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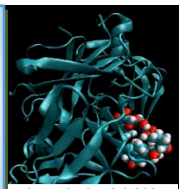

Characterization of Aniline Degradation by A Previously Isolated Molybdenum-reducing *Pseudomonas* sp.

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ABSTRACT

Microorganisms play an integral role in detoxification and removal of toxic compounds from the environment. Aniline is the simplest aromatic amine, consisting of a phenyl group attached to an amino group that is used as herbicide to control weeds. Aniline is detrimental to both environment and health. In this research, six previously isolated bacteria (isolate A-F) were screened on Bushnell Hass media for their potential to grow and utilize aniline as a sole carbon source. Isolate A (*Pseudomonas* sp.) was found to tolerate and grow best with aniline sole source of carbon. Optimum conditions for aniline degradation by this isolate were found to be pH 6.0, temperature between 30 and 37 °C, inoculums size of 600 µL, aniline concentration of 200 mg/L and incubation time of 96 h. The capacity of this isolate to reduce toxic aniline to less toxic form is novel and makes the bacterium important instrument for bioremediation of this pollutant.

INTRODUCTION

Environmental pollution rate has risen in the past few decades due to increased human activities on energy reservoirs, questionable agricultural practices and swift industrialization [1]. Aniline is an environmental pollutant that results from the manufacture of products such as dyes, additives, paints, plastics and herbicides [2,3]. Aniline is formed from the biotransformation of nitroaromatic compounds or other aniline based pesticides by microorganisms [4]. It is a toxic compound that causes oxidative stress and mechanisms that contributes to cell death [5]. It is a weak aromatic base that in alkaline condition remains stable and significantly affect aquatic lives [6]. Aniline is also an intermediate in the biodegradation of herbicide butachlor by *Bacillus altitudinis* [7].

The accumulation of these toxic substances in water bodies and soil has led to initiation of various research on ways to remove or eradicate the environments of these toxicants as the use of biological treatment has proven to be the most effective method of achieving this goal. However, high concentrations of destructive chemicals affects the ability of these organisms in the removal of the toxic compounds [8]. Fate of organic pollutants in the environment depends on the biochemical and physical aspects of the pollutants as well as its environment [9]. Nevertheless, suitable physical factors like pH and temperature as observed enhances the biodegrading ability of microorganisms [8]

Over the years, researchers have developed various techniques that can be used to rid environment of its pollutants, but to date, there is no single procedure that serve as a single-shot to remediating all type of pollutants [1]. Bioremediation as a process involves the use of microorganisms in the restoration of contaminated environment to their original form. The organisms residing in the polluted areas holds the key to removing pollutants [10]. Until present, literature on the isolation and characterization of aniline-degrading bacteria are scarce and often not from this region. Hence, this research will focus on finding indigenous isolate with potential to degrade aniline.

MATERIALS AND METHODS

Sample Collection and Reagents
Aniline was a product of Sigma-Aldrich procured from the Department of Biochemistry, Bayero University, Kano state, Nigeria.

Media Preparation
All chemicals used in this research were of analytical grade. Bushnell Haas media containing (gL^{-1}): $MgSO_4$, 0.2; $CaCl_2$, 0.02; KH_2PO_4, 1.0; K_2HPO_4, 1.0; NH_4NO_3, 1.0 and $FeCl_3$, 0.050 were added to 700 ml of distilled water in a 1000 ml conical flask and autoclaved at 121 °C for 45 minutes. 0.1 gL^{-1} of aniline was added to the media to study aniline degradation by bacterial isolates. Nutrient broth was used for growth of bacterial culture.

Screening of isolate for aniline degradation
Screening of isolates was carried out in petri dish. In each plate, 20 ml of prepared solid media was added under a lamina flow and allowed to solidify. To all the petri dish, bacterial isolates labelled (A-F) were inoculated and incubated for 72 h. Screening for maximum growth was done macroscopically.

