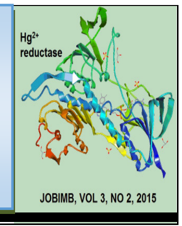

Toxicity, Pollution and Biodegradation of Acrylamide – A Mini Review

Kusnin, N., Syed, M.A. and Ahmad, S.A.*

Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Dr. Siti Aqlima Ahmad
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia, 43400, UPM Serdang,
Selangor, Malaysia.
Email: aqlima@upm.edu.my / aqlimaahmad@gmail.com

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ABSTRACT

Acrylamide is a monomer to polyacrylamide; a polymer with diverse application in basic research, industries and agriculture. The monomer is highly toxic while the polymeric form is slowly degraded to its monomeric form in the environment. In this mini review, the toxicity, uses, pollution and biodegradation of this important monomer are discussed. An important aspect of this review is to highlight the application of microorganisms as remediating agent for the removal of this compound from the environment.

INTRODUCTION

Thousands of different toxic compounds enter the environment as a result of human activities. Toxic compounds can be present in water, soil, air, dust, food, and consumer products. Every year, industries around the world pump billions of pounds of toxic chemicals into the air, soil, and water. In the air, toxic compounds such as hydrocarbons enter the environment through the consumption of fuels in cars and factories [1]. Some air toxicants are also released from natural sources such as volcanic eruptions and forest fires. Effects of air pollution have increased premature mortality and morbidity from asthma, bronchitis, emphysema, pneumonia, coronary artery disease and abnormal heart rhythms [2,3].

In water and soil pollution, toxic compounds occur when pollutants are discharged directly or indirectly into the water or land without adequate treatment to remove harmful compounds. Industrial facilities without proper ways to control runoff contribute toxic chemicals such as heavy metals, poly-aromatic hydrocarbons, pesticides, hydrofluoric acid and methyl alcohol to the environment [4–7]. It is a leading cause of water quality problems in rivers, streams and lakes. In fact, water and soil pollution of toxicants including acrylamide and polyacrylamide are major problems throughout the world, and the potential negative health effects on the human population is large [8].

Acrylamide

Acrylamide is an organic compound with the chemical formula C_3H_5NO. Other names of acrylamide are acrylic amide, propenamide, 2-propenamide, acrylic amide (50%), ethylene carboxamide and vinyl amide [9]. Acrylamide is a compound widely used in the industry to generate polyacrylamide, polymer that is utilized in numerous products in today's modern life. Acrylamide can be found as monomers (single units) or polymers. Single unit of acrylamide are harmful to the central nervous system, has been shown to be carcinogenic to laboratory animals, and are suspected to be carcinogens in humans.

Properties of acrylamide

Acrylamide can occur in two forms which are crystalline solid and liquid forms. The solid monomer is a white colorless crystal that is soluble in water and polar solvents (e.g., methanol, ethanol, dimethyl ether, and acetone), but are insoluble in non-polar solvents (e.g., benzene, heptane, and carbon tetrachloride). Acrylamide has a molecular mass of 71.08 Da, boiling point of 84.5°C at 25 mmHg and melting point of 84.5°C [9]. The chemical structure of polyacrylamide and acrylamide are shown in **Fig.** 1.

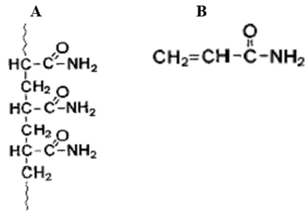

Fig. 1. Chemical structure of polyacrylamide (A) and acrylamide (B), from [10].

Commercial acrylamide monomer contains residual levels of acrylonitrile (1 to 100 mg/kg). The specific gravity of acrylamide is 1.122 g/cm^3 at 30°C and it readily polymerizes if heated to melting point or if exposed to ultraviolet radiation [10]. The major site of reaction is sulfhydryl groups contained on proteins.

Industrial uses of acrylamide and polyacrylamide

Acrylamide is widely used for various chemical and environmental applications. It is also used in the production of high molecular weight polyacrylamides for a variety uses in industrial applications. It is reported that up to 99.9% of acrylamide is used in the production of polyacrylamide in the European Union [11].

