JOBIMB, 2021, Vol 9, No 1, 37-41
https://doi.org/10.54987/jobimb.v11i1.804

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JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

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Characterizing the Potentials of *Alcaligenes* sp. for Acrylamide Degradation

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HISTORY

Received: 1st Jan 2023
Received in revised form: 23rd Feb 2023
Accepted: 4th April 2023

KEYWORDS

Acrylamide
Alcaligenes sp.
Bioremediation
Acrylamde-degrading bacterium
Detoxification

ABSTRACT

Microorganisms play a crucial role in the detoxification and elimination of harmful substances from the environment. Acrylamide is a major pollutant in soil from the breaking down of pesticide addictive. Slowly but steadily, the used of microbe-mediated acrylamide breakdown as a bioremediation approach has gained attention all around the world. This research was aimed to characterize the potential of previously isolated bacteria for acrylamide degradation. In this research five previously isolated bacteria were screened on minimal salt media (MSM) to assess their potential for utilizing acrylamide as the sole nitrogen source. Of all the isolates, isolate D, identified as *Alcaligenes* sp., displayed the highest growth on acrylamide supplemented MSM agar medium. The optimum conditions for acrylamide degradation by this bacterium were acrylamide concentration of 600 mg/L, pH 7.0, an inoculum size of 400 µL, an incubation time of 96 h, glucose concentration of 4 g/L and temperature of 35 °C. The ability of this isolate to convert toxic acrylamide into less harmful form is a novel finding, highlighting its significance as a valuable tool for bioremediation of this pollutant.

INTRODUCTION

Water and land contamination by industrial chemicals, waste products, and biocides is a major issue for human civilization [1]. The world's progress nowadays is gradually making the environment unpleasant owing to contamination and pollution. Among the most pervasive sources of pollution include oil spills, chemical wastes, and heavy metals. One of the most cost-effective and potentially fruitful methods for cleaning the environment of harmful contaminants is the use of biological agents and their mechanisms [2]. Polyacrylamide, which is made from acrylamide, is used to purify water as well as in the printing, plastics, and adhesives industries [3,4]. As a result of its widespread usage in industry, acrylamide has been linked to environmental contamination caused by its careless disposal [5]. Carbohydrate-rich meals that are cooked at high temperatures through a mechanism called the Maillard reaction [6] produce acrylamide, which is among the neurotoxic and carcinogenic chemicals. The creation of acrylamide, which is carcinogenic and neurotoxic [4,7], can occur during the cooking process known as the Maillard reaction. Due to its neurotoxic, carcinogenic, and teratogenic qualities [5,8] mentioned previously, acrylamide poses a significant environmental risk [8].

The neurotoxic, carcinogenic, and teratogenic effects of acrylamide have been well-documented [9]. In Shingu, Japan, in 1974, there was a case of severe acrylamide poisoning that gained widespread attention [10]. The grouting activity was only 2.5 meters away, yet it was enough to pollute the well water with high amounts of acrylamide (400 mg/L). After drinking and bathing in acrylamide-tainted well water for a week, five members of the same family had symptoms of intoxication [11]. These symptoms included confusion, disorientation, memory difficulties, hallucinations, and truncal ataxia. Getting rid of acrylamide is crucial. Since acrylamide monomer was used by practically every industry. It's crucial that acrylamide and its derivatives be removed from industrial effluents before they're

released into the environment [5]. Therefore, it is necessary to address this issue by concentrating on biological acrylamide remediation [6]. Thermal, photolytic, chemical, mechanical, and biological degradation are the five main methods currently available for acrylamide breakdown. Chemical treatment is typically used in wastewater treatment to breakdown acrylamide [5,12]. Bioremediation is a method of biological degradation being utilized to address acrylamide contamination as an alternative to physicochemical treatments. Remediation technology that employs the microbial process to clean contaminated places has been shown to be successful and reputable [13]. Despite the widespread reports of microorganisms having acrylamide-degrading capacity, nothing is known about the characterisation of naturally occurring bacteria in Nigeria.