Effect of Aniline Concentration
Prepared liquid media (Bushnell Haas) was amended with different concentrations of aniline (50, 100, 200, 400, 600, 800 mg/L) in 250 ml conical flacks each was inoculated with 100 µL of *Pseudomonas* sp. It was incubated at 37 °C in triplicate and also control without inoculums kept in triplicate and analysed for the growth of *Pseudomonas* sp. The optical densities were measured at 24 h intervals using spectrophotometer at wavelength of (600 nm).

Effect of pH
Prepared liquid media (Bushnell Haas) supplemented with 0.1 g/L of aniline 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 *Pseudomonas* sp. and incubated at 37 °C, A triplicate control, without inoculation of *Pseudomonas* sp. was kept under similar conditions. The optical densities were determined at 24 h interval using a spectrophotometer at a wavelength of 600 nm to observe the growth rate of the isolate.

Effect of Inoculums Size
Prepared liquid media (Bushnell Haas) supplemented with 0.1 g/L of aniline was inoculated with different inoculums sizes (100, 200, 400, 600,800 and 1000 µL) each in triplicate, controls without inoculation were kept under similar condition. Optical density (OD) was measured using spectrophotometer at 600 nm at 24 h interval to determine the optimum inoculums size of the *Pseudomonas* sp. on aniline degradation.

Effect of Temperature

Prepared liquid media (Bushnell Haas) supplemented with 0.1 g/l of aniline was inoculated with 100 µL of *Pseudomonas* sp. and incubated at different temperatures (25, 30, 37 and 40 °C) in triplicate. Also controls, without inoculation of *Pseudomonas* sp. was kept under similar conditions. Optical densities (OD) were measured at 24 h interval using a spectrophotometer at 600 nm to determine the optimum growth temperature for aniline biodegradation.

Effect of incubation Time
Prepared liquid media (Bushnell Haas) supplemented with 0.1 g/L of aniline was inoculated with 100 µL of *Pseudomonas* sp. in triplicate. Also controls, without inoculation of *Pseudomonas* sp. was kept under similar conditions. Optical densities (OD) were measured at regular intervals of time 24 h up to 120 h using a spectrophotometer at 600 nm to determine the optimum growth temperature for aniline biodegradation.

RESULTS

Screening of Isolates for Aniline Biodegradation
A total of six (6) previously molybdenum-reducing bacteria isolated from Agricultural soils in Kano state were screened for their potential to degrade aniline and utilize it as sole source of carbon. Out of the six isolates, isolate A (*Pseudomonas* sp.) was observed to tolerate and grow best on Bushnell Haas media containing aniline as sole carbon source following 48 h of incubation at 37 °C, thus was chosen as a better candidate for further analysis.

Characterization of Aniline Degradation

Effect of incubation time on aniline degradation
The result of the effect of incubation time shows that *Pseudomonas* sp. was found to grow exponentially attaining optimum after 96 h of incubation, while a decline in growth was observed beyond the optimum incubation time, signifying its death phase (**Fig 1**).

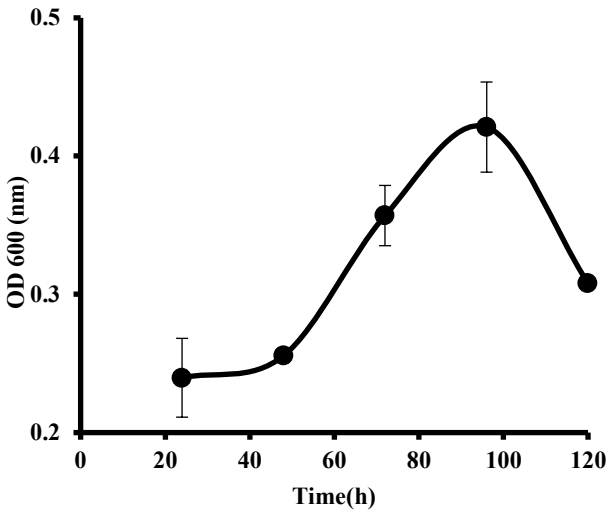

Fig. 1. Effect of incubation time on aniline degradation by *Pseudomonas* sp. Data represent: Mean ± standard deviation of triplicate determination.