Polyacrylamide is a polymer that is commonly used as a sewage-flocculating agent [12–14], as soil conditioning agents [15], in the manufacture of paper as strengthener [12], and in numerous biomedical applications [16]. It can be manufactured as an cationic, anionic, and nonionic polymer for various uses. Polymerisation (copolymerization and homopolymerisation) occurs through a free-radical mechanism in aqueous solution. Anionic polyacrylamides have a negative charge, and is controlled by their charge density, which is the percentage of OH^- groups substituted for NH_2 groups on the polymer (hydrolysis) [17]. Cationic polyacrylamides is presently is not used in erosion control as it may have adverse effects on aquatic life and [18].

Polyacrylamide is a highly reactive and water-soluble polymer that is commonly used in both industries and laboratories. Recently, the usage of polyacrylamides to lessen soil erosion has brought raising attention. Probably the most extensively published uses is in furrow irrigation systems, by which polyacrylamides is included with the irrigation water in order to avoid deterioration of the furrows.[19]. This action leads to the reduction of furrow erosion by up to 94%. Polyacrylamide when introduced through a sprinkler irrigation system has also been shown to reduce soil erosion [17,20]. In the pulp and paper industry, polyacrylamides are widely used as binders and retention aids of the fibers and to maintain the pigment in the paper fibers. The paper industry consumes about 12,000 tons of polyacrylamides per year in the United Kingdom [10].

Polyacrylamide is also used as a thickening agent in pesticides. In herbicides, polyacrylamides are used to increase its surfactants capabilities and to reduce spray drift [10]. Other uses of polyacrylamides are as a binder of bone cement and as a medium for hydroponically grown crops. Apart from that, polyacrylamide is also used in small quantities for molecular biology applications, permanent press fabrics, adhesive manufacture, electrophoresis, cosmetic additives, food processing and photographic emulsions [21]. The pattern of acrylamide usage in USA is shown in **Fig.** 2.

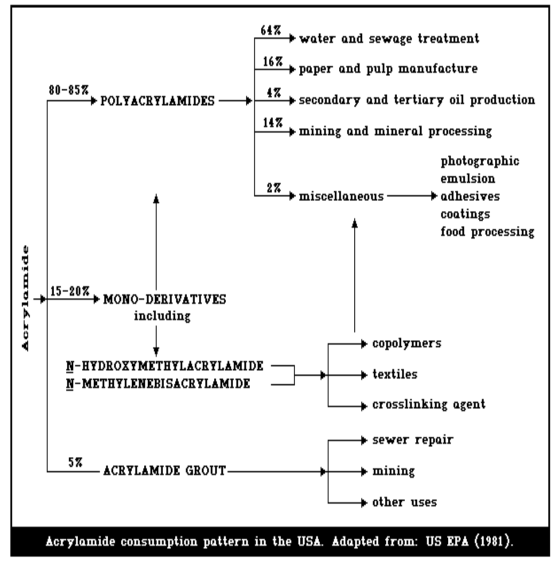

Fig. 2. Acrylamide uses pattern in the USA [22].

In Malaysia, glyphosate is the most popular herbicides used in oil palm plantations and for eradicate weeds problem [23]. It is used for the control of a wide range of grasses and broad-leaved weeds such as *Eleusineindica* (goosegrass). This pesticide contained polyacrylamide which is used as an additive (25-30% solutions) and as the gel-based support for the hydroponics vegetable sector. The Malaysian Agricultural Directory and Index estimated that at least 2 million liters of polyacrylamide are dumped into the soil per year and eventually into rivers. This is because the use of pesticides is increasing up to 8 million liters in the year 2000 [24].

It was reported that acrylamide will remain in water after flocculation with polyacrylamides [25] . Some may be entrained in the sediment although it tends not to be absorbed by sediment and sludge. Therefore, environmental and applicator safety considerations are needed to limit the usage of acrylamide. The acrylamide monomer must be < 0.05% (w/v) in the polyacrylamides application and will consider as hazard if the residues exceed 0.05% (w/v). Based on the report by [26], the acrylamide residue from industries that using polyacrylamide is in the range of 0.05 - 5.0% after polymerization.

