

Determination of Bio-kinetic Parameters and Elucidation of Butachlor Metabolic Pathway by *Serratia ureilytica* Sp. AS1

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ABSTRACT

In the present study, biotransformation of butachlor by a highly tolerant, butachlor degrading *Serratia ureilytica* strain AS1 isolated previously from the pesticide-contaminated agricultural soil has been investigated. Under optimal conditions, the strain AS1 has the capacity to degrade up to 500 mg/L of butachlor completely within 240 hours in aqueous medium. Since, the substrate is inhibitory in nature, fitting the biodegradation data in to Haldane model, the following bio-kinetic parameters are obtained: $q_{\max} = 3.83$ mg/L/h, $K_s = 99.109$ mg/L and $K_i = 434.16$ mg/L ($R^2 = 0.9906$). EI-MS analysis to determine the butachlor degradation pathway reveals that biodegradation of butachlor by the strain involves production of intermediates such as Alachlor, 2-chloro-N-(2,6-dimethylphenyl) acetamide, DEA, etc. Bioremediation of butachlor by the strain AS1 in agricultural soil has also been evaluated which shows complete removal of butachlor within 288 hours at a rate constant of 0.2 day^{-1} following a first-order rate kinetics proving to be a promising candidate for the biodegradation of butachlor.

BACKGROUND

- Butachlor, also known as, N-(butoxymethyl)-2-chloro-2',6'-diethyl acetanilide is a widely recommended chloroacetanilide class of pesticide used mostly to control pre-emergent or early post-emergent broadleaf weeds, annual grasses, and submerged macrophytes in corn, rice, soybean and other crops.
- It is one of the most predominantly used herbicides in South America, Africa, and Asia and one of the top three in China.
- Asia reportedly consumes more than 4.5×10^7 kg of butachlor per annum.
- On account of its persistent nature, several studies have been undertaken to determine the harmful effect of butachlor.
- In-vitro studies suggests that butachlor is both mutagenic as well as carcinogenic in nature.
- A very few studies regarding the removal of butachlor from the environment by both physio-chemical and biological techniques have been reported.
- In the present study, biodegradation of butachlor and other herbicides by the microbial strain *Serratia ureilytica* AS1, isolated previously from pesticide contaminated agricultural soil has been discussed. The microorganism demonstrates superior degradation efficiency of tolerating up to 1000 mg/L of butachlor and metabolizing it completely.

