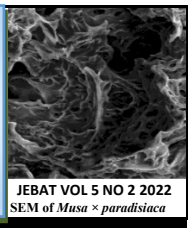
JEBAT, 2022, Vol 5, No 2, 1-6
https://doi.org/10.54987/jebat.v5i2.737

HIBISCUS PUBLISHER

JOURNAL OF ENVIRONMENTAL BIOREMEDIATION AND TOXICOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JEBAT/index



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

Mohd. Fadhil Rahman[1], Mohd Badrin Hanizam[1], Isam M. Abu Zeid[2] and Mohd Yunus Shukor[1]*

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia.
[2]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.

*Corresponding author:

Mohd Yunus Shukor
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
UPM 43400 Serdang,
Selangor,
Malaysia.
Email: mohdyunus@upm.edu.my

HISTORY

Received: 24th Oct 2022
Received in revised form: 3rd Dec 2022
Accepted: 18th Dec 2022

KEYWORDS

Acrylamide
Bacillus sp.
Bioremediation
Biodegradation
Characterization

ABSTRACT

Acrylamide is a major pollution in soil from the breakdown of pesticides additive. Slowly but steadily, the use of microbe-mediated acrylamide breakdown as a bioremediation approach has gained attention all around the world. At room temperature, the effect of the initial pH on bacterial growth shows that the optimum pH range was discovered to be between 6.5 and 7.5. The optimal growing temperature at pH 7.5 ranged from 25 to 35 ºC. In a series of experiments using a 1.0 percent (w/v) starting concentration of various organic carbon sources, it was determined that fructose, glucose and sucrose all supported the greatest amount of cellular growth on acrylamide. Acrylamide concentration of 500 mg/L promoted the most rapid expansion of growth, while levels of 1500 mg/L and higher entirely stopped growth. Mercury at 2 ppm caused 83% of inhibition whilst other metal ions such as copper, cadmium, lead and chromium showed minimal inhibition of less than 20% . The concentration of acrylamide and the time it took for this bacterium to start growing show an inverse relationship. A lag time of 1-3 days was found as the content of acrylamide was raised from 100 to 1000 mg/L while growth was abolished at 1500 mg/L. The maximal growth rate increased as acrylamide concentrations increased, indicating an overall trend of increased toxicity.

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, acylamide 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]. Igisu 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 ℃ on an incubator shaker (Certomat 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, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 6.8 g/L (buffering species and source of phosphorous), $FeSO_4 \cdot H_2O$ 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 millilitre were diluted one by one. So that the number of bacteria could be counted, samples of one millilitre 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 ºC 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].

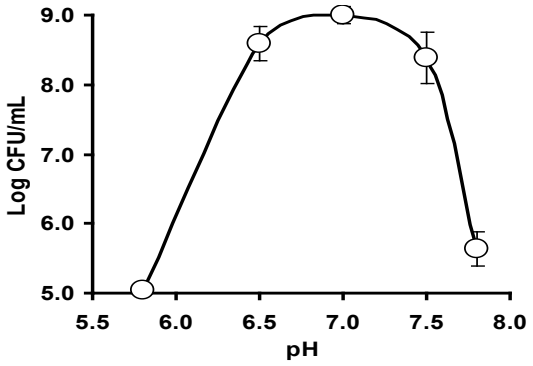


Fig. 1. Growth of the bacterium at various pH. Each data point represents the mean ± SD.

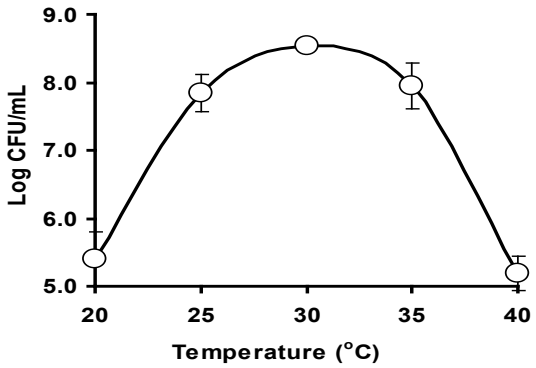


Fig. 2. Growth of the bacterium at various temperatures. Each data point represents the mean ± SD.

