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Biodegradation of Atrazine by Bacteria Isolated from Lotic Water

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Authors' contributions

This work was carried out in collaboration between both authors. Author CNA contributed during conception and design, analysis and interpretation of results and write-up of the manuscript. Author AA contributed during design, sample collection, analysis and acquisition of data. Both authors read and approved the final manuscript.

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ABSTRACT

Background: The persistence of toxic herbicides in water and soil has been considered to be potential environmental concern. As atrazine is still used in Nigeria as a major herbicide, a continuous search for atrazine degrading microorganisms is required.
Objectives: The effects of incubation period on growth and atrazine degradation by bacteria isolated from a lotic water in the Niger Delta were evaluated and also determined were the effects of temperature and anaerobic incubation on atrazine removal by the most efficient isolate.
Methods: The bacteria capable of degrading atrazine were isolated from the lotic water using enrichment technique. The optimal incubation period for growth and atrazine degradation by the isolates was assessed by growing the isolates in Mineral salts medium containing 100 mg/L atrazine for 30 days at 35ºC. The flasks inoculated with the most efficient isolate were also incubated anaerobically and at varying temperatures (25, 30, 35 and 40ºC). Cultures were withdrawn every 5 days and growth and atrazine concentration measurements were carried out using standard plate count and HPLC respectively.
Results: Atrazine degrading bacteria of the genera *Pseudomonas*, *Bacillus* and *Micrococcus* were isolated with *Pseudomonas* sp. as the most efficient isolate. Incubation time of 20 days was

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observed as optimum for growth of the three isolates. *Pseudomonas* sp. gave the highest atrazine degradation rate of 82.67% followed by *Bacillus* sp. (75.33%) and *Micrococcus* sp. (69.33%) at the end of 30 days. Atrazine was degraded at reduced rate under anaerobic condition and temperature of 35ºC was optimum for atrazine degradation by *Pseudomonas* sp.
Conclusion: These atrazine degrading strains may be useful in bioremediation of contaminated wastewater.

Keywords: Herbicide; atrazine; biodegradation; bacteria.

1. INTRODUCTION

Pesticides, including herbicides, have been used in agriculture with the aim to get greater productivity in crops. However, only small amounts of the released agrochemical reach the specific target while the rest of the application has the potential to move into the soil and may contaminate surface waters [1].

Atrazine (2-Chloro-4-ethylamino-6-isopropylamino-s-triazine) is a herbicide widely used to kill weeds globally. Although several countries gave up the use of atrazine because of its toxicity, it is still one of the most popular herbicide in many countries [2]. It is still being used in Nigeria as the herbicide of choice and hence there is a high possibility of soil and water contamination in various parts of the country. The average half-life of atrazine in soil range from 13 to 261 days [3], in river water, it is more than 100 days [4], in seawater it is around 10 days [5] and nearly 660 days in case of anaerobic degradation [6]. However, half-life of atrazine has been previously reported to be 32 days in anaerobic soil and 86 days in the aqueous phase above the soil [7]. As a consequence of its high persistence and mobility atrazine and its metabolites can be detected in surface water, groundwater, drinking water supplies and even in fog [8].

The atrazine molecule consisting of N-alkylated and chlorinated heterocyclic aromatic ring, is a pollutant of environmental concern due to its low biodegradability. Once in aquatic environment atrazine may alter the structure and function of the communities [9]. But microorganisms have demonstrated the ability to metabolize the molecule partially or completely, leading to the formation of NH_3 and CO_2 [10]. Microbial degradation process aids the elimination of atrazine from the environment in a cost effective way [6]. The search of microbial strains capable of degrading atrazine in the environment is fundamental to the development of bioremediation processes [9].

Therefore, the aim of this present study was to isolate bacteria from a lotic water in the Niger Delta that have the ability to degrade atrazine and to evaluate the impact of some parameters on bacterial growth and atrazine degradation.

2. MATERIALS AND METHODS

2.1 Sample Collection

Freshwater sample was collected in sterile plastic bottle from a stream in Akpajo village, Eleme, Rivers State, Nigeria.