OUTCOME OF THE STUDY

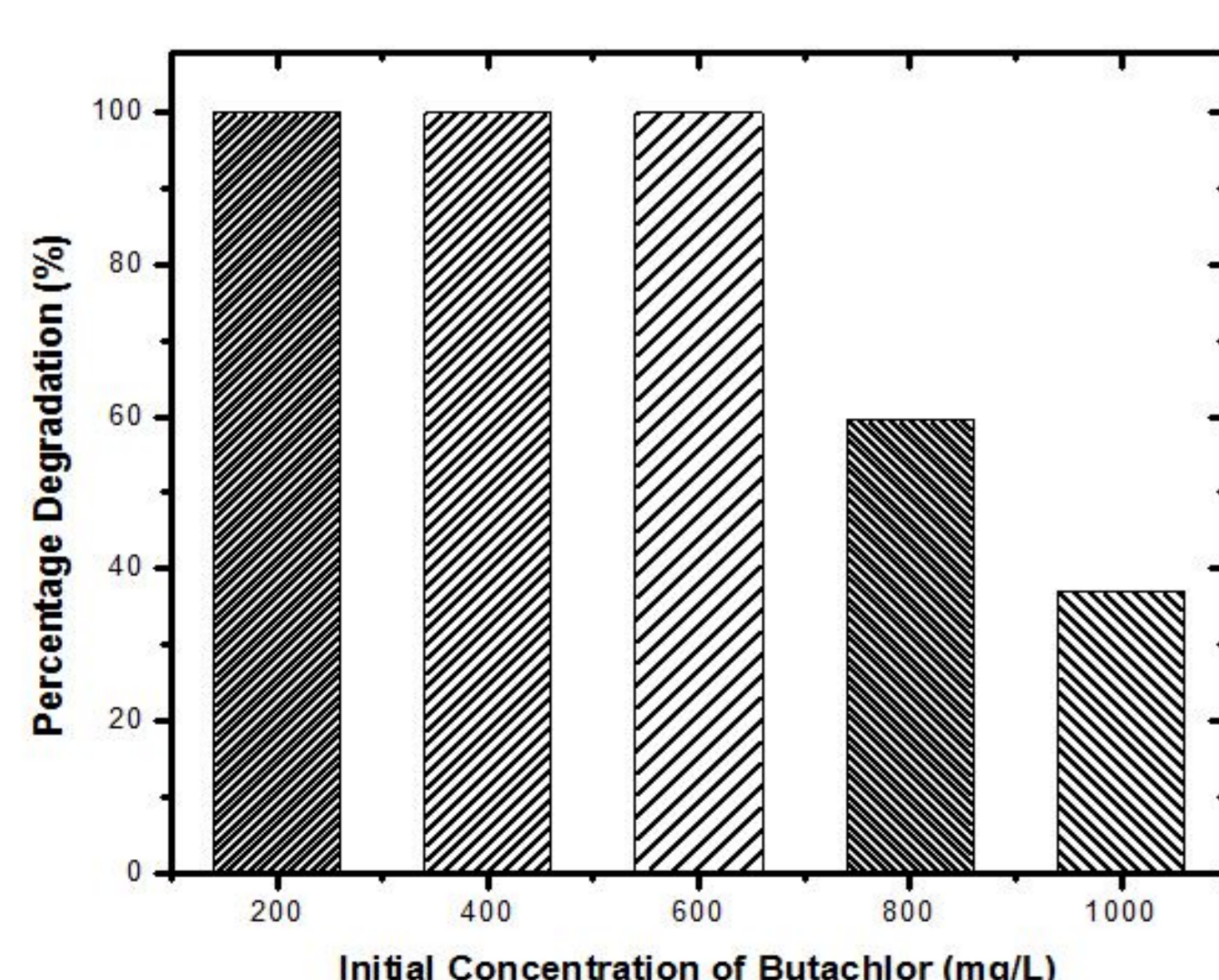


Figure 1: Butachlor Degradation percentage by using different initial butachlor concentrations by the isolated strain *S. ureilytica* AS1.