Effects of Carbon Sources on Growth

The effects of a 1% (w/v) initial concentration of organic carbon sources like fructose, glucose, lactose, maltose, mannitol, citric acid, and diesel on bacterial growth on acrylamide were studied in detail. 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 that 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 thermophilic* [26] whilst salad oil was the sole carbon source by *pseudomonas aeruginosa* [30].

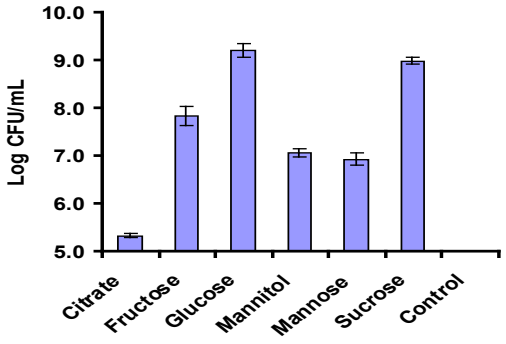


Fig. 3. Growth of the bacterium at various carbon sources and 0.5 g/L acrylamide. The error bars represents the mean ± SD and n=3.

Effect of Acrylamide Concentration on Growth

As a single nitrogen supply, acrylamide doses up to 2000 mg/L were investigated. The highest growth occurs at 500 mg/L of acrylamide, resulting in a nett growth of 3.84 log CFU/mL (**Fig. 4**). This study shows that the acrylamide-degrading bacterium is resistant to acrylamide concentrations of up to 1000 mg/L, with optimal growth occurring at 500 mg/L, in a manner analogous to that of a consortium of bacteria isolated from volcanic soil [21,21]. The *A. oryzae* fungus was able to break down acrylamide concentrations of roughly 100 mg/L by using nitrate and sucrose as nitrogen and carbon sources, respectively. This amount is regarded to be minimal [31]. [32] reported that *Ralstonia eutropha* TDM-3 and *Ralstonia eutropha* AUM-01 can utilize up to 780-1990 mg/L acrylamide as the sole carbon and nitrogen source while *Pseudomonas stutzeri* and *Pseudomonas* sp. strain DRYJ7 require between 440 and 500 mg/L, respectively, for optimal growth [28,33].

The highest tolerant and degrader so far is *Cupriavidus oxalaticus*, which can degrade up to 60 mM or 4260 mg/L acrylamide [18]. The breakdown of acrylamide is usually to acrylic acid, which can be metabolized by numerous bacteria via the Kreb's cycle. For instance, acrylate metabolism in aerobic acrylate-using bacteria has been found to proceed via hydroxylation to -hydroxypropionate, which is then oxidized to carbon dioxide [31]. Heavy metals have a considerable influence on the breakdown of acrylamide, with stronger inhibition occurring in the presence of mercury, copper, and silver than in the presence of other metals [14–17,19–22].

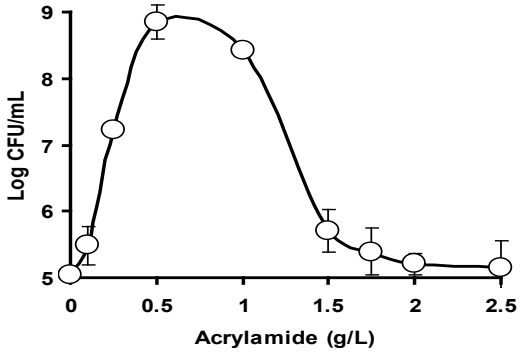


Fig. 4. Growth of the bacterium at various concentrations of acrylamide. Each data point represents the mean ± SD n=3.

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 sulfhydryl 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.

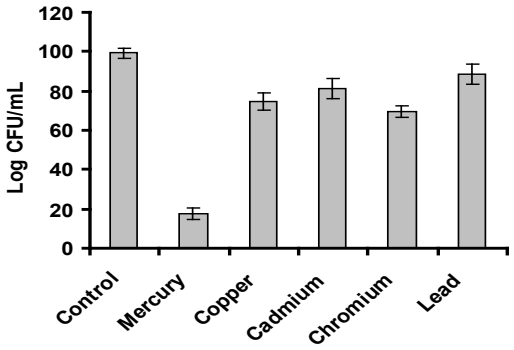


Fig. 5. The effect of heavy metals on acrylamide degradation by acrylamide-degrading bacterium. Each data point represents the mean ± SD.