2.2 Source of Atrazine

Atrazine used was commercial Multrazine 50 SC (Active ingredient – Atrazine 50%). It was obtained from an agrochemical shop in Port Harcourt, Nigeria.

2.3 Enrichment and Isolation of Atrazine-Degrading Bacterial Strains

The atrazine-degrading bacteria were isolated using enrichment culture technique. The mineral salt medium according to [11] contained (g/L of distilled water): K_2HPO_4 0.8 g, KH_2PO_4 0.2 g, NaCl 0.5 g, $MgSO_4$ 0.1 g, $CaCl_2$ 0.4 g, $FeSO_4$ 0.02 g and $MnSO_4$ 0.01 g. The final pH was adjusted to 7.2. The medium was autoclaved at 121ºC for 15 minutes. Ten millilitres of water sample per 100 mL of medium supplemented with 10 mg/L of atrazine as the sole source of carbon and nitrogen in a 250 mL Erlenmeyer flask was incubated at 35ºC for 7 days. The enrichment cultivation was performed with different concentrations (10, 50, 100 and 150 mg/L) of atrazine in the media.

The bacteria present in the enrichment culture were isolated on mineral salt agar plates supplemented with 100 mg/L atrazine using spread plate technique. Isolates with distinct colonial morphology were picked and streaked repeatedly on nutrient agar plates until pure. The

purified isolates were identified to generic level based on their morphological and physiological characteristics [12].

2.4 The Effect of Incubation Period on Atrazine Degradation in Liquid Medium by the Bacterial Isolates

The bacterial isolates were tested for their ability to remove atrazine. The isolates were grown in 100 mL of liquid medium containing sterile mineral solution as described in 2.3 and atrazine in the concentration of 100 mg/L as the single source of carbon and nitrogen. The experiment was performed in triplicate and each flask received 0.1 mL of a 24 h culture of each isolate tested. The flasks and the uninoculated control flask were incubated at 35ºC for 30 days. Cultures were withdrawn every 5 days and growth and atrazine concentration measurements were carried out using standard plate count and High-performance liquid chromatography (HPLC) respectively.

2.5 HPLC Analyses

At each sampling time, 1 mL of culture supernatant was extracted with 2 mL of ethyl acetate and further concentrated by air flow to 0.1 mL before analysis. The concentration of atrazine in the extracts were analysed using HPLC under the following conditions: column c-18 (150 x 4.6 mm), mobile phase of methanol: water (50:50, v/v), UV detector at 230nm, continuous flow of 1ml min^{-1}, oven temperature of 35ºC, runs of 15 min and injection volume of 20 µL. The percentage degradation of atrazine was calculated using the equation [Co – Cx /Co] X 100 where Co is concentration of atrazine (mg/L) in the uninoculated control medium. Cx is the concentration of atrazine (mg/L) in the medium that has atrazine degrading strain.

2.6 Effect of Incubation Temperature on Atrazine Degradation by *Pseudomonas* Strain

The optimal temperature for atrazine degradation by *Pseudomonas* strain was assessed by growing the isolate at various temperatures ranging from 25ºC to 40ºC. The isolate was grown in 100 mL of liquid medium containing sterile mineral solution as described in 2.3 and atrazine in the concentration of 100 mg/L as the single source of carbon and nitrogen. The experiment was performed in triplicate and each flask received 0.1 mL of a 24 h culture of *Pseudomonas* strain. The flasks and the uninoculated control flasks were incubated at varying temperatures (25, 30, 35, and 40ºC) for 30 days. Cultures were withdrawn every 5 days and growth and atrazine concentration measurements were carried out using standard plate count and HPLC respectively.

2.7 Effect of Anaerobic Incubation on Atrazine Degradation by *Pseudomonas* Strain

Pseudomonas strain was tested for its ability to degrade atrazine in the absence of oxygen (O_2). The isolate was grown in 100 mL of liquid medium containing sterile mineral solution as described in 2.3 and atrazine in the concentration of 100 mg/L as the single source of carbon and nitrogen. The experiment was performed in triplicate and each flask received 0.1 mL of a 24 h culture of *Pseudomonas* sp. The flasks and the uninoculated control flask were incubated anaerobically at 35ºC for 30 days. Cultures were withdrawn every 5 days and growth and atrazine concentration measurements were carried out using standard plate count and HPLC respectively.

