Growth Characterization of Bacillus sp. strain ZEID-14 on Acrylamide as the Sole Nitrogen Source

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INTRODUCTION

The Maillard reaction, which happens when food is cooked at high temperatures, can make acrylamide, a chemical that can cause cancer and damage nerve cells. Because of the Maillard process, people who eat a lot of carbs may find acrylamide in their bodies. The Maillard reaction takes place when sugars and amino acids are mixed together. There is a lot of acrylamide made by this method [1]. But acrylamide can be made from several different carbonyl chemicals [2]. Cows and fish in Sweden and Norway died because acrylamide got into nearby streams and made them sick. Acrylamide is mostly used to make polyacrylamide (PAM), which has many different uses in the glue, plastic, printing, and water treatment industries. In 2005, the safety of our food supply was affected in a big way by the widespread use of commercial polyacrylamides, which are often contaminated with acrylamide's lethal monomer. The use of Roundup, which has polyacrylamide in it at a rate of 30%, causes acrylamide pollution. To fix this problem, acrylamide must go through a process of remediation [3].

Acrylamide has been shown to bind to DNA and mouse protamine during all stages of spermiogenesis in mice, which suggests that it may damage genes [4]. Rats that were exposed to acrylamide had more prenatal deaths, mutagenicity, clastogenicity, endocrine-related cancers, and male reproductive toxicity [5]. When mice were given acrylamide intraperitoneally at a dose of 50 mg/kg, the number of chromosomal problems in their bone marrow went up. When acrylamide was injected into the abdomen of mice up to 125 mg/kg, the number of cells with...
chromosomal problems did not change much [6]. The way acrylamide affects the reproductive systems of male rats also changes the way the seminiferous tubules look. If you breathe in or put it on your skin, acrylamide can cause a burning sensation or a rash. Too much sweating, being tired and having a tongue that shakes are all signs of a problem with the nervous system [7]. Because acrylamide dissolves easily in water, it can get into the body through the lungs, stomach, placenta, and skin.

Acrylamide can get into the body when you breathe in dirty air or eat or drink something that has been tainted. This material can be taken in through the skin, the mucous membranes in the lungs, or the digestive system. It will leave the body, though, through the kidneys and urine. The impact of acrylamide is sped up by the fact that it is easily accessible in biological fluids and that it is spread out all over the body. Even though acrylamide is quickly broken down and eliminated after being exposed, it is still dangerous for both workers and consumers [8–10]. Tests can be done to see how much acrylamide adducts to haemoglobin the average worker is exposed to on the job. Using haemoglobin adducts as a biomarker, the study found that 41 people who worked at an acrylamide plant had higher levels of neurotoxicity.

At a Chinese acrylamide plant, workers' haemoglobin adduct levels went up, which shows that they were exposed to very high levels of acrylamide [11]. Igiši et al. [12] Because of pollution from grouting at a depth of 2.5 meters, the amount of acrylamide in the well was as high as 400 mg acrylamide/L. Five of the people who drank the polluted water and got sick with acrylamide had truncal ataxia and felt like they were in a different place. Bacteria continue to be the most common microorganisms discovered to be capable of degrading acrylamide [13–22]. The identification and characterization of another acrylamide-degrading strain with metal reduction capacity is described here.

MATERIALS AND METHODS

All of the materials utilized in this investigation were of analytical grade unless otherwise specified. Experiments were conducted in triplicates.

Growth and maintenance of acrylamide-degrading bacterium

The bacterium was previously isolated from Sudan’s soil as a molybdenum-reducing bacterium [23]. Characterization of this bacterium on acrylamide was conducted on minimal salts medium (MSM) supplemented with only acrylamide as the source of nitrogen and glucose as the sole carbon source. Revival of the bacterium from a 16% glycerol stock was carried out by growing overnight the pure culture in 10 mL of nutrient broth. From this, 0.1 mL was added into 45 mL of acrylamide enrichment medium in a 100 mL volumetric flask and the culture was incubated at 150 rpm for 48 h at 25 °C on an incubator shaker (Cerntomat R, USA). Minimal salt medium (MSM) for growth was supplemented with 0.5 g acrylamide g/L as the sole nitrogen source, glucose 10 g/L as the carbon source, MgSO₄·7H₂O 0.5 g/L, KH₂PO₄ 6.8 g/L (buffering species and source of phosphorous), FeSO₄·H₂O 0.005 g/L and 0.1 mL of trace elements [3].

The phosphate in the medium acts as a buffer, keeping the pH in a range that goes from 5.8 to 7.8. For the sterilization process, the only source of nitrogen was acrylamide, and 0.45-micron-sized PTFE syringe filters were used.