MATERIALS AND METHODS

Sample Collection

Acrylamide was procured from Biochemistry Laboratory, Bayero University Kano State, Nigeria. Bacterial isolates were obtained from a culture collection of locally isolated bacteria.

Media Preparation

All chemicals used in this research were of analytical grade. Minimal salt medium (MSM) containing (gL^{-1}): Glucose: 10 g, KH_2PO_4: 1.36 g, $MgSO_4.7H_2O$: 0.50 g, Na_2HPO_4: 2.13 g and 1 of trace element or metal solution was dissolved in 1.0 L deionized water. The metal solution contains $CaCl_2.2H_2O$: 0.40 g, H_3BO_3: 0.30 g, $CuSO_4.5H_2O$:0.04 g, $FeSO_4.7H_20$: 0.20 g, $MnSO_4.7H_2O$: 0.40 g was added to the medium and autoclaved at 121 ºC for 45 min. To investigate acrylamide breakdown by bacterial isolates, 0.5 g/L of acrylamide was introduced to the media. Bacteria were cultured in a nutrient broth medium.

Growth on solid medium

The autoclave setting was at 121 degrees Celsius for 45 min. To prepare the solid medium, 2.8 grams of nutritional agar were added to 100 milliliters of distilled water in a 250 milliliter conical flask. To test the viability of individual isolates, the sub-culturing of the bacterium was routinely carried out.

Screening of isolates for acrylamide degradation

Prepared MSM containing 1.5 g of agar-agar was used. The media was distributed into petri-dishes (20 each) under a biosafety cabinet and allowed to solidify. Bacterial isolates labeled N, D, S, R and H were streaked unto each petri-dish and incubated for 3 days. Macroscopic screening was performed to identify the isolate with maximum growth.

Characterization of acrylamide degradation

Effect of acrylamide concentration

Prepared liquid media (MSM) was amended with different concentration of acrylamide (100, 200, 400, 600, 800, 1000 mg/L) in a 250 mL conical flask each was inoculated with 100 µL of *Alcaligenes* sp. and incubated at 37 ºC in triplicate. The optical densities were measured 24 h intervals using spectrophotometer at wavelength of 600 nm [14].

Effect of pH

Prepared MSM broth supplemented with 0.5 g/L of acrylamide was adjusted to different initial pH (5.5, 6.0, 6.5, 7.0, 7.5, and 8.0) using HCl and NaOH. Each in triplicate was inoculated with 100 µL of *Alcaligenes* sp. and incubated at 37 ºC. Control without inoculum was kept under similar conditions. The optical densities (OD) were determined after 24 h up to 120 h using spectrophotometer at a wavelength of 600 nm to observe the growth rate of the isolate [14].

Effect of inoculums size

Different inoculum volumes (50, 100, 200, 400, 600, and 800 µL) were inoculated in triplicate into a prepared liquid media (MSM) enriched with 0.5 g/L of acrylamide. The inoculated group and the non-inoculation group were kept in identical conditions. The optimum inoculum size of *Alcaligenes* sp. on acrylamide degradation [14] was determined by measuring optical densities (OD) using a spectrophotometer at 600 nm at intervals of time ranging from 24 to 120 h.

Effect of incubation time

About 100 µL of *Alcaligenes* sp. was treated in triplicate with prepared liquid media (MSM) supplemented with 0.5g/L of acrylamide. *Alcaligenes* sp.-noninoculated controls were also maintained under the same conditions. The optimal growth period for acrylamide degradation [14] was determined by measuring optical densities (OD) at 600 nm at regular intervals from 24 to 120 h using a spectrophotometer.

Effect of glucose concentration

In a series of 250 conical flasks, 100 µL of *Alcaligenes* sp. was added to MSM that had been supplemented with glucose at doses of 1, 2, 4, 6, 8, and 10 g/L and it was incubated at 37 ºC in triplicate including the control without inoculum were kept under similar condition. The incubation period was from 24 to 120 hours. The growth rate of the isolate was monitored by measuring the optical density (OD) at a wavelength of 600 nm using a spectrophotometer. [14].