Studies done by several researchers have estimated that 10% of polyacrylamide degrades per year in soil through physical, chemical, biological and photochemical processes. However, the polyacrylamide breakdown rate may be faster than 10% per year if it was applied at the soil surface for erosion control because it is highly susceptible to natural UV degradation [20].

Globally, 93% of acrylamide capacity is located in China, the United States, Western Europe and Japan. The global acrylamide market was worth approximately $1.7 billion in 2004, and the consumption of acrylamide was about 450,000 metric tons [21].

In Malaysia, there are no data available for the usage of acrylamides and polyacrylamides, but several industries in

Malaysia also use polyacrylamides such as in water treatment, waste water treatment, paper and pulp processing [14]. Polyacrylamide is also used to strengthen the foundation of artificial lakes in golf courses in Malaysia. However, the use of this compound has caused contamination of underground water supplies. The effects such as skin diseases, irritations and other allergic reactions have been found in a number of golfers, caddies and residents [18].

Acrylamides in food

Since 2002, reports of acrylamide monomers in human foods are increasing. Potato and grain-based foods that are cooked at high temperatures (grilling, frying, and baking) were found to be one of the factors for acrylamide formation. Acrylamide levels as high as 3500 mg/kg have been reported in potato chips and french fries. Evidence to date suggests that the main mechanism of acrylamide formation in foods and drinks is the reaction between the amino acid asparagine and certain reducing carbohydrates which recognize as Maillard reaction [27]. This reaction is best known as the reaction that produces the tasty crust and golden color in fried and baked foods [28]. Therefore, studies about acrylamide mitigation in the final food products have been done by many researchers [27,28].

The acrylamide content of food items is directly related to the cooking temperature used. Acrylamide was not present in boiled foods or unheated control with level found is usually less than 5 mg/kg. Moderate amounts of acrylamide (5-50 mg/kg) were found in heated protein-rich foods while higher contents were found in carbohydrate-rich foods, such as beetroot, potato, and also certain heated crisp bread and commercial potato products. The level found was between 150 to 4000 mg/kg. Consumption habits reveal that the acrylamide levels in the studied heated foods can lead to a daily intake of a few tens of micrograms [28].

Acrylamide as pollutant

The extensive industrial usage of acrylamide and its polymers has led to contaminations in the environment [15]. The applications of polyacrylamides in China clay, paper industry, and water industry have been contaminating the soil and water [29]. In the past, there are several cases of acrylamide contaminations such as in Hallandas, Sweden where acrylamide and N-methyloacrylamide were used as chemical grouting agents [30]. As a monomer for the polymer, polyacrylamide, the acrylonitrile-acrylamide industries are reported to be the major sources of acrylamide contamination with their effluents containing acrylamide concentration as high as 1,000 mg/L [31].

Acrylamide monomers may enter the environment from many sources. In modern acrylamide manufacturing, the use of closed systems has led to safer practices, but accidental events such as a leak from reactors may cause acrylamide contamination. Other sources like acrylamide-based grouting and wastepaper recycling also can cause the acrylamide to be released into waterways and finally become water pollution. However, presence of acrylamide in drinking water is typically a result of leaching during treatment processes [32].

Acrylamide is not expected to be an airborne contaminant due to its low vapor pressure and high water solubility. However, this high water solubility had caused contamination of drinking-water when used in water treatment processes [20,25]. The high chronic toxicity of acrylamide makes it an undesirable contaminant in potable waters. Therefore, regulatory limitations states that polyacrylamide monomers must not exceed 0.05% (w/v) in potable water after water treatment. Residual acrylamide concentrations in polyacrylamide flocculants approved for water treatment plants are in the range of 0.5 to 600 mg/L. It was reported that acrylamide monomers may not be removed in many water treatment processes and remain stable for more than two months in tap water [25]. A potential source of acrylamide pollution in Malaysia is from the large amount of polyacrylamide used in drinking water treatment, where about one ton of polyacrylamide is annually used in the Sarawak state alone [14].

