Examining the Role of Material Science in Atrazine Herbicide Biodegradation by Pseudomonas putida MTCC 2252

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Abstract. Atrazine is a chlorinated herbicide of the triazine class. It is used to prevent pre-emergence broadleaf weeds in crops such as maize (corn), soybean and sugarcane and on turf, such as golf courses and residential lawns. Atrazine's primary manufacturer is Syngenta and it is one of the most widely used herbicides in the United States, Canadian, and Australian agriculture. The bacteria used for the bio degradation of Atrazine is *Pseudomonas putida*. In this study, we report the biodegradation of Atrazine at high initial concentrations. The biodegradation of this Atrazine was investigated using *Pseudomonas putida*. For *Pseudomonas putida* optimization parameters like Contact time, Ph, Initial concentration, Temperature, Inoculum volume, Carbon source, Nitrogen source

Keywords: Atrazine, Herbicide*, Pseudomonas putida*, Biodegradation

1 Introduction

Atrazine, developed in 1958, is a 1,3,5-triazine herbicide synthesized from cyanuric chloride, ethylamine, and isopropyl amine. It disrupts photosystem II's plastoquinone-binding protein, causing plant death via electron transport disruption and oxidative damage, particularly in intense light. Atrazine acts as an endocrine disruptor in humans and animals, potentially causing hormonal imbalances and binding to mammalian proteins.

It persists in soil for months to several years, with migration potential to groundwater, particularly in heavily used regions, raising contamination concerns noted by the U.S. Environmental Protection Agency. Microbial action primarily degrades it in soil, through

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hydrolysis of C-Cl bonds catalyzed by hydrolase enzymes, yielding cyanuric acid, or via dealkylation, yielding 2- chloro-4-hydroxy-6-amino-1,3,5- triazine, facilitated by Pseudomonas species and other bacteria.

2 Experimental

The bacterial strain *Pseudomonas putida* MTCC 2252 was procured from Pune, India.

2.1 Maintenance of pure cultures:

The *Pseudomonas putida* colonies which appeared different from one another to naked eye (surface texture), were transferred to SCA petri dishes and incubated at 28°c for 2 weeks. The isolates which appear identical to the naked eye in respect of colour of aerial mycelium, reverse colour, soluble pigment and colony texture were eliminated.

Pure cultures were maintained on SCA medium in a petri dish for better binary fission. The pure cultures from petri dish with SCA were inoculated into slants, incubated at 28°c for seven to eight days until good growth was observed and stored in refrigerator at 4°c. The cultures were sub cultured for every 4 weeks.

2.2 Preparation of chemical reagent solutions

2.2.1 Preparation of pyridine reagent

50 mg of Atrazine was weighed and dissolved in 100ml of ethanol. The prepared stock solution is kept aside and stored for further use.

2.2.2 Preparation of pyridine reagent

3 ml of HCl was measured and dissolved in 18ml pyridine and 12 ml distilled water. The prepared stock solution is kept aside and stored for further use.

2.2.3 Preparation of NaOH solution

8 gm of NaOH was measured and dissolved in 100 ml of distilled water. The prepared stock solution is kept aside and stored for further use.

2.2.4 Preparation of 1% 4- amino acetanilide solution

1 gm of 4-amino acetanilide was measured and dissolved 100 ml distilled water. The prepared stock solution is kept aside and stored for further use.

2.2.5 Preparation of HCl solution

14.58 ml of HCl was measured and dissolved 100 ml distilled water. The prepared stock solution is kept aside and stored for further use.

2.2.6 Preparation of 1.4 moll/lit SLS solution:

0.004 gm of SLS was measured and dissolved 100 ml distilled water. The prepared stock solution is kept aside and stored for further use.

2.3 Procedure for estimation of atrazine

Prior to experiment collect the sample of 1ml in a test tube and add 0.2 ml of pyridine reagent into the test tube containing sample. Then the test tube is kept in water bath for heating upto 15min at 70°c. After heating in the test tube containing sample and pyridine reagent cool the test tube to room temperature.

After cooling to room temperature add 1ml of 2 molar or lit NaOH and 2 ml of 1% 4- amino acetanilide solution to the test tube and stir for 2 minutes, immediately add 2 ml of 4N HCl to the mixture in test tube. Then make up the volume to 20ml by adding 1.4 N, SLS solution i.e., 13.8 ml of 1.4 N, SLS solution is required.