3. RESULTS AND DISCUSSION

A total of three atrazine-degrading bacterial strains identified as *Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp. were isolated from lotic water using enrichment techniq.ue (Fig. 1) with *Pseudomonas* sp. as the most efficient isolate (Fig. 2).

Studies have shown that many microorganisms in water and soil have the ability to degrade atrazine. They include the members of the genera *Pseudomonas* [13,14,15], *Rodococcus rhodochrous* [16], *Acinetobacter* spp. [17], *Aerobacterium* sp., *Microbacterium* sp., *Bacillus* sp., *Micrococcus* sp., *Deinococcus* sp. and *Delftia acidovorans* [11] as well as species consortia such as *Agrobacterium tumefaciens*, *Caulobacter crescentus*, *Pseudomonas putida*, Sp*hingomonas yaniokuyae*, *Nocardia* sp., *Rhizobium* sp., *Flavobacterium oryzihabitans* and *Variovorax paradoxus* [18].

Incubation time of 20 days was observed as optimum for the growth of the three isolates (Fig. 1). Atrazine maximum degradation rate of 82.67% for *Pseudomonas* sp., 75.33% for

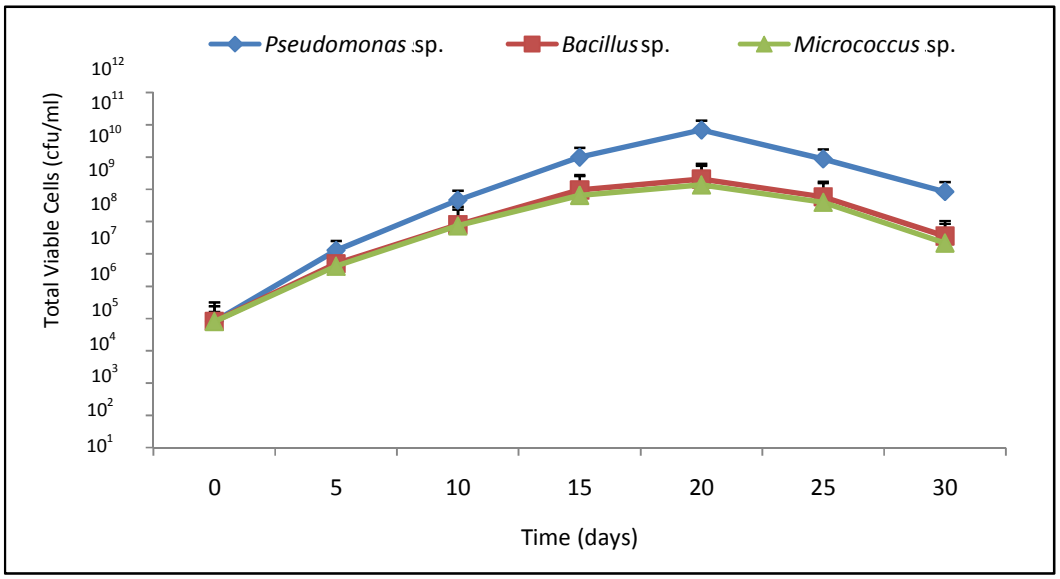

Fig. 1. Effect of incubation time on growth of atrazine degrading bacteria

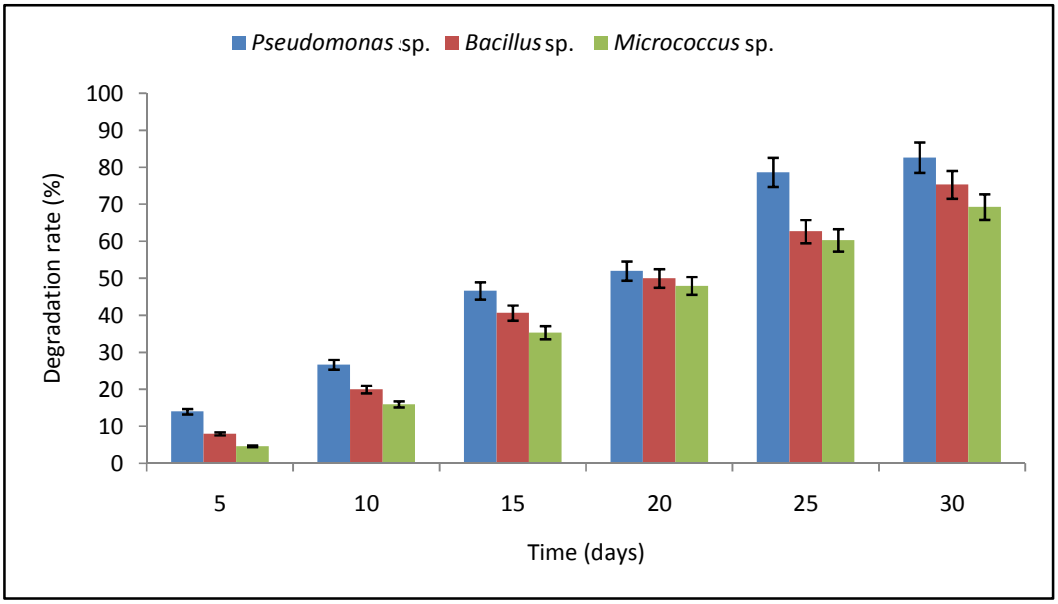

Fig. 2. Effect of incubation time on atrazine degradation by the bacterial isolates

Bacillus sp. and 69.33% for *Micrococcus* sp. were observed at the end of the 30 days (Fig. 2). The loss of atrazine in uninoculated sterile controls was not evident indicating that the observed growth (Fig. 1) occurred at the expense of atrazine (Fig. 2). The atrazine degradation rates for *Microbacterium* sp. and *Arthrobacter* sp. reached 77.7% and 