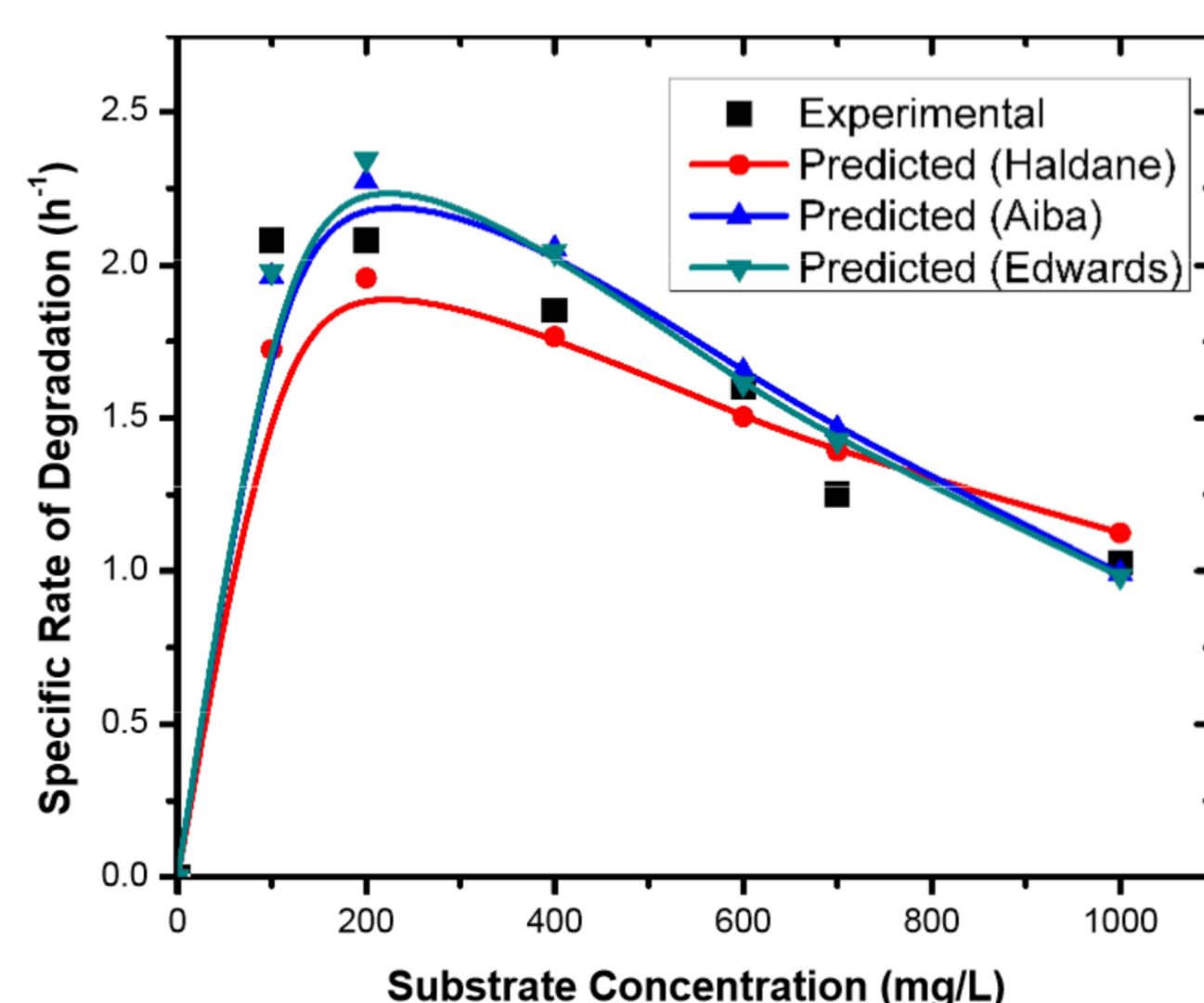


Figure 2: Fitting experimental data with the various substrate inhibition models depicting the relationship between the initial butachlor concentration with the specific rate of degradation.

| Mathematical model | q_{\max} | K_s | K_i | R^2 |
|--------------------|------------|--------|--------|--------|
| Haldane Model | 3.83 | 99.109 | 434.16 | 0.9906 |
| Aiba Model | 4.539 | 100.6 | 702.2 | 0.9041 |
| Edwards Model | 3.398 | 83.39 | 806.9 | 0.8925 |

Table 1: Bio-kinetic parameters for biodegradation of butachlor by the isolated strain *S. ureilytica* AS1 obtained by various models.