After the addition of 1.4 N, SLS solution the reaction mixture forms yellow orange product.

The formed mixture or product must pass through a carbon cartridge which is cleaned or sterilized by 3 ml methanol and 3 ml water solution before the filtration process. After the carbon cartridge is sterilized then the formed product is passed through the carbon cartridge and 5 ml of methanol is added to the filtered mixture and the absorbance of the mixture is analysed at 460nm in UV spectrophotometer.

2.3.1 Culture Maintenance

The Pseudomonas species are sub cultured and maintained on nutrient agar slants at a low temperature which is suitable to the species and utmost care is taken in order to avoid the contamination.

The below methods are adopted for the bio degradation of Atrazine.

1. Optimization for the extraction Atrazine solution.
2. Bio degradation of Atrazine using *Pseudomonas putida* MTCC 2252
3. Immobilization of *Pseudomonas putida* MTCC 2252for the bio degradation of Atrazine - Column Chromatography.

2.3.2 Determination of % degradation

% degradation of Atrazine using the Pseudomonas species was estimated using the below formulae by obtaining the absorbance by UV-Visible-Spectrophotometry at the corresponding nm.

$$\% \text{ Degradation} = \frac{(\text{Initial concentration} - \text{Final concentration})}{\text{Final concentration}} \times 100$$

3 Results and discussions

The present study has come out on the studies of biodegradation of Atrazine by using Pseudomonas aeruginosa and *Pseudomonas putida*. Various physico-chemical process parameters contact time, effect of fermentation temperature, effect of pH effect of inoculum volume, effect of inoculum volume were studied and optimized to improve the percent biodegradation because of their large impact on production Nutrient supplements such as carbon and nitrogen source impact on degradation of Atrazine.

3.1 Bio degradation of Atrazine using *Pseudomonas putida* MTCC 2252

In the present study an attempt was made to evaluate the ability of bacteria *Pseudomonas putida* MTCC 2252 for the degradation of Atrazine pesticide. The present degradation studies were carried out in two different 250ml conical flasks containing 50ml nutrient medium. The medium was inoculated with completely grown culture of *Pseudomonas putida* MTCC 2252 under proper sterilized conditions.

The degradation was carried out by adding 3% inoculum of *Pseudomonas putida* MTCC 2252 to the sample of 1ml in a test tube and add 0.2 ml of pyridine reagent into the test tube containing sample. Then the test tube is kept in water bath for heating up to 15min at 70^0C. After heating in the test tube containing sample and pyridine reagent cool the test tube to room temperature. After cooling to room temperature add 1ml of 2 mol/lit NaOH and 2 ml of 1% 4- amino acetanilide solution to the test tube and stir for 2 min, immediately add 2 ml of 4N HCl to the mixture in test tube.

Then make up the volume to 20ml by adding 1.4 N, SLS solution i.e., 13.8 ml of 1.4 N, SLS solution is required. After the addition of 1.4N, SLS solution the reaction mixture forms yellow orange product. The formed mixture or product must pass through a carbon cartridge which is cleaned or sterilized by 3 ml methanol and 3 ml water solution before the filtration process. After the carbon cartridge is sterilized then the formed product is passed through the carbon cartridge and 5 ml of methanol is added to the filtered mixture and the absorbance of the mixture is analysed at 460nm in UV spectrophotometer.

The effects of various parameters were studied:

1. Effect of Contact time
2. Effect of pH
3. Effect of initial concentration of Atrazine
4. Effect of Temperature
5. Effect of inoculum volume
6. Effect of Carbon source
7. Effect of Nitrogen source

Fig. 1. Atrazine powder

Fig. 2. *Pseudomonas putida* MTCC 2252

3.1.1 Effect of contact time for the % degradation of Atrazine by Pseudomonas putida

Experiments were conducted to study the effect of varying time intervals from 0-60 hr. for the degradation of Atrazine using *Pseudomonas putida* MTCC 2252, keeping the other parameters constant i.e. temperature 35°C, pH 7. The percentage of degradation increased from 0 to 55.32% with an increase in contact time from 0 to 60 hr. The degradation has been observed from 12 hours and the maximum degradation was observed at 36 hours for *Pseudomonas putida* which has remained constant. The results obtained are shown below.