Growth profile

The growth of this bacterium at different doses of acrylamide indicates that an increasing lag time occurs as the acrylamide concentrations are elevated. The lag time was anything from one to three days depending on the quantity of acrylamide, which varied from one hundred to one thousand five hundred milligrams per liter. At a concentration of 1500 mg/L acrylamide, no signs of growth were seen (**Fig. 6**). As the amounts of acrylamide were raised, there was a general tendency toward an increase in toxicity, which was shown by the maximum growth being reduced. Acrylamide is hazardous to the development of many microorganisms, and at concentrations of 1000 mg/L or more, growth will often be stopped [14–17,19–22].

Because certain microorganisms have the enzyme amidase, it is possible for them to grow at these significantly higher concentrations [18–46].

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].

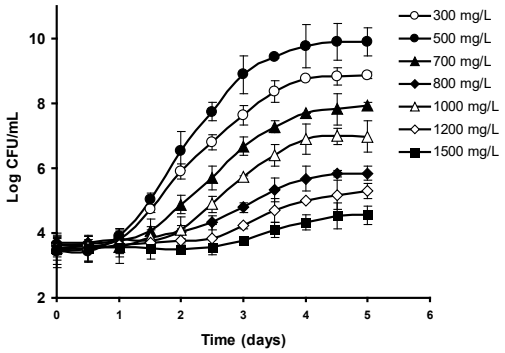


Fig. 6. The growth profile over time of the bacterium on various concentrations of acrylamide. Each data point represents the mean ± SD n=3.