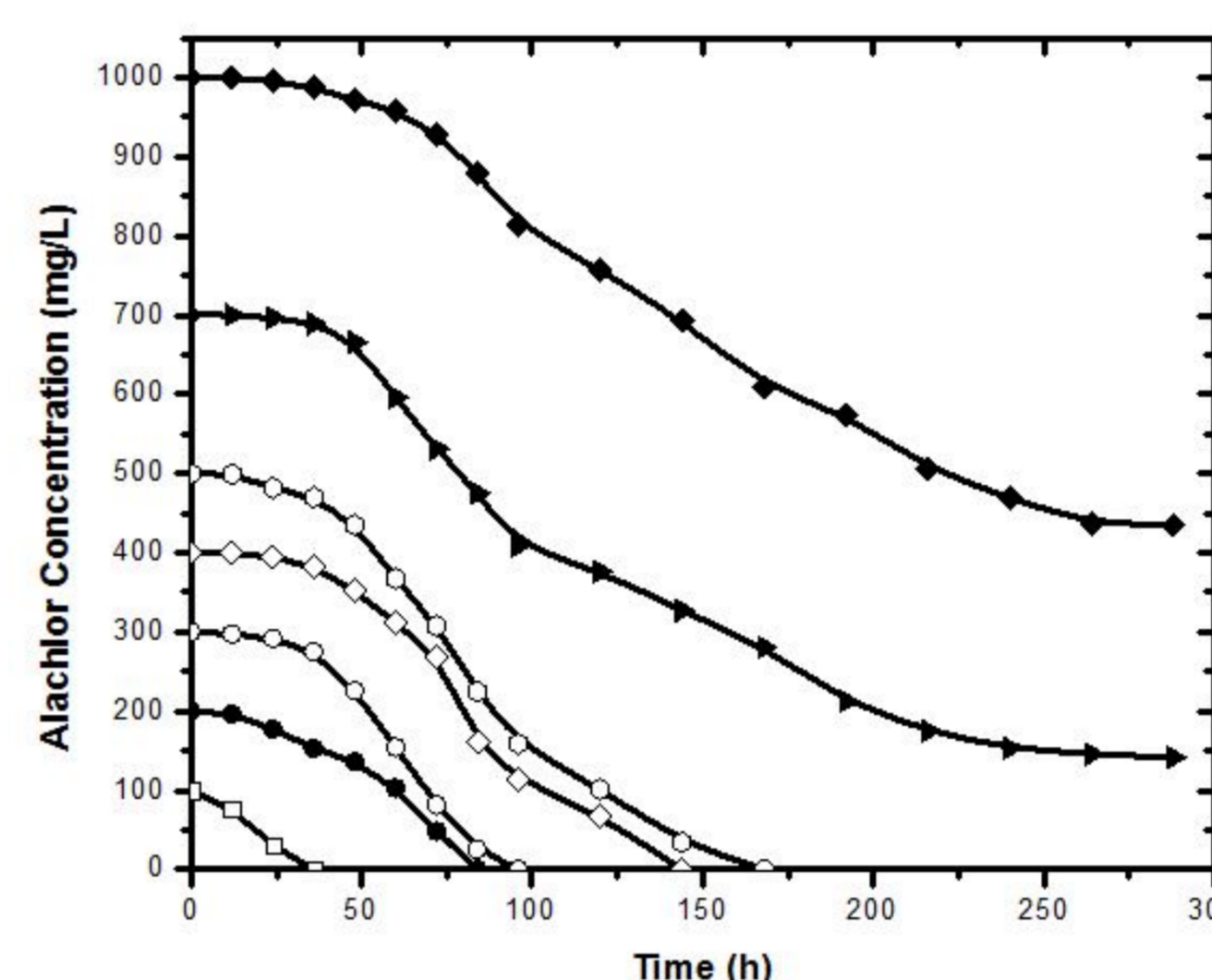


Figure 3: Degradation profile of the isolate *Serratia ureilytica* strain AS1 at a different initial concentration of Alachlor.