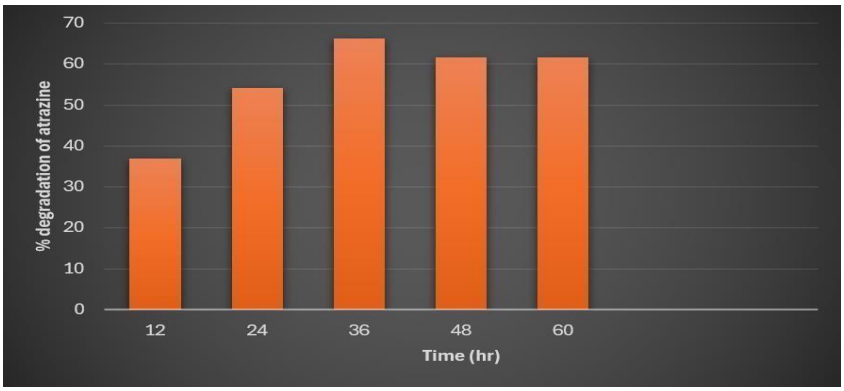

Fig. 3. Different time intervals for the % degradation of Atrazine by *Pseudomonas putida*

From the above graph in Fig. 3 it was observed that *Pseudomonas putida* has degraded 66.36%. It was found that when the contact time increases the degradation seems to be falling down. Hence 36 hours is the maximum contact time for the degradation of Atrazine.

3.1.2 Effect of pH for the % degradation of Atrazine by Pseudomonas putida

Experiments were conducted to study the effect of pH (5-9) for the degradation of Atrazine using *Pseudomonas putida* MTCC 2252 keeping the other parameters constant apart from time. As the optimized time is 36hr. The other parameters are kept constant i.e. temperature 30°C, pH 5 to 9, initial concentration 300 ppm, Inoculum 1% (v/v).

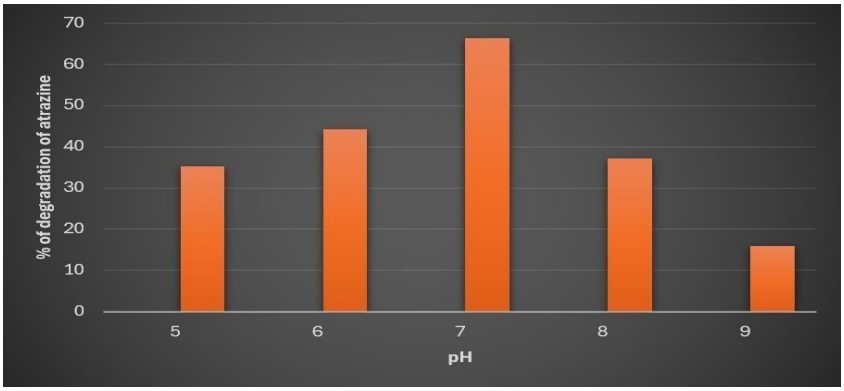

Fig. 4. Different pH conditions for the % degradation of Atrazine by *Pseudomonas putida*

The obtained plots show that the percentage of degradation of Atrazine has not shown any variation from the first optimized parameter as the highest degradation has obtained at pH 7 apart from the others i.e. 5,6,7, 8&9. Hence the % degradation has remained constant i.e. (66.36% for *Pseudomonas putida* MTCC 2252) The pH tolerance plays a vital role for the growth.

3.1.3 Effect of initial concentrations for the % degradation of Atrazine by Pseudomonas putida

Experiments were conducted to study the effect of initial concentrations i.e., 100,200,300,400,500 (ppm) for the degradation of Atrazine using *Pseudomonas putida* MTCC 2252 keeping the other parameters constant apart from time. As the optimized time is 36hr. The other parameters are kept constant i.e. temperature 30°C, pH 7, Inoculum 1% (v/v).