65.6% respectively after 14 days culture in a liquid medium with an atrazine concentration of 100 mg/L [19]. In granular activated carbon column filters inoculated with *Rodococcus rhodochrous*, atrazine degradation achieved 72.6% after 39 days [16]. Atrazine degradation in media containing atrazine as sole carbon and nitrogen source showed maximum degradation of 80% by *Cryptococcus laurentii* [20]. These atrazine degrading strains through their metabolism process can reduce or even eliminate the toxicity caused by atrazine as an environmental pollutant so as to decrease the harms to human health and the ecosystem.

The effect of temperature on atrazine degradation by *Pseudomonas* sp. is presented in Fig. 3. The degradation efficiency was found to be maximum (82.67%) at 35ºC. A decline in the degradation efficiency was observed for temperatures below and above 35ºC. This shows that the isolate is a mesophile and that temperature plays active role in bacterial metabolism and atrazine degradation. Wang and Xie [21] studied atrazine removal from contaminated soil and water by *Arthrobacter* sp. and the results showed that this strain of bacteria was capable of removing atrazine in a wide range of temperature (25-35ºC). For bacterial strain L-6, maximum biomass and best course of degradation was observed at incubation temperature of 30ºC [22]. The environmental fate of atrazine is largely dependent on various factors such as pH, temperature and atrazine concentration [23].

Pseudomonas sp. slowly degraded atrazine in the absence of oxygen. A 61.33% loss of atrazine was observed after 30 days anaerobic incubation (Fig. 4).