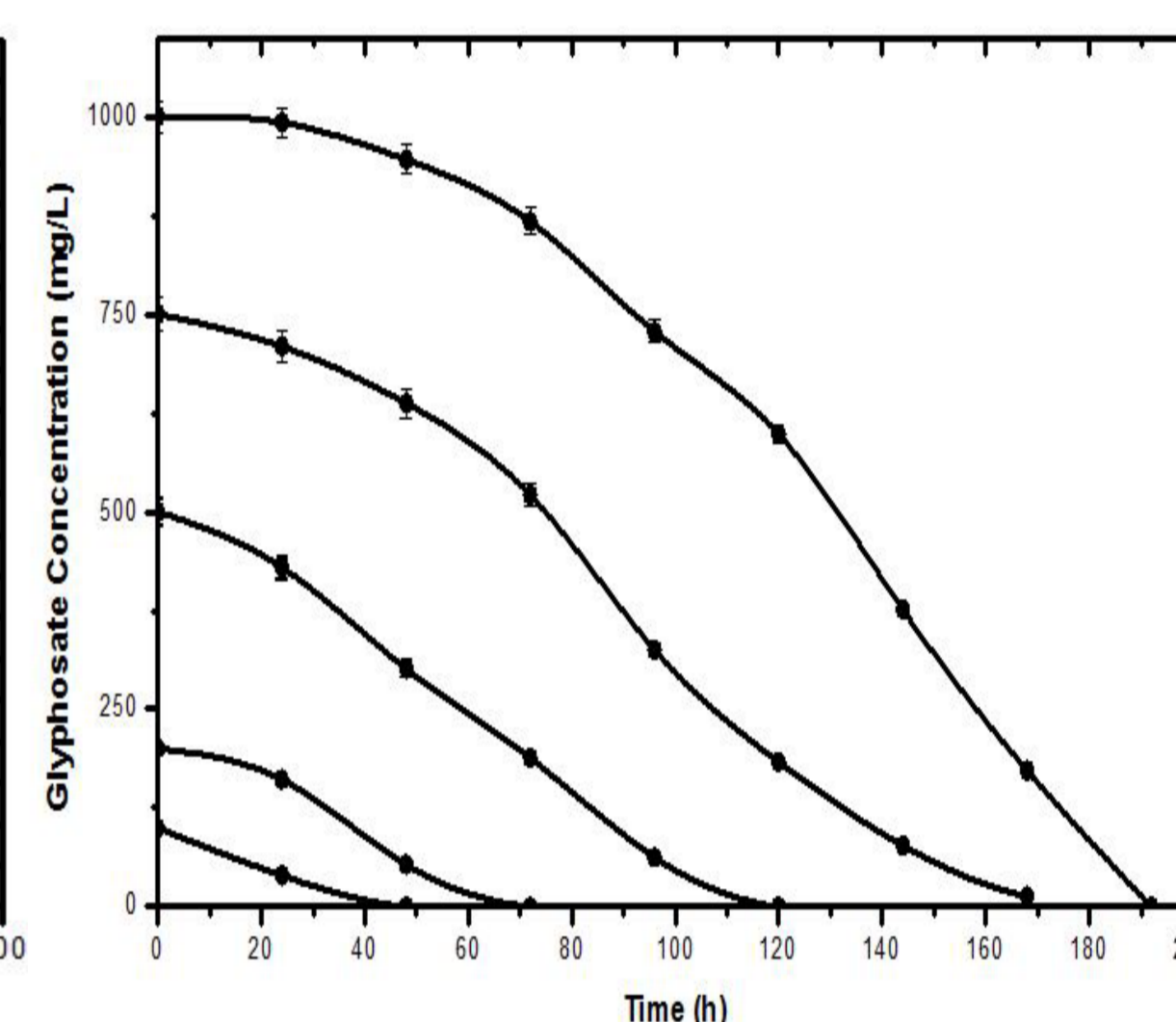


Figure 4: Degradation profile of the isolate *Serratia ureilytica* strain AS1 at a different initial concentration of Glyphosate.