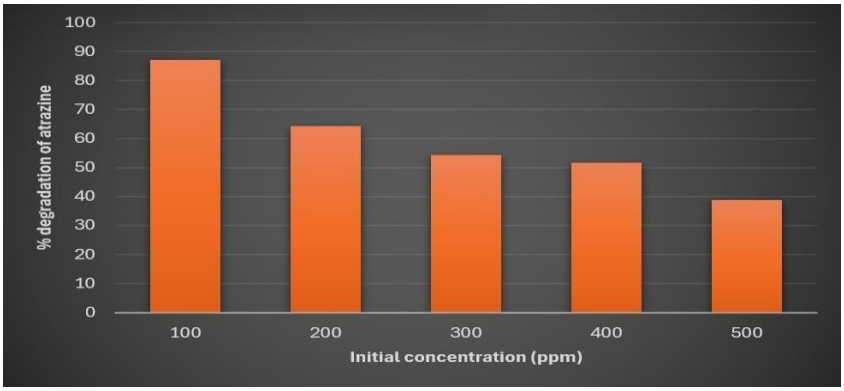

Fig. 5. Different Initial concentrations for the % degradation of Atrazine by *Pseudomonas putida*.

From the above graph in Fig. 5 it was observed that *Pseudomonas putida* has degraded maximum Atrazine at 100 ppm initial concentration.

3.1.4 Effect of temperature for the % degradation of Atrazine by Pseudomonas putida

In order to explore temperature, experiments were performed at various temperatures like 25,30,35,40°C for the degradation of Atrazine using *Pseudomonas putida* MTCC 2252 and maintaining their operational parameters as per the above two optimized conditions.

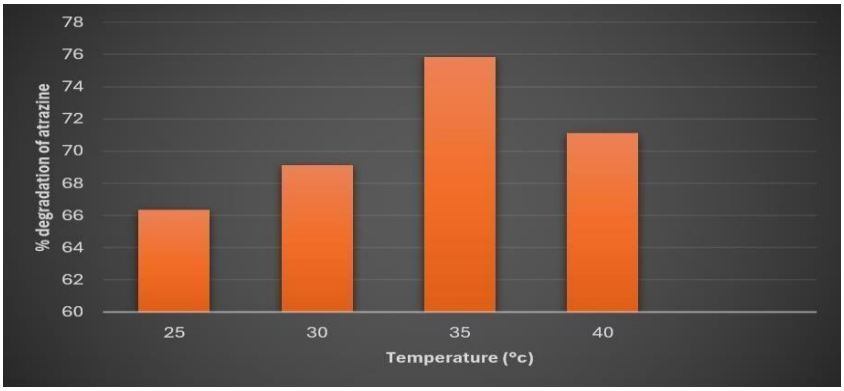

Fig. 6. Different temperatures for the % degradation of Atrazine by *Pseudomonas putida*

From the above graph in Fig. 6, it was observed that *Pseudomonas putida* has degraded maximum Atrazine at 35°C.

3.1.5 Effect of Inoculum volume for the % degradation of Atrazine by Pseudomonas putida

Experiments were conducted to study the effect of inoculum volume i.e., 1,2,3,4%for the degradation of Atrazine using *Pseudomonas putida* MTCC 2252 keeping the other parameters constant apart from time. As the optimized time is 36hr. The other parameters are kept constant i.e. temperature 30°C, pH 7, Initial concentration 100ppm.

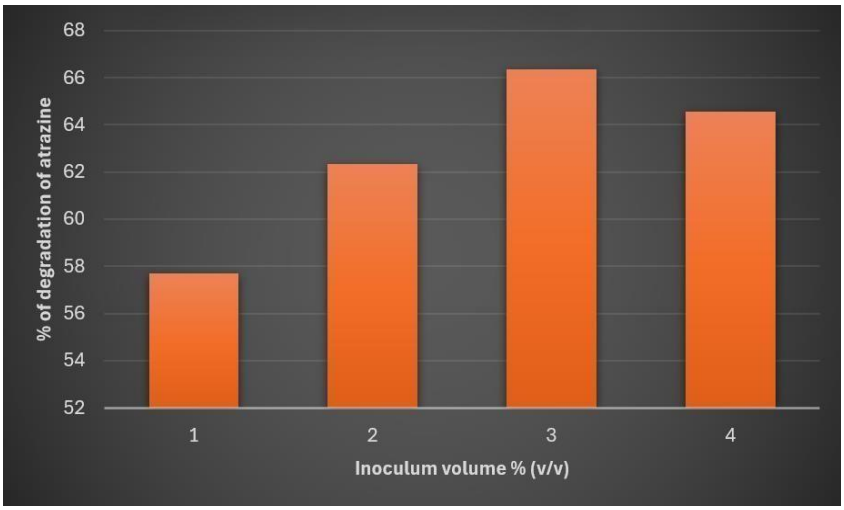

Fig. 7. Different Inoculum volumes (v/v) for the % degradation of Atrazine by Pseudomonas putida

From the above graph in Fig. 7, it was observed that *Pseudomonas putida* has degraded maximum Atrazine 3% Inoculum volume.