Effect of Aniline Concentration
The effect of aniline concentrations on the degradation capability of *Pseudomonas* sp. was assessed between 50 – 800 mg/L. An optimum growth was observed at 200 mg/L concentration, with a sharp decline in growth observed as the concentration was increased between 400 to 600 mg/L after 48 h of incubation (**Fig. 2**).

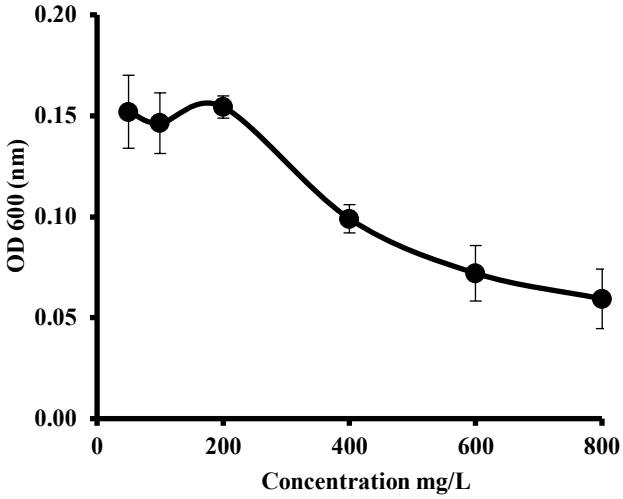

Fig. 2. Effect of aniline Concentrations (carbon source) on aniline degradation by *Pseudomonas* sp. after 48 h of incubation. Data represent mean ± standard deviation of a triplicate concentration.

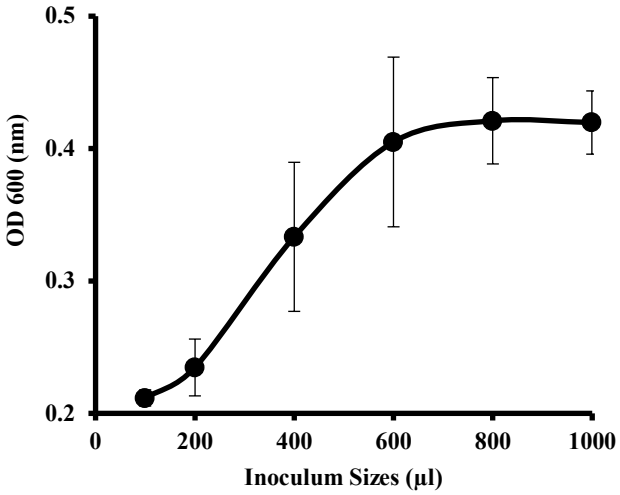

Fig. 4. Effect of inoculums sizes on aniline degradation by *Pseudomonas* sp. after 96 h of incubation. Data represent: mean ± standard deviation of triplicate determination.

Effect of pH on Aniline degradations

The effect of various initial pH on Aniline degradations was evaluated at different initial pH range from 5.5 – 8.0. The result obtained showed that the growth was found to be optimum at pH 6.0 for aniline degradation. However, growth decreases significantly ($p<0.05$) at pH above 6.0 over 96 h of incubation (**Fig. 3**).

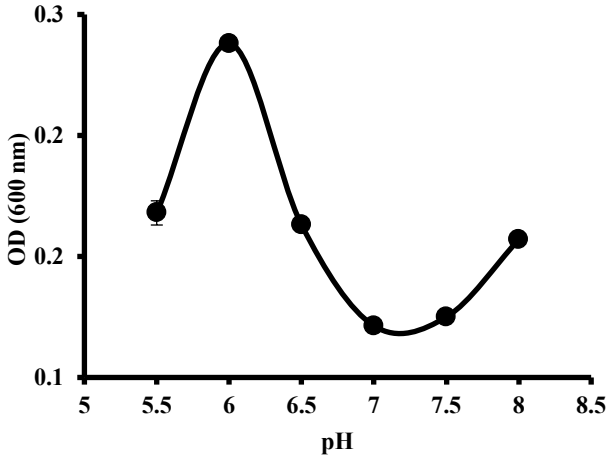

Fig. 3. Effect of various initial pH on aniline degradation by *Pseudomonas* sp. after 96 h Incubation Data represent: Mean ± Standard Deviation of triplicate determination.