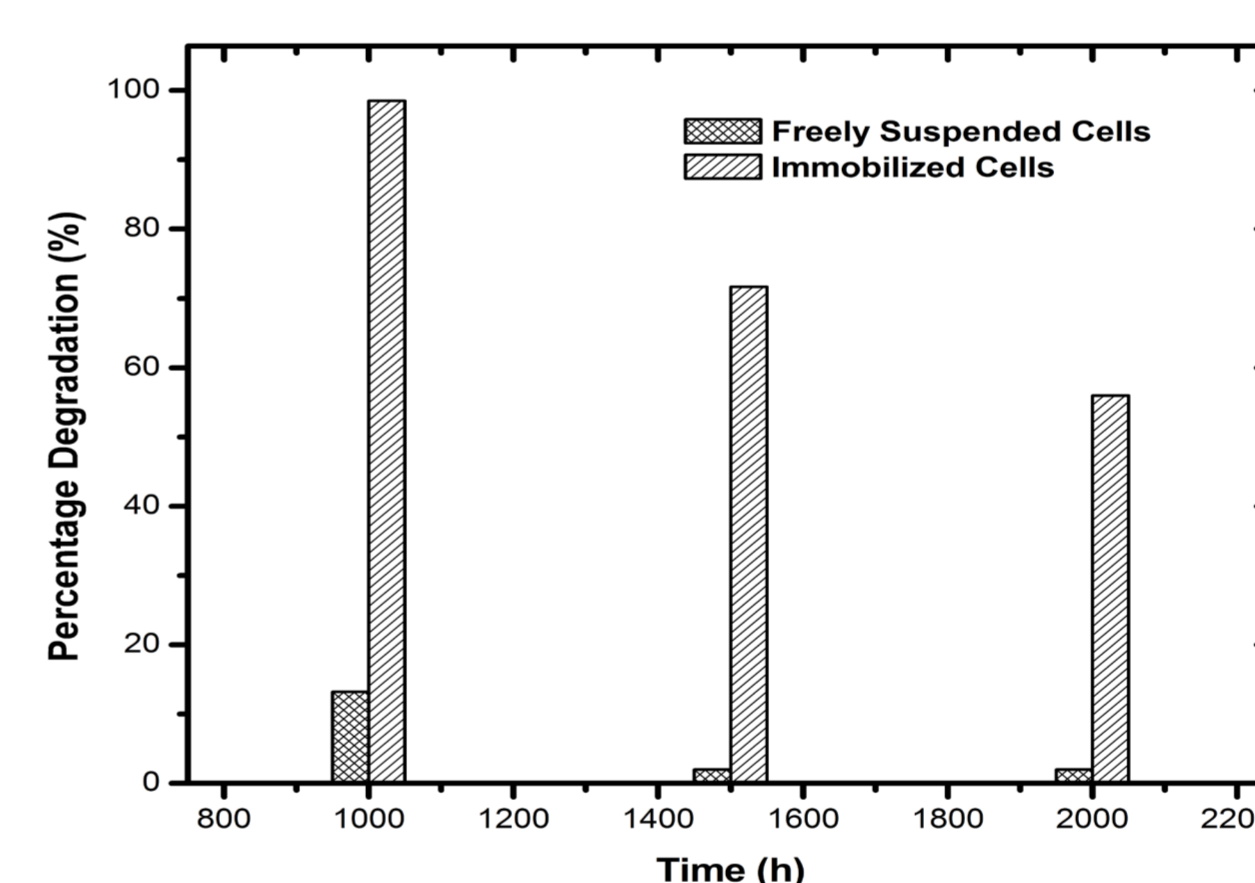


Figure 5: Comparative study of butachlor biodegradation profile by the freely suspended and immobilized strain AS1 at various initial concentration of butachlor.

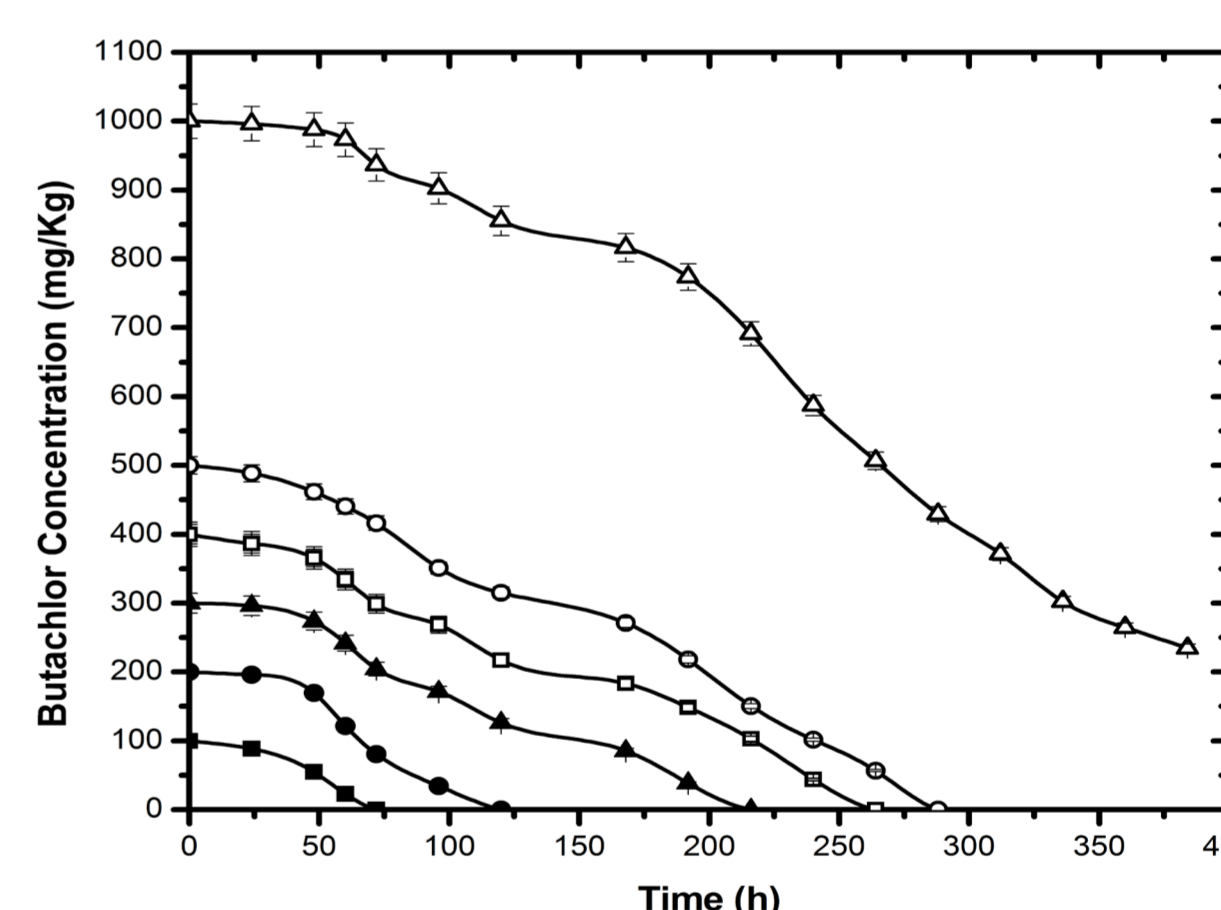


Figure 6: Degradation of butachlor in the soil by the isolate *Serratia ureilytica* strain AS1.