Acrylamide levels were detected less than 5 µg per liter in both tap water and river in an area where polyacrylamides were used in the treatment of potable water. In West Virginia (USA), 0.024 – 0.041 µg of acrylamide per liter was found in the public water supply [25]. The recommended standard for drinking water in Malaysia was set on the basis of WHO Guidelines (1976) for drinking water. Based on National Technical Committee, the maximum allowable level of acrylamide content in drinking water in Malaysia is 0.5 µg per liter [33].

Exposure to acrylamide may occur in the workplace or in the environment. Small amounts of polyacrylamides may be lost to the environment when it is used. In soil, acrylamide mainly comes from plastics and dye industries. The exposure into acrylamide monomer appears to be dermal absorption of the monomer from solution and inhalation of dry monomer or aerosols of acrylamide solution. Moreover, the exposure also occurs during acrylamide and polyacrylamide manufacture, during preparation of polyacrylamide gels and during acrylamide grouting [30,34].

Individuals most potentially exposed to acrylamide are workers in the construction, cosmetics, paper and pulp, plastics, mining, agricultural industries, foundry, textiles, oil drilling and food processing industries. It is reported that acrylamide and polyacrylamides are also used in the manufacture of consumer products such as building materials, contact lenses, soap preparations, gelatin capsules, textiles, food, and appliances [10].

There are several factors such as heat, light, and outdoor environmental conditions (except pH), that promotes depolymerization of polyacrylamide to acrylamide ([35]. The photolytic effect is a major factor in environmental degradation. Under high heat conditions or exposure of polyacrylamide to ultraviolet radiation, the polyacrylamide can be degraded to acrylamide as a result of scission on the polyacrylamide chain [35][36]. However, a study has shown that adding 1% (w/w) of polyacrylamide soil conditioner to tomato and wheat plantings did not pollute the soil with enough acrylamide monomers arising from depolymerization to form a potential hazard [20].

Acrylamide pollution around the world

Incidents of acrylamide pollution are increasing worldwide. Accidental spillage of xenobiotics during transport and storage has become one of the main routes for accidental pollution [37]. According to the Department of Minerals and Western Australia, (1996), an acrylamide storage incident occurred in Kalamunda Road of South Guildford, Western Australia caused the evacuation of people within 500 m of a transport yard and eight people were sent to hospital. This incident was due to the long-term storage of acrylamide at temperatures as high as 42.4°C which caused one of six large shipping containers containing acrylamide to polymerize and release toxic fumes into the air. As a result, 30,000 kg of acrylamide were spilled from a total of 90,000 kg stored acrylamide [37].

Another notable case of acute acrylamide poisoning occurred in 1974 in Shingu, Japan [38]. High levels of acrylamide (400 mg/L) were found in well water that had been contaminated from a grouting operation 2.5 meters away. The agent used in the grouting was NITTO-SS30R which consisted of acrylamide in addition to small amounts of 1,3-diacrylamidemethyl-2-imidazolidone and dimethylaminopropionitrile. Five family members who were exposed through ingestion and external use of well water contaminated with acrylamide began to show signs of intoxication included confusion, disorientation, memory disturbances, hallucinations, and truncial ataxia after one week of water intake. All exposed persons recovered completely within 4 months [38].

During the building of a Swedish tunnel in 1997, concerns were raised on the use of polyacrylamide and N-(methyl)-acrylamides (NMA) as grouting agent in a sealant called Rhoca-Gil to seal the tunnel walls and prevent water flow into the tunnels [39]. In that year, a leakage incident at tunnel project in Sweden had caused the release of unpolymerized acrylamide and NMA into the water system resulting in paralyzed cows and dead fish [39]. Also, [40] reported that exposed tunnel workers have suffered impaired peripheral nervous system, impaired color vision and light sensitivity. These incidents were caused by the dilution of grouting material due to a sudden high rate of water leakage and time pressures of project deadline that affected the gel polymerization process [40]. The polluted ground water from the tunnel in southern Sweden has been estimated to contain 7 to 21 tonnes of unpolymerized acrylamide and NMA. The maximum level of acrylamide leakage can reach 5% due to slow or incomplete polymerization at low temperature less than 5°C [40].