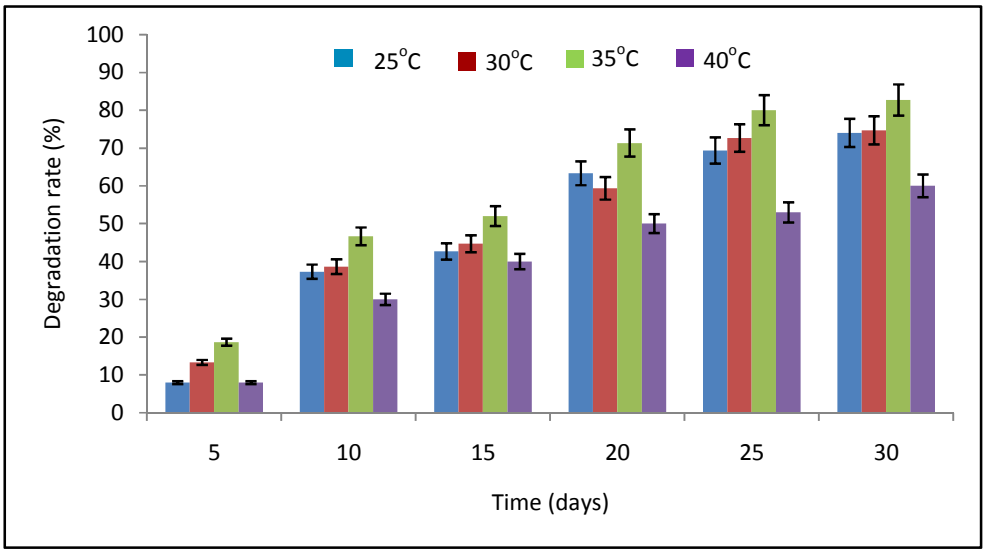

Fig. 3. Effect of temperature on atrazine degradation by *Pseudomonas* sp.

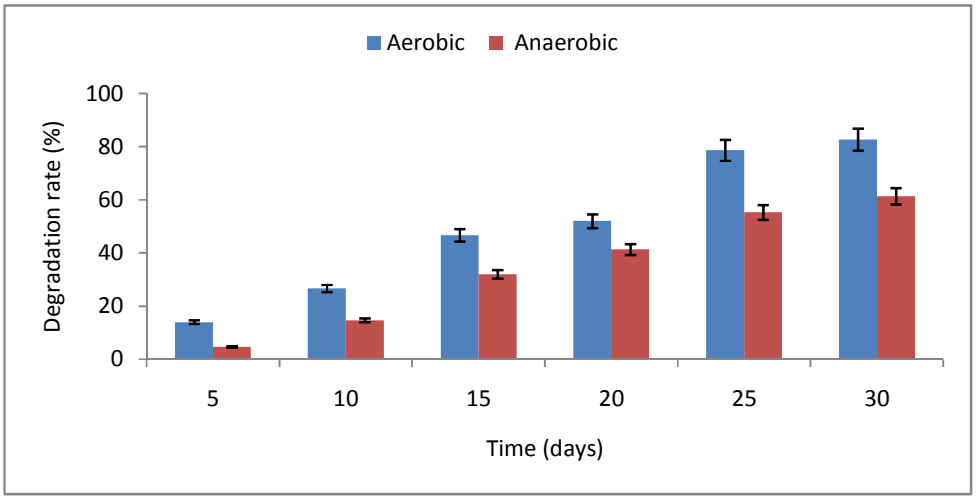

Fig. 4. Effect of anaerobic incubation on atrazine degradation by *Pseudomonas* sp.

There was no loss of atrazine in uninoculated control. Biodegradation of atrazine in absence of oxygen by pure culture has been reported in literature [24,25]. A facultative anaerobic, gram-negative bacterium *Ralstonia basilensis* (M91-3) capable of using atrazine under anaerobic conditions has been reported [26]. The authors stated that atrazine was degraded by *Ralstonia basilensis* (M91-3) at reduced rates and the degradation was completely inhibited when the medium was supplemented with NH_4^+. They suggested that the dealkylation and subsequent oxidation of atrazine side chains are coupled with denitrification under anaerobic conditions.

4. CONCLUSION

In this study, three atrazine-degrading bacterial strains identified as *Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp. were isolated from lotic water using enrichment technique with *Pseudomonas* sp. as the most efficient isolate. The degradation rates of *Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp. at the end of 30 days reached 82.67%, 75.33% and 69.33% respectively. Their optimum growth occurred on the 20th day. Atrazine degradation efficiency by *Pseudomonas* sp. was found to be influenced by incubation time, temperature and anaerobic incubation. The degradation of atrazine by these strains may have application in bioremediation of atrazine contaminated environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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