Effect of temperature

Prepared liquid media (MSM) supplemented with 0.5 g/L of acrylamide was inoculated with 100 µL of *Alcaligenes* sp. and incubated at different temperature (25, 30, 35, 37, 40 ºC) in triplicates. The inoculum-free control group was also maintained in the same way. The optimal growth temperature for acrylamide degradation [14] was determined by measuring optical densities (OD) at 600 nm at regular intervals from 24 to 120 h using a spectrophotometer as previous.

RESULTS AND DISCUSSION

Characterization of acrylamide degradation

Effect of acrylamide concentration

The effect of acrylamide concentration on growth of *Alcaligenes* sp. showed that the growth increases linearly as the concentration increases from 100 mg/L to 600 mg/L. An optimum acrylamide concentration was observed at 600 mg/L, with a significant decreased in growth at concentrations above the optimum (800 mg/L and 1000 mg/L) as showed in **Fig. 1**.

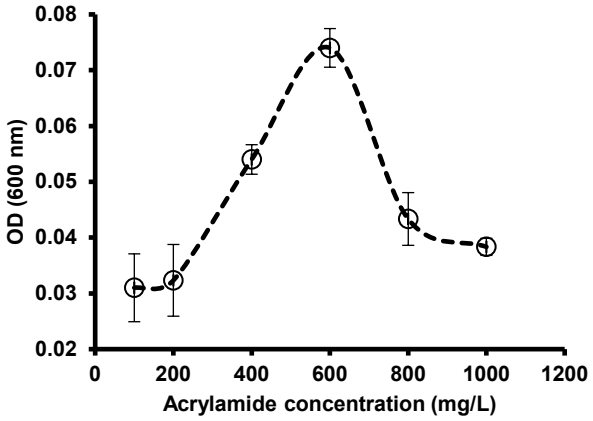

Fig. 1. Effect of acrylamide concentrations (nitrogen source) on growth of acrylamide-degrading *Alcaligenes* sp. after 96 h of incubation. Data represent mean ± standard deviation of triplicate.

Effect of pH on acrylamide degradation

The initial effect of pH on the growth of *Alcaligenes* sp. showed that the growth increases sequentially as pH increases from pH 5.5 to pH 7.0. An optimum pH was notice at pH 7.0, with a significant decrease in growth at pH above the Optimum (pH 7.5 and 8.0), as depicted in **Fig 2.**

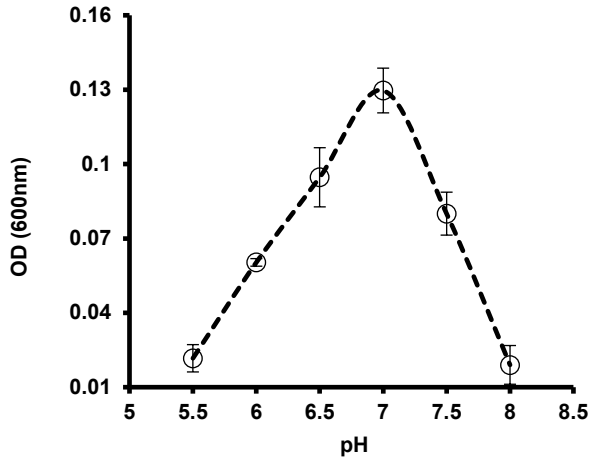

Fig. 2. Effect of pH on acrylamide degradation on growth of acrylamide degrading *Alcaligenes* sp. after 96 h of incubation. Data represent mean ± standard deviation of triplicate.

Effect of inoculum sizes on acrylamide degradation

Fig. 3 shows the effect of inoculum sizes on growth of *Alcaligenes* sp. that the growth increases as the inoculum size increases progressively from 50 µL to 400 µL. An optimum inoculum size was seen at 400 µL with no significant decrease in growth at inoculum size above the optimum (600 µL and 800 µL).