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

1. Mottram, DS, Wedzicha BL, Dobson AT. Acrylamide is formed in the Maillard reaction. Nature. 2002;419:448–9.
2. Zamora R, Delgado RM, Hidalgo FJ. Strecker aldehydes and □-keto acids, produced by carbonyl-amine reactions, contribute to the formation of acrylamide. Food Chem. 2011;128(2):465–70.
3. Shukor MY, Gusmanizar N, Azmi NA, Hamid M, Ramli J, Shamaan NA, et al. Isolation and characterization of an acrylamide-degrading *Bacillus cereus*. J Enviromental Biol. 2009;30(1):57–64.
4. Sega GA, Valdivia Alcota RP, Tancongco CP, Brimer PA. Acrylamide binding to the DNA and protamine of spermiogenic stages in the mouse and its relationship to genetic damage. Mutat Res Mutagen Relat Subj. 1989 Aug 1;216(4):221–30.
5. Tyl RW, Friedman MA. Effects of acrylamide on rodent reproductive performance. Reprod Toxicol. 2003 Jan 1;17(1):1–13.
6. Backer LC, Dearfield KL, Erexson GL, Campbell JA, Westbrook-Collins B, Allen JW. The effects of acrylamide on mouse germ-line and somatic cell chromosomes. Environ Mol Mutagen. 1989;13(3):218–26.
7. Spencer P, Schaumburg HH. Nervous system degeneration produced by acrylamide monomer. Environ Health Perspect. 1975 Jun 1;11:129–33.
8. Eikmann T, Herr C. How dangerous is actually acrylamide exposure for the population. Umweltmed Forsch Prax. 2002;7(6):307–8.
9. Pruser KN, Flynn NE. Acrylamide in health and disease. Front Biosci - Sch. 2011;3 S(1):41–51.
10. Pennisi M, Malaguarnera G, Puglisi V, Vinciguerra L, Vacante M, Malaguarnera M. Neurotoxicity of acrylamide in exposed workers. Int J Environ Res Public Health. 2013;10(9):3843–54.
11. Hagmar L, Törnqvist M, Nordander C, Rosén I, Bruze M, Kautiainen A, et al. Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. Scand J Work Environ Health. 2001;27(4):219–26.
12. Igisu H, Goto I, Kawamura Y, Kato M, Izumi K. Acrylamide encephaloneuropathy due to well water pollution. J Neurol Neurosurg Psychiatry. 1975;38(6):581–4.
13. Wampler DA, Ensign SA. Photoheterotrophic metabolism of acrylamide by a newly isolated strain of Rhodopseudomonas palustris. Appl Environ Microbiol. 2005;71(10):5850–7.
14. Buranasilp K, Charoenpanich J. Biodegradation of acrylamide by *Enterobacter aerogenes* isolated from wastewater in Thailand. J Environ Sci. 2011;23(3):396–403.
15. Charoenpanich J, Tani A. Proteome analysis of acrylamide-induced proteins in a novel acrylamide-degrader *Enterobacter aerogenes* by 2D electrophoresis and MALDI-TOF-MS. Chiang Mai Univ J Nat Sci. 2014;13(1):11–22.
16. Gusmanizar N, Shukor Y, Ramli J, Syed MA. Isolation and characterization of an acrylamide-degrading *Burkholderia* sp. strain DR.Y27. J Ris Kim. 2015 Feb 11;2(1):34.
17. Yu F, Fu R, Xie Y, Chen W. Isolation and characterization of polyacrylamide-degrading bacteria from dewatered sludge. Int J Environ Res Public Health. 2015;12(4):4214–30.
18. Bedade DK, Singhal RS. Biodegradation of acrylamide by a novel isolate, *Cupriavidus oxalaticus* ICTDB921: Identification and characterization of the acrylamidase produced. Bioresour Technol. 2018 Aug 1;261:122–32.
19. Aisami A, Gusmanizar N. Characterization of an acrylamide-degrading bacterium isolated from hydrocarbon sludge. Bioremediation Sci Technol Res. 2019 Dec 28;7(2):15–9.
20. Othman AR, Rahim MBHA. Modelling the Growth Inhibition Kinetics of *Rhodotorula* sp. strain MBH23 (KCTC 11960BP) on Acrylamide. Bioremediation Sci Technol Res. 2019 Dec 28;7(2):20–5.
21. Rusnam, Gusmanizar N. An Acrylamide-degrading Bacterial Consortium Isolated from Volcanic Soil. J Biochem Microbiol Biotechnol. 2021 Dec 31;9(2):19–24.
22. Rusnam, Gusmanizar N. Characterization of An Acrylamide-degrading Bacterium Isolated from Volcanic Soil. J Environ Bioremediation Toxicol. 2022 Aug 5;5(1):32–7.
23. Adnan M, Abu Zeid I, Ahmad SA, Effendi Halmi M, Abdullah S, Shukor M. A Molybdenum-reducing *Bacillus* sp. Strain Zeid 14 in Soils from Sudan that Could Grow on Amides and Acetonitrile. Malays J Soil Sci. 2016 Jan 1;20:111–34.
24. Jonston JJ, Borden RC, Barlaz MA. Anaerobic biodegradation of alkylbenzenes and trichloroethylene in aquifer sediment down gradient of a sanitary landfill. J Contam Hydrol. 1996;23(4):263–83.
25. Baek SH, Kim KH, Yin CR, Jeon CO, Im WT, Kim KK, et al. Isolation and characterization of bacteria capable of degrading phenol and reducing nitrate under low-oxygen conditions. Curr Microbiol. 2003;47(6):462–6.
26. Egorova K, Trauthwein H, Verseck S. Purification and properties of an enantioselective and thermoactive amidase from the thermophilic *actinomycete Pseudonocardia thermophila*. Appl Microbiol Biotechnol. 2004;38–45.