3.1.5 Effect of carbon source for the % degradation of Atrazine by Pseudomonas putida

To explore the carbon effect on degradation of Atrazine using *Pseudomonas putida*, experiments were performed with different carbon sources (glucose, sucrose, fructose, and starch) at a concentration of 3%(v/v) and keeping the other operational parameters as per the above optimization i.e. (contact time 36 hrs, initial concentration at 35°C- *Pseudomonas putida*, pH 7). The results obtained. The obtained plots show that the higher deterioration 78.09% for *Pseudomonas putida* was attained for Glucose.

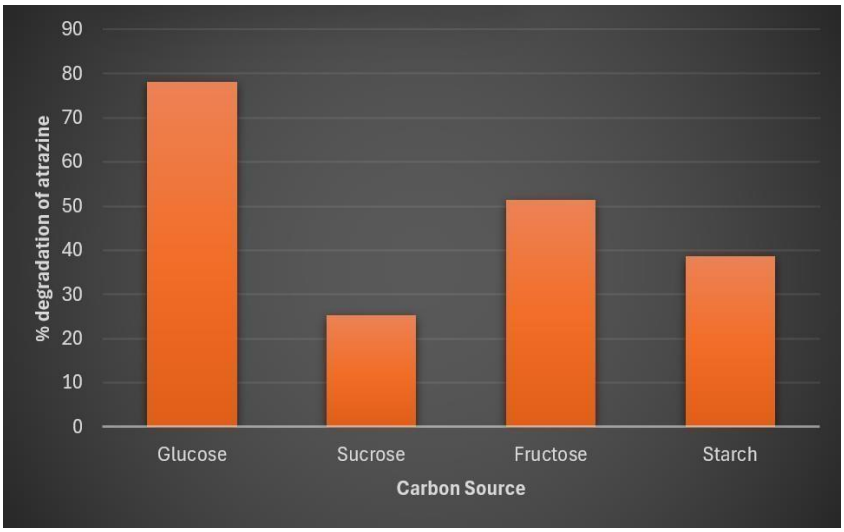

Fig. 8. Different carbon sources for the % degradation of Atrazine by *Pseudomonas putida*

The above results proved that glucose is the best carbon source utilized by Pseudomonas species which has given the best results apart from all other carbon sources. Apart from this Pseudomonas species utilize organic compounds as the source of carbon and energy for their growth.

3.1.6 Effect of Nitrogen sources for % the degradation of Atrazine by Pseudomonas putida

To explore the nitrogen effect on degradation of Atrazine using *Pseudomonas putida*, experiments were performed with different nitrogen sources (urea, ammonium sulphate, ammonium nitrate and ammonium chloride) at a inoculum of 3%(v/v) and keeping the other operational parameters as per the above optimization i.e. (contact time 36 hrs, temperature 35°C- *Pseudomonas putida*, pH 7, Carbon source-Glucose for the organism). The results obtained are shown below.

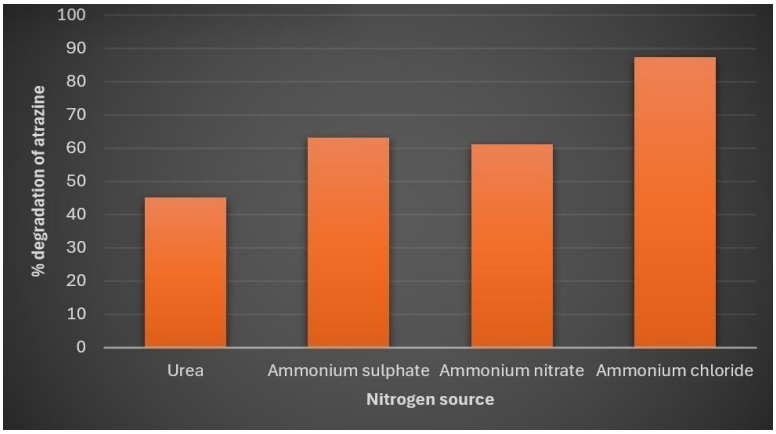

Fig. 9. Different nitrogen sources for the % degradation of Atrazine by *Pseudomonas putida*