Acrylamide toxicity

Acrylamide continues to be substantially researched because of its toxicity on people and the atmosphere. Research laboratory analyses utilizing microorganisms to gauge the toxicity of acrylamide revealed the mutagenic capacity of acrylamide for Salmonella strains TA98 and TA100 [41]. Another study showed that intraperitoneal injection of acrylamide in cells of mice at doses of $\geq$ 50 mg/Kg, chromosomal aberrations incidence in bone marrow was found to be increased [42]. However, there were no significant increases in the frequency of chromosomal aberrations in splenocytes from mice that received 100 mg/kg [43] and in lymphocytes of mice which was exposed intraperitoneally to 125 mg/kg of acrylamide [44]. Acrylamide also shows adverse effects at doses over 100 mg/kg, in which neurotoxic effects begin to appear in many species. Acrylamide not simply leads to a number of histopathological lesions in the seminiferous tubules but additionally exhibits the toxicological outcomes on reproductive system in male rats. Acrylamide monomers can be easily taken on in human body and affect human health via inhalation, intact skin, and the gastrointestinal tract [45]. It can cause injury to the central nervous system, leading to weakness and numbness in the hands and feet, along with a burning sensation or rash upon make contact with. Acrylamide also contributes to loss of balance, slurred speech, and heavy sweating. Skin contact could cause eye burns and skin rash. Roughly twenty thousand American workers have been probably exposed to acrylamide since 1976 as stated by the National Institute of Occupational Safety and Health, [45].

As previously mentioned, acrylamide is readily absorbed through the digestive tract, the lungs, the skin and the placental barrier. Scientific studies on the biomarkers (hemoglobin adducts) which are utilized to approximate internal dose in occupationally-exposed people have been carried out. In this research, 163 workers employed at the construction of a railway tunnel through Hallandas (Sweden) were exposed to an acrylamide that was used for water leakage. All 163 workers had hemoglobin adducts levels up to maximum of 17.7 nmol/g globin [40,46]. However, hemoglobin adducts levels as high as 33.8 nmol/g globin was detected in the Chinese workers who had been manufacturing acrylamide in China [40].

Scientific studies carried out to evaluate the neurotoxic outcomes of acrylamide in occupationally-exposed workers demonstrated that roughly 73% of the workers displayed the signs of acrylamide poisoning. Early signs of acrylamide exposure are skin peeling from the hands accompanied by numb hands, weak legs and feet, and loss of ankle reactions and incapacity of the vibration feeling in the toes [41].

Acrylamide metabolism

The mechanism of acrylamide can be enhanced through body fluids and distributed fairly throughout body organs. It wills rapidly metabolized by glutathione conjugation and despite the rapid metabolism, its high reactivity with proteins is the reason it is hazardous to the workers. The content of α, β-unsaturated amide in acrylamide product cal also react with nucleophilic compounds via a Michael addition. The major site of reaction is sulfhydryl groups containing proteins and amino acids [41].

The amidase enzyme is one of the important enzymes involved with acrylamide degradation. These enzymes are often found in bacteria related to *Nocardia*-like Actinomycetes (*Nocardia*, *Rhodococcus*and *Arthrobacter*). It was reported that the hydrolysis of acrylamide to acrylic acid and ammonia can be catalyzed by intracellular amidase [47]. The structure of acrylamide and its metabolism is shown in **Fig.** 3.

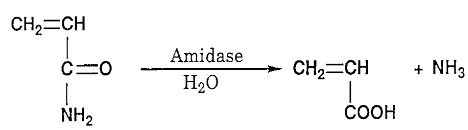

Fig. 3. Acrylamide and its metabolism [47].

Treatment of acrylamide pollution

Industrial effluents containing acrylamide and their derivatives require suitable treatment before being discharged into the environment. Currently, biological treatment processes are practiced in acrylamide treatment in the field of environmental pollution control.