27. Rahim MBH, Syed MA, Shukor MY. Isolation and characterization of an acrylamide-degrading yeast *Rhodotorula* sp . strain MBH23 KCTC 11960BP. J Basic Microbiol. 2012;52(5):573–81.
28. Rogacheva SM, Ignatov OV. The Respiratory Activity of *Rhodococcus rhodochrous* M8 Cells Producing Nitrile-Hydrolyzing Enzymes. Appl Biochem Microbiol. 2001;37(3):282–6.
29. Shukor MY, Ahmad SA, Nadzir MMM, Abdullah MP, Shamaan NA, Syed MA. Molybdate reduction by *Pseudomonas* sp . strain DRY2. J Appl Microbiol. 2010;108:2050–8.
30. Shen S min, Wan T jou, Hwang H yuan. Biocatalysis and Agricultural Biotechnology Enhancement of degradation of acrylamide coupled with salad oil by *Pseudomonas aeruginosa* DS-4 using incubation periods. Biocatal Agric Biotechnol. 2012;1(2):110–4.
31. Wakaizumi M, Yamamoto H, Fujimoto N, Ozeki K. Acrylamide degradation by filamentous fungi used in food and beverage industries. J Biosci Bioeng. 2009;108(5):391–3.
32. Cha M, Chambliss GH. Characterization of Acrylamidase Isolated from a Newly Isolated Acrylamide-Utilizing Bacterium , *Ralstonia eutropha* AUM-01. Curr Microbiol. 2011;671–8.
33. Shukor MY, Gusmanizar N, Ramli J, Shamaan NA, Maccormack WP, Syed MA. Isolation and characterization of an acrylamide-degrading *Antarctic* bacterium. J Enviromental Biol. 2009;30(1):107–12.
34. Amor L, Kennes C, Veiga MC. Kinetics of inhibition in the biodegradation of monoaromatic hydrocarbons in presence of heavy metals. Bioresour Technol. 2001 Jun 1;78(2):181–5.
35. Gopinath KP, Kathiravan MN, Srinivasan R, Sankaranarayanan S. Evaluation and elimination of inhibitory effects of salts and heavy metal ions on biodegradation of Congo red by *Pseudomonas* sp. mutant. Bioresour Technol. 2011;102(4):3687–93.
36. Roane TM, Josephson KL, Pepper IL. Dual-Bioaugmentation Strategy To Enhance Remediation of Cocontaminated Soil. Appl Environ Microbiol. 2001 Jul;67(7):3208–15.
37. Hettiarachchi GM, Pierzynski GM, Ransom MD. In situ stabilization of soil lead using phosphorus and manganese oxide. Environ Sci Technol. 2000;34(21):4614–9.
38. Babich H, Stotzky G. Effect of Cadmium on Fungi and on Interactions Between Fungi and Bacteria in Soil: Influence of Clay Minerals and pH. Appl Environ Microbiol. 1977 May;33(5):1059–66.
39. Kamel Z. Toxicity of cadmium to twoStreptomyces species as affected by clay minerals. Plant Soil. 1986 Jun 1;93(2):195–203.
40. Kulkarni NH, Muley AB, Bedade DK, Singhal RS. Cross-linked enzyme aggregates of arylamidase from Cupriavidus oxalaticus ICTDB921: process optimization, characterization, and application for mitigation of acrylamide in industrial wastewater. Bioprocess Biosyst Eng [Internet]. 2019; Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85075085765&doi=10.1007%2fs00449-019-02240-4&partnerID=40&md5=12e064000a111764698781f8642894
41. Bedade DK, Muley AB, Singhal RS. Magnetic cross-linked enzyme aggregates of acrylamidase from Cupriavidus oxalaticus ICTDB921 for biodegradation of acrylamide from industrial waste water. Bioresour Technol. 2019;272:137–45.
42. Bedade DK, Singhal RS. Isolation and Characterization of Acrylamidase from Arthrobacter sp. DBV1 and Its Ability to Biodegrade Acrylamide. Appl Biochem Biotechnol. 2017;182(2):570–85.
43. Lakshmikandan M, Sivaraman K, Elaiya Raja S, Vasanthakumar P, Rajesh RP, Sowparthani K, et al. Biodegradation of acrylamide by acrylamidase from Stenotrophomonas acidaminiphila MSU12 and

analysis of degradation products by MALDI-TOF and HPLC. Int Biodeterior Biodegrad. 2014;94:214–21.
44. Emmanuel Joshua Jebasingh S, Lakshmikandan M, Rajesh RP, Raja P. Biodegradation of acrylamide and purification of acrylamidase from newly isolated bacterium *Moraxella osloensis* MSU11. Int Biodeterior Biodegrad. 2013;85:120–5.
45. Syed MA, Ahmad SA, Kusnin N, Shukor MYA. Purification and characterization of amidase from acrylamide-degrading bacterium *Burkholderia* sp. strain DR.Y27. Afr J Biotechnol. 2012;11(2):329–36.
46. Cha M, Chambliss GH. Characterization of acrylamidase isolated from a newly isolated acrylamide-utilizing bacterium, *Ralstonia eutropha* AUM-01. Curr Microbiol. 2011;62(2):671–8.
47. Rahman MFA, Yasid NA, Ahmad SA, Shamaan NA, Shukor MY. Characterization of molybdenum-reduction by an acrylamide-degrading Antarctic bacterium. In 10-3 Midori-cho, Tachikawa, Tokyo, Japan: National Institute of Polar Research (NIPR); 2018. Available from: http://id.nii.ac.jp/1291/00015258/