The obtained plots show Ammonium chloride with degradation of 87.40% for *Pseudomonas putida*. The effective degradation may be due to the fact that bacteria utilized the ammonium chloride as a supportive material along with the nutrients present in it for growth. From the above perceptions it was observed that *Pseudomonas putida* MTCC 2252 have the capability to degrade Atrazine up to an extent of 82%.

3.1.7. Effect of time for the degradation Atrazine using Immobilized Pseudomonas putida

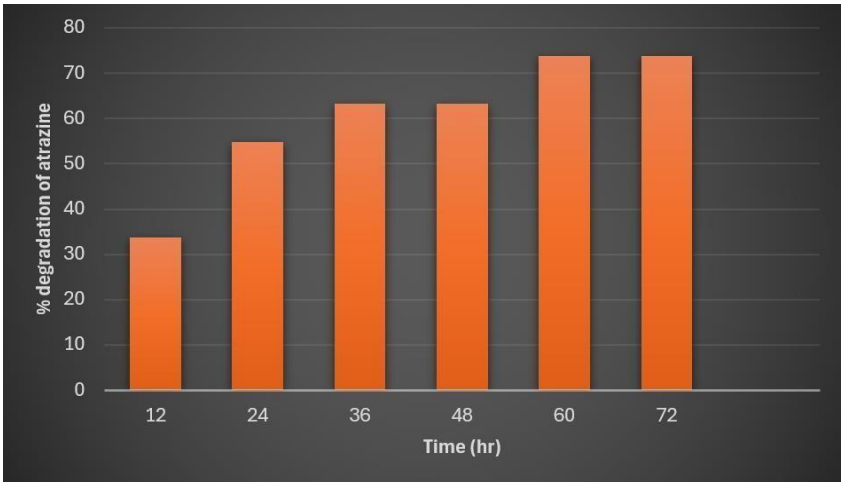

Fig. 10. Effect of time for the % degradation Atrazine using Immobilized *Pseudomonas putida*

From the above graph it was observed that the degradation has been initiated to the maximum at 12 hr. and it has been continued till 60 hr. where the degradation has ended at 60 hr. and became stable further.

The above graphical representation, Time Vs Concentration of Atrazine (µg/ml) is the comparative study of the bio degradation Atrazine using immobilized beads of *Pseudomonas putida*. It was observed that *Pseudomonas putida* has the capability to degrade the Atrazine

where *Pseudomonas putida* (73.92% for Atrazine) has given a result in the degradation for Atrazine.

4 Summary and Conclusion

The bacteria used for the bio degradation of Atrazine is *Pseudomonas putida*. In this study, we report the biodegradation of Atrazine at high initial concentrations. The bio- degradation of this Atrazine was investigated using *Pseudomonas putida*.

4.1 Bio degradation of Atrazine using *Pseudomonas putida* MTCC 2252:

In the present study an attempt was made to evaluate the ability of *Pseudomonas putida* MTCC 2252 for the degradation of Atrazine pesticide.

Effect of different parameters on atrazine degradation by *Pseudomonas putida*:

- **Contact time:** Maximum percentage degradation of Atrazine is **66.36** for **36hrs.**
- **pH:** Highest degradation percent of atrazine is **66.36** at pH **7**
- **Initial concentration:** Maximum percentage degradation of Atrazine obtained is **87.06 at 100 ppm**
- **Temperature:** Maximum percentage degradation of Atrazine is **75.83** at **35^{0}C**
- **Inoculum volume:** Maximum percentage degradation of Atrazine is **66.36** at **3%(v/v)**
- **Carbon Source:** Maximum percentage degradation of Atrazine is **78.9** for **Glucose**
- **Nitrogen source:** Maximum percentage degradation of Atrazine is **87.4** for **Ammonium Chloride**

Effect of different parameters on atrazine degradation by Immobilized *Pseudomonas putida*:

- **Contact time:** Maximum percentage degradation of Atrazine is **73.92** for **60hrs.**

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