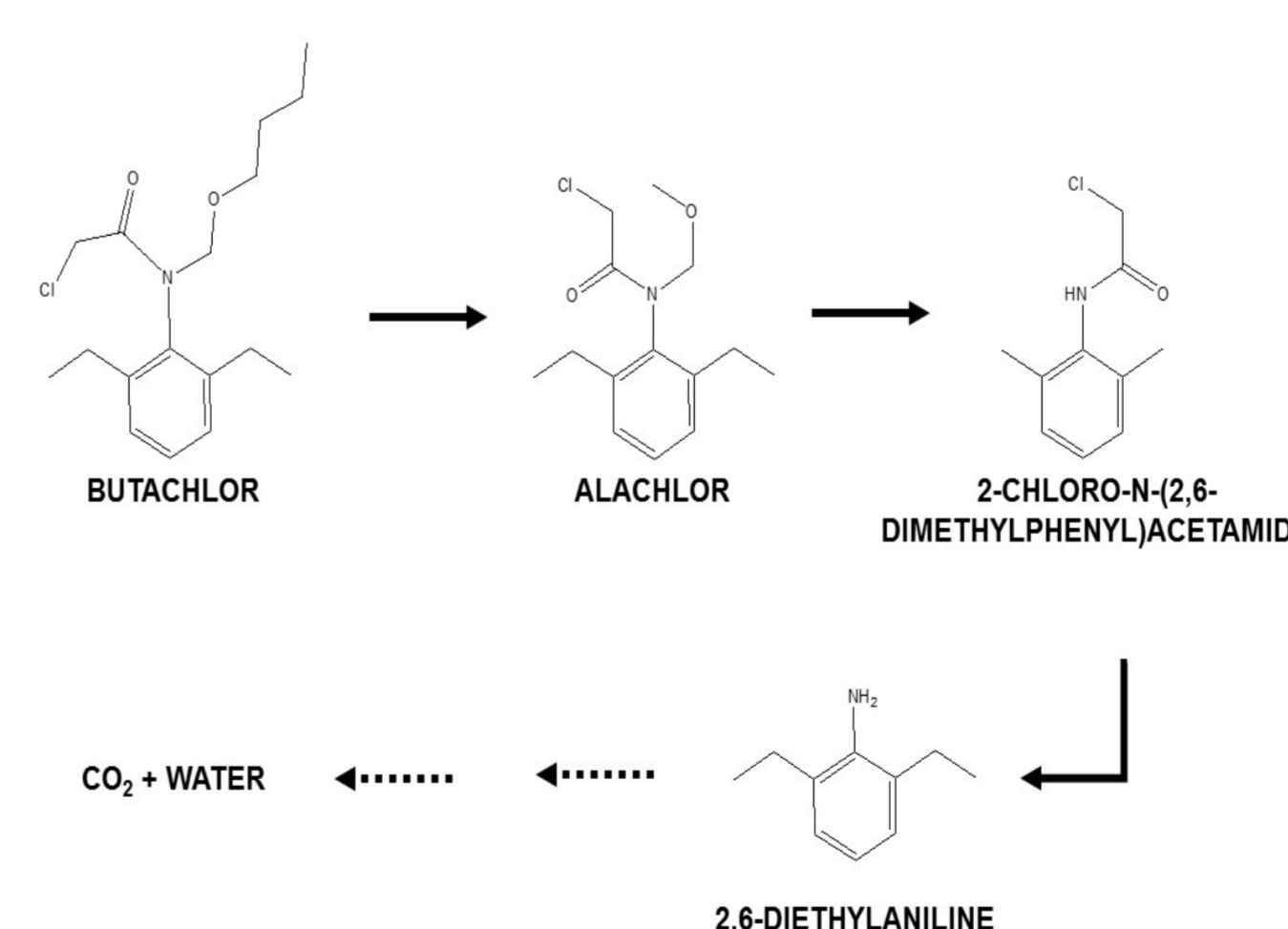


Figure 7: The proposed degradation pathway of butachlor by the bacterial strain *S. ureilytica* AS1.