Biological treatment (also known as bioremediation) is actively being explored for acrylamide-contaminated wastes. Bioremediation is defined as the restoration of the contaminated environment to its original condition using biological agents such as microorganisms, fungi, green plants or their enzymes [48]. On thermodynamic grounds, every organic compound can serve as a possible energy source for aerobic microorganisms [49–56]. This is the principle that supports the approach of many researchers to employ microorganisms for the bioremediation of many compounds. The main advantage of bioremediation is its economical perspective where it is believed that bioremediation requires less cost compared to conventional methods. Bioremediation is also a promising approach because it offers a permanent solution when complete mineralization of the contaminants is possible. Unlike physical or chemical methods, bioremediation is non-invasive or at least less invasive to the environment. This will prevent secondary pollution and keep the environment intact. Various microorganisms are capable of efficiently degrading xenobiotics and toxic compounds in the environments. Effective bioremediation is dependent upon the growth of specific microorganisms competent at degrading the targeted toxic contaminants [23,57–63,63–69].

As mentioned earlier, large amounts of acrylamide compounds are released into the environment, either deliberately in the application of acrylamide, or accidentally as in the case of spills. Bioremediation technology has been demonstrated to function in field pilot or full scale applications [15]. Since microbial oxygenases play a key role in the biodegradation processes of acrylamide compounds, most field and laboratory trials have been carried out in aerobic conditions [70–72].

Bacteria, fungi, yeast and plants have evolved various means of extracting essential nutrients from the environment and can be used to degrade or treat acrylamide pollutants. Microorganisms with the ability to grow on acrylamide and other amides have been isolated as potential candidates for bioremediation and include *Variovorax boronicumulans* CGMCC 4969 [73], *Pseudomonas azotoformans* [74], *Stenotrophomonas acidaminiphila* MSU1 [75], *Pseudomonas putida* [76], *Pseudomonas* sp. [33], *Pseudomonas aeruginosa* [77], *Pseudomonas acidovorans* [78], *Thermococcus hydrothermalis* [79], *Pseudomonas chlororaphis* [80], *Pseudonocardia thermophilia*, *Bacillus cereus* [81], *Rhodococcus* sp. [47], *Burkholderia* sp. [82], *Kluyvera georgiana* [59], *Enterobacter aerogenes* [83], *Kluyvera georgiana* [59], the yeast *Rhodotorula* sp. [84] and the plant growth-promoting bacterium *Variovorax boronicumulans* CGMCC 4969 [73]. The most recent isolate is the molybdenum-reducing and acrylamide-degrading *Burkholderi cepacia* strain Neni-11 [85].

The isolated acrylamide-degrading microorganisms exhibit a range of physiological properties. The optimum temperature for acrylamide degradation by the majority of the microorganism is in the mesophilic range. For instance, *Rhodotorula* sp. strain MBH23 is able to grow on acrylamide between 25 and 30ºC while other degraders prefer 30 ºC such as *Aspergillus Orizae* KBN1010 [27], *Pseudomonas stutzeri* [86], *Ralstonia Eutropha* [22]. The only low temperature acrylamide degrader is the bacterium *Pseudomonas* sp. strain DRYJ7 isolated from Antarctica [33].

CONCLUSION

Acrylamide is a toxic compound that forms a silent pollution worldwide. Its neurotoxic properties are a testament of its extreme toxicity to organisms when present in the environment. One important hurdle to acrylamide mineralization is the toxicity of acrylamide itself. It can crosslink biological molecules rendering them inactive. As they are more than one form of toxic amides present in polluted water and industrial effluents another challenge is to isolate multiple amide degraders not to mention that resistance to heavy metals would also be needed if heavy metals pollutant is present together with these amides. Addition of multiple amide-degrading microorganisms to the current repertoire of amide-degrading microorganisms is urgently needed. Simulated bioremediation studies in various types of soils and aquatic bodies may reveal the extent of remediation taking into account the half-life of acrylamide in the soils.

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