Effect of inoculums size on aniline degradation

Inoculums of 100 - 1000 µL were used to determine the effect of inoculums sizes. The result showed that the growth increases as size of the inoculum increases (**Fig. 4**). Though the increase in growth was insignificant ($p>0.05$) from 600 – 1000 µL.

Effect of temperature on Aniline degradation

The effect of temperatures on aniline degradation by *Pseudomonas* sp. was assessed ranging from 25 to 40 °C. Optimum growth was seen at 37 °C, though insignificantly ($p>0.05$) vary from 30 °C, providing wide range of optimum temperature to be between 30 and 37 °C (**Fig. 5**).

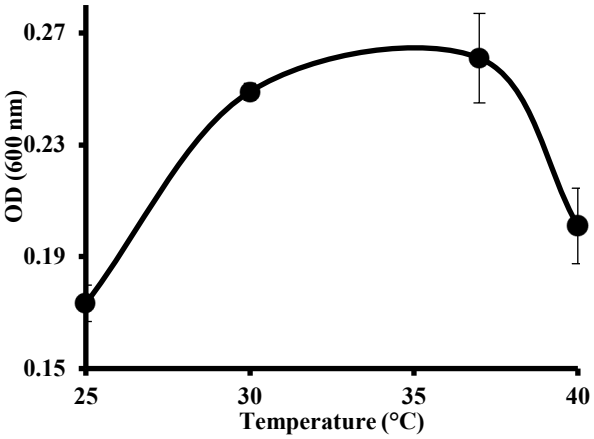

Fig. 5. Effect of temperature on aniline degradation by *Pseudomonas* sp. Data represent: mean ± standard deviation of triplicate determination.

DISCUSSION

Bioremediation taps into the ability of microorganisms to metabolize and convert organic contaminants into less toxic form. It has been an efficient method, economic and environmentally friendly biological treatment technique [11]. In the present study, different concentrations were used to determine the tolerance of the bacterium to aniline.

The result revealed a lower optimum concentration of 200 mg/L, compared to other literature. [2] reported a tolerable concentration by *Enterobacter ludwigii* KH-5 of up to 700 mg/L. [12] reported a concentration of 2000 mg/L degraded in 22 h by *Delftia* sp. XYJ6. [13] reported an optimum concentration of 3200 mg/L by *Delftia tsuruhatensis* 14S. [14] also reported an off-the-chart tolerable concentration of up to 5000 mg/L. Considering some of these findings, it can be safe to say, despite the fact that this isolate tolerated and utilize aniline as sole carbon source, but it is less efficient degrader of the toxicant compared to *Delftia* sp. strains.

In another study set to test the effect of pH on the degradation potential of this bacterium, a pH of 6.0 was found to be optimum. This means that the bacterium is likely to be an acidophile. The findings contradict the work on *Rhodococcus erythropolis* AN-13 with an optimum pH between 5.0 to 9.0 [15]. Additionally, growth of the bacterium in the media was observed to increase as the inoculum sizes increases attaining maximum growth at 600 µL, from which a steady state was maintained. This can be explained looking at the fact that a larger healthy inoculum multiplies exponentially much better than small inoculum.

Temperature exerts a strong selective pressure on microbial communities and can affect the degradation of organic compounds through direct effects on enzyme activity [16]. Optimum temperature was observed in this study to between 30 and 37 °C. A similar study on the degradation of aniline by *Pseudomonas* strain KI reported 30 °C optimum temperature for biodegradation [17]. It was also demonstrated in another study that growth and metabolic activity of *Pseudomonas* sp. B10 was also best at 30 °C hence effective for degradation [18].