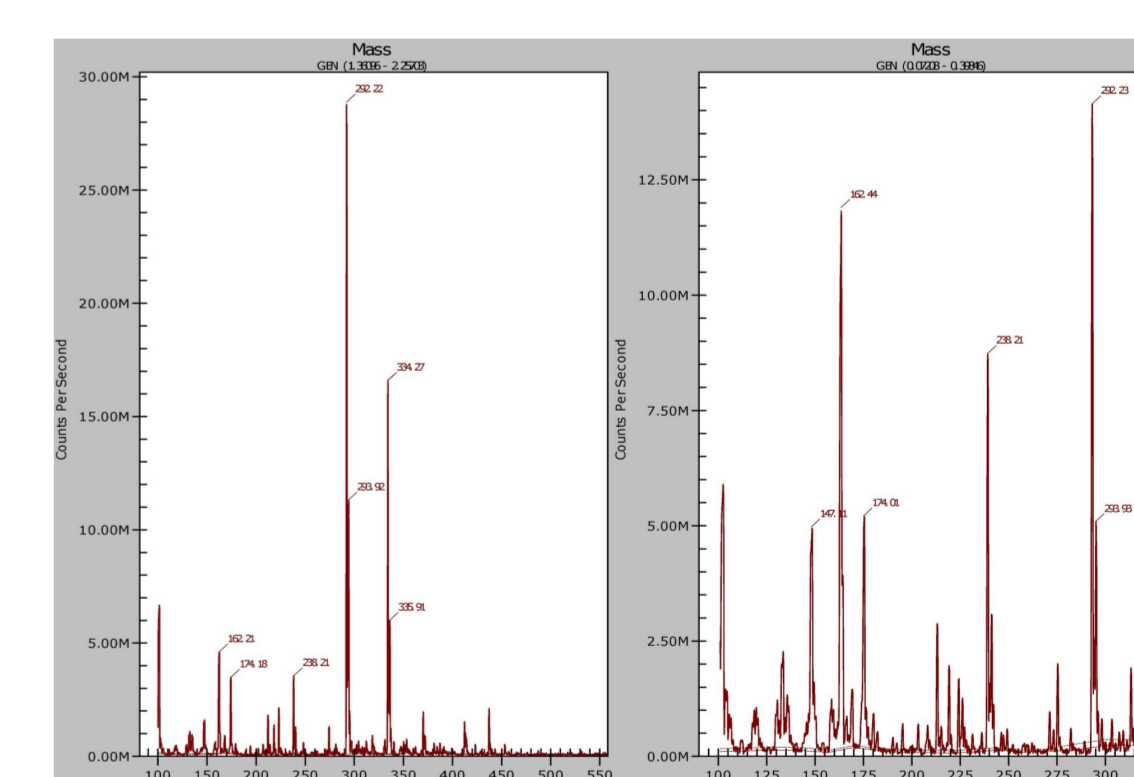


Figure 8: ESI-MS profile of the metabolite produced during butachlor degradation by the strain AS1 demonstrating the change in the abundance of the ion mass spectra of the metabolite with time.

CONCLUSION

- The bacterial strain *Serratia ureilytica* AS1 has the capability of degrading the pesticide to a concentration as high as 600 mg/L completely which is the highest till date to be reported.
- The bacterial strain is capable of complete removal of butachlor in agricultural soil 500 mg/Kg at laboratory scale within 288h at a rate constant of 0.2 day^{-1} following a first-order rate kinetics.
- Further, the microbial strain AS1 has the ability to degrade other herbicides of global concern such as alachlor and glyphosate efficiently. Till date no microbial strain was evaluated for such high concentration of alachlor and glyphosate.
- The cells are immobilized within the calcium alginate beads and the batch experimental study suggests that immobilization enhances the tolerance and degradation efficiency of the microbial strain significantly.
- Decrease in the intermediate concentration with time as evidenced from the ESI-MS analysis establishes the fact that the isolated strain AS1 degrades butachlor completely without adding any toxic intermediates as the end product.
- The bacterial strain would be a highly efficient and potential addition to the already existing repertoire of microbes for the remediation of chloroacetanilide and other herbicide-contaminated soil and wastewater.