So that the number of bacteria could be counted, samples of one millilitre were diluted with sterile tap water and spread on nutrient agar. The phosphate in the medium acts as a buffer, keeping the pH level in the range of 5.8 to 7.8. Acrylamide was sterilized with a filter made of PTFE syringe filters with 0.45 micron-sized holes. So that the number of bacteria could be counted, samples of one milliliter were diluted one by one. So that the number of bacteria could be counted, samples of one milliliter were diluted with sterile tap water and put on nutrient agar plates overnight.

Statistical Analysis

One-way analysis of variance (with post hoc analysis by Tukey’s test) or Student’s t-test was used to compare between groups. Values are means ± standard deviation (SD) of triplicate experiments. P-value of < 0.05 was considered was significant.

RESULTS AND DISCUSSION

Effects of Initial pH and Temperature on Growth

The effect of the initial pH on bacterial growth was analyzed between pH 5.7 and 8. A growth rate was measured after 48 hours of incubation. The optimum pH range as analyzed using ANOVA was between 6.5 and 7.5 pH values determined (Fig. 1). Outside of this range, the rate of cell growth slowed down a lot. Fig. 2 shows that acrylamide grows best when the temperature is between 25 and 35 °C. The results of this study agree with what has been learned before about how pH affects the formation of acrylamide. Researchers have found that many microbes that break down acrylamide like a pH of around 7.0 [13–22].

Strong metabolic activity in tropical soils makes organic acid and carbon dioxide. This usually means that the pH of the soil is lower, or that it is acidic. So, chemicals that control pH should be given so that the water is close to neutral for the best cleaning [24]. The temperature has a big effect on how bacteria break down acrylamide. Many microorganisms that break down acrylamide said that a temperature near 30 °C was the best place for them to grow [13–22]. On the other hand, thermoactive bacteria require a greater temperature for optimum growth such as in Pseudonocardia thermophilic and Brevibacillus borstelensis BCS-1, where temperatures of 50 °C and 55°C, respectively, were required [25,26].

![Fig. 1. Growth of the bacterium at various pH. Each data point represents the mean ± SD.](https://doi.org/10.54987/jebat.v5i2.737)
Pseudomonas aeruginosa* can be grown on carbon sources like starch for optimal growth. Other than simple carbon sources, complex carbon sources require glucose at concentrations ranging from 0.5 to 2.0% (w/v). Acrylamide, a monomer used in the production of acrylic acid, can be metabolized by bacteria via the Kreb’s cycle. In aerobic conditions, acrylamide can be broken down into -hydroxypropionate, which is then oxidized to carbon dioxide.

**Effects of Acrylamide Concentration on Growth**

Fig. 2. Growth of the bacterium at various temperatures. Each data point represents the mean ± SD.

The effects of a 1% (w/v) initial concentration of organic carbon sources like fructose, lactose, maltose, mannitol, citric acid, and diesel on bacterial growth on acrylamide were studied. After 72 hours, fructose, glucose, and sucrose all had the best growth, with 9.2 log CFU/mL, which was better than the other carbon sources and the control. Compared to the control, the results showed that all carbon sources made cells grow faster (Fig. 3).

Carbon sources are very important for bacteria to grow on acrylamide in a low-salt medium because most bacteria break down acrylamide use it as their only source of nitrogen, so they need to be supplemented with carbon sources that are easy to use. Most scientists agree that glucose is the best source of carbon. Bacillus clausii and Burkholderia sp. [27], Rhodococcus rhodochrous [28], Bacillus cereus [3] and Pseudomonas sp. [29] require glucose at concentrations ranging from 0.5 to 2.0% (w/v) for optimal growth. Other than simple carbon sources, complex carbon sources such as starch were used by Pseudonocardia thermophila [26] whilst salad oil was the sole carbon source by *Pseudomonas aeruginosa* [30].

**Effect of Acrylamide Concentration on Growth**

As a single nitrogen supply, acrylamide doses up to 2000 mg/L were investigated. Heavy metals like copper, lead, cadmium, chromium, and mercury can slow the growth of bacteria on acrylamide in different ways. Mercury caused the most severe inhibition, at 83 percent, while other metal ions caused less than 20 percent inhibition (Fig. 5).

Heavy metals affect how *Acrylamide* grows and breaks down. Heavy metals at the polluted site are one of the main things that make bioremediation harder to do. This is because many types of bacteria can’t live in places with a lot of heavy metals, so they lose their ability to break down target compounds. A study found that at a concentration of 2 parts per million, heavy metals like copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg) slow the growth of bacteria on acrylamide in different ways. Mercury caused the most severe inhibition, at 83 percent, while other metal ions caused less than 20 percent inhibition (Fig. 5).