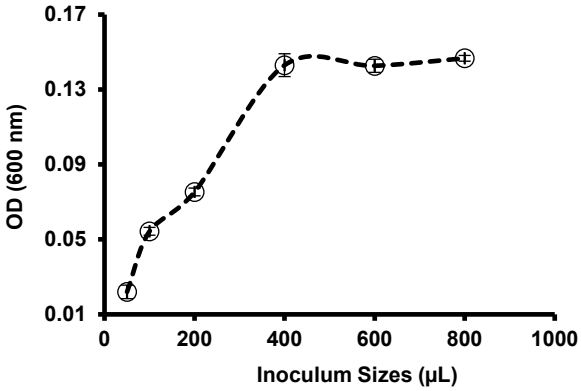

Fig. 3. Effect of inoculum sizes on acrylamide biodegradation on growth of acrylamide-degrading *Alcaligenes* sp. after 96 h of incubation. Data represent mean ± standard deviation of triplicate.

Effect of incubation time on acrylamide degradation

The effect of incubation time on acrylamide degradation by *Alcaligenes* sp. revealed on an exponential growth from 24 – 96 h of incubation in minimal salt medium (MSM). The growth attained peak (maximum) at 96 h and gradually decline afterwards (as depicted in **Fig. 4**).

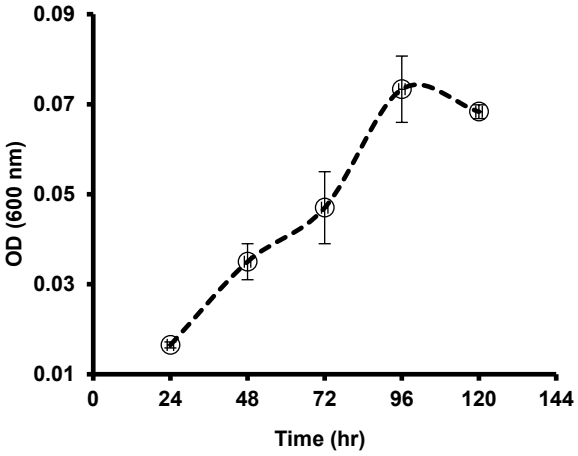

Fig. 4. Effect of incubation times on acrylamide degradation on growth of acrylamide degrading *Alcaligenes* sp. Data represent mean ± standard deviation of triplicate.

Effect of glucose concentration on acrylamide degradation

The effect of glucose concentration on growth of *Alcaligenes* sp. showed that the growth increases as the concentration progressively increase from 1 g/L to 4 g/L. An optimum glucose concentration was observed at 4 g/L with a significant decrease in growth at concentration above the optimum (6, 8 and 10 g/L), as depicted in **Fig. 5**.

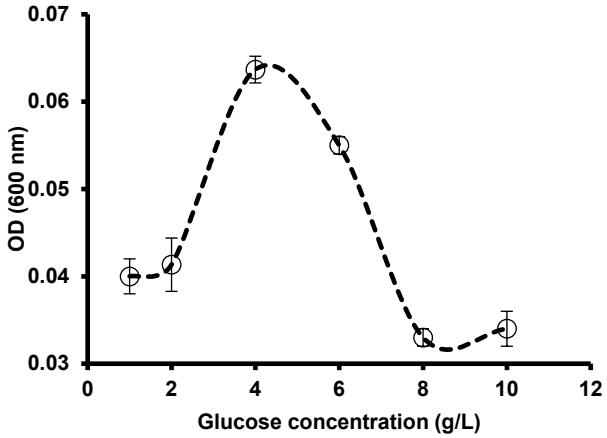

Fig. 5 Effect of glucose concentrations (carbon source) on acrylamide degradation on growth of acrylamide-degrading *Alcaligenes* sp. after 96 h of incubation. Data represent mean ± standard deviation of triplicate.