CONCLUSION

In this study, the potentials of a previously isolated molybdenum reducer to degrade aniline was determined. The bacterium was found to grow optimally at pH 6.0, temperature 37 °C, incubation time of 96 h and aniline concentration of up to 200 mg/L with inoculums size of 600 µl. Hence this isolate could be suitable organism for the future bioremediation of this pollutant.

REFERENCES

1. Azubuike CC, Chikere CB, Okpokwasili GC. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol. 2016;32(11).
2. Kafilzadeh F, Khezri A. Biodegradation of aniline by *Enterobacter ludwigii* kh-5 isolated from the soil around shiraz refinery, Iran. Global NEST J, 2016;18(X):1–11.
3. Yen J-H, Tsai P-W, Chen W-C, Wang Y-S. Fate of anilide and aniline herbicides in plant-materials-amended soils. J Environ Sci Heal Part B. 2008 Jun;43(5):382–9.
4. Mujahid M, Sasikala C, Ramana VC. Aniline is an inducer, and not a precursor, for indole derivatives in *Rubrivivax benzoatilyticus* JA2. PLoS One. 2014;9(2):1–10.
5. Wang Y, Gao H, Na X, Dong S, Dong H, Yu J, et al. Aniline Induces Oxidative Stress and Apoptosis of Primary Cultured Hepatocytes. Int J Environ Res Public Health, 2016;5–7.
6. Sarwade V, Gawai K. Biodegradation of aniline by alkaliphilic strain *Bacillus badius* D1. IOSR J Environ Sci Toxicol Food Technol, 2014;8(5):71–8.
7. Kaur R, Goyal D. Biodegradation of Butachlor by Bacillus altitudinis and Identification of Metabolites. Curr Microbiol, 2020;76:2602–2612.
8. Khan SA, Hamayun M, Khan AL, Ahmad B, Lee I. Influence of pH , temperature and glucose on biodegradation of 4-aminophenol by a novel bacterial. Afr J Biotechnol, 2009;8(16):3827–31.
9. Boyd SA, Shelton DR, Berry D, Tiedje JM. Anaerobic Biodegradation of Phenolic Compounds in Digested Sludge. Appl Environ Microbiol, 1983;46(1):50–4.
10. Verma JP, Jaiswal DK. Book Review: Advances in Biodegradation and Bioremediation of Industrial Waste. Front Microbiol. 2016;6(January):2.
11. Feidieker D, Kampfer P, Dott W. Microbiological and chemical evaluation of a site contaminated with chlorinated aromatic compounds and hexachlorocyclohexanes. FEMS Microbiol Ecol,1994;15: 265–78.
12. Chengbin X, Jun N, Hai YAN, Xudong SUN. Biodegradation of Aniline by a Newly Isolated *Delftia* sp. XYJ6. Chin J Chem Eng, 2009;17(3):500–5.
13. Sheludchenko MS, Kolomytseva MP, Travkin VM, Akimov VN, Golovleva LA. Degradation of Aniline by *Delftia tsuruhatensis* 14S in Batch and Continuous Processes. Appl Biochem Microbiol. 2005;41(5):465–8.
14. Liu Z, Yang H, Huang Z, Zhou P, Liu S-J. Degradation of aniline by newly isolated, extremely aniline-tolerant *Delftia* sp. AN3. Appl Microbiol Biotechnol. 2002;58(5):679–82.
15. Aoki K, Ohtsuka K, Shinke R, Nishira H. Isolation of Aniline-assimilating Bacteria and Physiological Characterization of Aniline Biodegradation in *Rhodococcus erythropolis* AN-13. Agr Biol Chem, 2014;1369.
16. Pettersson M, Baath E. Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. FEMS Microbiol Ecol, 2003;45: 13–21.
17. Knight D, Turco RF. Characterization of a *Pseudomonas* sp. Capable of Aniline Degradation in the Presence of Secondary Carbon Sourcest Df. Appl Environ Microbiol, 1989;55(2):385–9.
18. El-Deeb BA. Plasmid-Mediated Aniline Assimilation by *Pseudomonas* sp. B10. J Microbial Biotech, 2001; 11(1):79–84.