Because heavy metals are so common in rivers that have been polluted by industry, research using metal inhibition models is important, but it isn’t talked about enough in the story. Researchers are doing a lot of work to find out how bacteria can live and grow in very dangerous places. With the help of the Andrews model, it was possible to figure out how toxic metals affect how fast *Pseudomonas* sp. and Bacillus sp. break down monoaromatic hydrocarbons [34]. Heavy metals likely stop enzymes from working because they bind to the sulphhydryl group that is often found in enzyme active sites. [35].

When it comes to heavy metals stopping biodegradation, there are a few things to think about. By introducing metal-resistant bacteria, you can lower the amount of metal that is bioavailable, which speeds up biodegradation in the presence of a dangerous metal. [36]. Combining a main bacterial degrader with a metal-resistant bacterium can make it easier for bacteria to break down acrylamide. In a soil microcosm experiment, a
cadmium-resistant Pseudomonas H1 that stores cadmium in its cells and 2,4-D-degrading bacteria were added to soil that was contaminated with both cadmium (60 mg total cadmium/kg) and 2,4-D (500 mg/kg). This caused the xenobiotic to be broken down more quickly. Treatment additives like calcium carbonate, manganese oxide, cement, phosphate, and magnesium hydroxide can make metals less bioavailable and mobile. This makes it easier to clean up metal contamination [37]. Clay minerals can be used as an alternative. Clay minerals have been shown to help reduce both the bioavailability of metals and the harm they cause when they are present. For example, the toxicity of cadmium was reduced when kaolinite (1–20%) or montmorillonite (1–5%) was added to a cadmium-containing agar medium that yeasts, bacteria, and an actinomycete could use [38].

In solution tests, it was found that 3 percent bentonite and vermiculite made 150 mg total cadmium/L less harmful to Streptomyces bottropensis. Even though kaolinite could reduce cadmium's toxicity, it needed a higher concentration (6 percent instead of 3 percent) and gave less overall protection than the other clays [39]. There isn't a lot of information in the published literature about how heavy metals affect how acrylamide and other xenobiotics break down. Because there isn't much written about how well microorganisms can handle heavy metals, the results of this study will have a big effect on how bioremediation is used in the future.

According to the findings of this research, one fact worthy of note is that the lag period is lengthened when development occurs at a very high concentration of acrylamide. By applying primary growth models such as modified Gompertz or logistics or even other existing models [20,47], one may derive essential growth characteristics such as the specific growth rate, the maximum growth rate, and the lag time. The particular growth rate that was acquired is a valuable parameter that may be further modeled using secondary models such as Monod, Haldane, Teissier (Tessier), Yano, Aiba, etc. [19,20].

Fig. 6. The growth profile over time of the bacterium on various concentrations of acrylamide. Each data point represents the mean ± SD n=3.

CONCLUSION

We have examined the ability of a bacterium that was previously classified as a metal reducer to degrade acrylamide. This bacterium was used in our study. Early studies indicated that the optimal conditions for development were a pH range of 7 to 7.5, a temperature range of 30 to 35 degrees Celsius, a concentration of acrylamide at 0.5 g/L, and glucose as the optimum carbon source. These conditions were great for the growth of the fungus. The formation of acrylamide was inhibited by the presence of toxic heavy metals such as mercury, copper, chromium, and cadmium, with mercury serving as the most effective inhibitor. When the concentration of acrylamide was increased from 300 mg/L to 1000 mg/L, the lag period for this bacterium's growth rose from 1 day to 3 days. However, when the concentration of acrylamide was increased to 1500 mg/L or 1.5 g/L, development was entirely stopped. The current research involves conducting an experiment with a two-level factorial design in order to find important qualities that boost growth. Once these parameters have been identified, they will be used in an RSM-based experiment to increase the growth on acrylamide. Primary and secondary models are being used to simulate the development of the bacteria over time in response to varying amounts of acrylamide. In the available research, there is scant evidence to support the hypothesis that heavy metals play a role in the degradation of acrylamide and other xenobiotics. Because there is so little previous research on the topic of microbial tolerance to heavy metals, the results of this study will have a significant impact on the development of future bioremediation techniques. The employment of these bacterium, in particular in metal-polluted soils, presents a substantial window of opportunity for the process known as bioremediation, which is used to remove acrylamide from the environment.
REFERENCES