Effect of temperature on acrylamide degradation

The effect of temperature on growth of *Alcaligenes* sp. shows that the growth increases as the temperature increases linearly from 25 ºC to 35 ºC. An optimum temperature was observed at 35 ºC, with a significant decrease in growth at temperature above the optimum (37 ºC to 40 ºC). But the growth is insignificant between 37 ºC and 40 ºC, as showed in **Fig 6.**

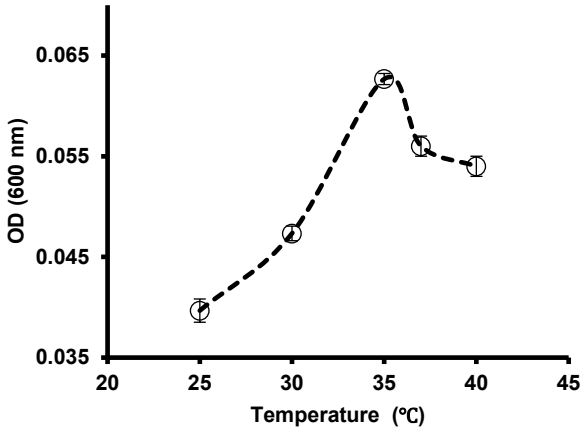

Fig. 6. Effect of temperature on acrylamide degradation on growth of acrylamide-degrading *Alcaligenes* sp. Data represent mean ± standard deviation of triplicate.

DISCUSSION

The reported optimum concentration that supported the growth of *Alcaligenes* sp. was 600 mg/L. This works agrees with the work of [4]. However, it contradicts the works of [1,6,15,16]. These differences may be attributable to the difference in degrading ability of the various hydrocarbon degradation bacteria used in the studies. The higher the concentration of acrylamide, the lower the enzymatic activity of the degradation bacteria, hence, a decrease or even a halt in the degradation ability of the bacteria. From the findings of this study, the reported optimum pH of 7.0 correspond with findings of several other research such as [1,4,15,17–19]. Similarly, the reported optimum pH of this work contradicts that of [20] who recorded 7.5 – 8.5 as the optimum pH for acrylamide degradation. From the findings, most acrylamide-degrading bacteria are neutrophils and a slight alteration in the pH condition may inhibit their growth as the degrading bacteria rarely thrive at acidic or alkaline conditions. This is due to the denaturation effect on the degrading bacteria, that occurs under highly acidic or strong alkaline pH which may alter their enzymatic action.

An inoculum 400 µL was found to optimally support to the growth of this bacterium. The higher the inoculum size, the higher the optical density, hence the growth of *Alcaligenes* sp. The reported optimum glucose concentration in this work, indicated that the optimum growth of *Alcaligenes* sp. occurred at glucose concentration of 4 g/L. This finding suggested that this bacterium grow favorably at 4 g/L of glucose. At lower concentration the availability of glucose may be insufficient to support optimal growth. On the other hand, higher glucose concentration might have created metabolic imbalance or inhibitory effect, negatively impacting growth.

The reported optimum temperature of 35 ºC in this work agrees with the works of [15,16,21]. However, contradicts the works of [1,4,9]. From the finding, the reported temperature showed that the bacteria belong to mesophiles, that can thrive at moderate temperature and any slightly decrease or increase in temperature may inhibit the growth of the bacteria due to their enzymatic activity. Enzyme activity is temperature-dependent, change in temperature can affect the enzymatic reaction, either accelerating or inhibiting them, and it can also lead to denaturation of their proteins.

CONCLUSION

Previously isolated bacteria were successfully screened for acrylamide degradation, an isolate (D) corresponding to *Alcaligenes* sp. was found to be the best acrylamide degrader. The study demonstrated that the bacterium thrived or grew best at acrylamide concentration of 600 mg/L, pH 7.0, inoculum size of 400 µL, incubation time of 96 h, glucose concentration of 4 g/L and temperature of 35 ºC. Hence this isolate could be a better candidate for the future bioremediation of this pollutant.

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