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Optimization of atrazine degradation process by *Bacillus paramycoides* strain in a batch system, and assessment of growth kinetics, bacterial toxicity, phytotoxicity and chlorophyll

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The herbicide atrazine is commonly to boost the agricultural production but also raises serious environmental concerns. For a safe environment from the point of effective mineralization, a potentially atrazine-degrading bacteria is required. In this regard, potential bacterial species was isolated from the agricultural field where atrazine was being used as herbicide from long time and batch experiment was performed to evaluate its potential to degrade atrazine. Various biodegradation-affecting parameters such as atrazine concentration (1-25 mg/L), pH (5.0-9.0), and inoculum dose (5-10%) were optimized with the help of the Central composite design of the Response surface methodology (RSM). The maximum atrazine degradation was obtained at pH 7.0, Inoculum concentration of 10 %, and atrazine concentration of 13 mg/L. Various kinetic growth models were also studied and based on the Andrew-Haldane model, the inhibition constant (K_i) and maximum specific growth rate (μ_{max}) of atrazine were found to be 5.5 mg/L and 0.18 h^{-1} respectively. Chlorophyll content and Phytotoxicity assessment were also carried out for *Vigna radiata* seeds. To evaluate the toxicity of Bacteria, *Pseudomonas fluorescens* was utilized, and it was found that the toxicity was lower in the case of treated wastewater.

Keywords: Biodegradation, Central composite design (CCD), Herbicide, Inhibition constant, Mung bean, Response surface methodology (RSM), *Vigna radiata*

Nomenclature: μ, Specific growth rate (mg/mg^{-h}); μ_{max}, Maximum specific growth (mg/mg^{-h}); K_s, half saturation constant (mg/h); S, substrate concentration (mg/L); K_i, inhibition constant (mg/L); K, constant in the Yano and Koga model

Introduction

Human exposure to pesticides can result in serious health problems like anxiety, cancer, Parkinson's disease, asthma allergic reactions, irritation in the eyes, and excessive sickness[1,2]. Highly harmful pesticides even in low quantities, kill 0.2 million people annually worldwide, with developing countries accounting for 95% of all fatalities[3]. Punjab is an agriculture hub of India. In Punjab's Jalandhar, the quality of groundwater was studied and found that various agrochemicals such as Chlorpyrifos (0.0-0.03 µg/L), atrazine (0.0-0.9 µg/L), Monocrotophos (0.0-0.14 µg/L) and Phorate (0.0-0.03 µg/L) were present in the groundwater[4]. Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-1, 2, 3-triazine) has been used in more than 80 nations, and one of the most used herbicide worldwide[5]. It is mainly used to control pre-emergence and post-emergence broadleaf weed in a sugarcane field, maize field, lawns, gardens, rod sides, etc. Atrazine herbicide is a member of the s-Triazine group and many studies show that it may be carcinogenic[8,9]. Atrazine has a half-life of about 30-740 days but it may vary depending on different environmental conditions such as temperature, moisture content, microbial activity, and pH. Due to excessive use of atrazine, and its high mobility, atrazine, and its degraded products are migrated underground and surface water bodies, hence it has been found in rivers, groundwater lakes, and water supply points[10-13]. Atrazine residual levels as high as 22 mg/L were found in the well close to the mixing and loading locations exceeding the permitted limit for atrazine in potable water[14].

Atrazine has serious health issues including Birth abnormalities and reproductive cancers[15,16]. Because of its affordability and efficiency, atrazine is still produced and utilized extensively in several underdeveloped nations despite its negative effects. Therefore, it is imperative to look for ways to clean up to clean up the atrazine polluted environment. The

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Environmental Protection Agency (EPA) established the Maximum Contamination Level (MCL) as 3 µg/L for atrazine under the Safe Drinking Water Act. Individual pesticide concentration in drinking water is limited by European Economic council (EEC) legislation to 0.1 µg/L. The Biodegradation of atrazine was reported in previous studies[17-19]. limited attention was carried out towards the optimization using CCD.

In the present work, atrazine degrading bacterial species was isolated and performed an optimization study using new software of design expert (Version13, Stat-Ease Inc., Minneapolis, USA). Response surface methodology (RSM) is an extensively useful tool in the optimization study of a particular response using several input variables[20-22]. The different variables are termed as factors in RSM and the range is referred to as labels. It is used for making statistical designing experiments, building different models, and finding out the effect of different parameters on a particular response[23]. For studies on multivariable optimisation in numerous biotechnological processes, such as catalysed reaction, oxidation, fermentation, Production, media process, food technology, environmental engineering, etc., RSM is frequently utilized[24]. It has several advantages over other methods like it reduces the number of experiments, time, saving randomness of experiments, laboratory chemicals, and it gives optimum response with less response[25]. Various studies have information about pollutant optimization using RSM[26]. To establish the relationship among variables and response to fit the real replies, the polynomial model is employed with several ideal responses.

The Central composite design (CCD) is a design that comes under the type of RSM. CCD requires three levels for each variable[27]. Due to its simplicity and wide uses in different studies, it is used in our experiment. Here, we investigated the batch biodegradation of atrazine by *Bacillus paramycoides strain* using CCD. The Central composite design which depends on RSM was utilized with the new version (13) of the Design Expert software-RSM tool to identify the ideal environmental circumstances for atrazine degradation and growth by the *B. paramycoides* strain. This bacterial strain which can more effectively break down atrazine, was found in the pesticide-contaminated soil of the Banaras Hindu University agriculture field in Varanasi, Uttar Pradesh, India. The potential application of the *B. paramycoides* strain HL 1 for atrazine clean-up *in situ* is also highlighted in this research.

Materials and Methods

Chemicals and Mineral salt medium (MSM) used

Atrazine was purchased from Sigma Aldrich India having purity more than 99 percent. The mineral salt medium (MSM) with composition in g/L (KH_2PO_4: 0.5, NaCl: 1, $MgSO_4 \cdot 7H_2O$: 0.2, K_2HPO_4: 1.5, Sucrose: 1) and micronutrient solution with composition in mg/L of H_3BO_3; 600, $CoCl_2$;400, $ZnSO_4$. $7H_2O$; 200, $MnCl_2$;60, $Na_2MoO_4 \cdot 7H_2O$:60, $NiCl_2$;40, $CuCl_2$: 20)[28,29] were bought from Merck, Mumbai, India and utilized for maintenance and growth of bacterial species in the conical flask. At 15 psi and 120°C, MSM was autoclaved for 15 min. For maintaining the pH of synthetic wastewater at a particular level Sodium hydroxide (0.1 M) and Sulphuric acid (0.1 M) were used.

Site selection, soil sampling, and isolation of bacterial species

Soil contaminated with atrazine was collected from an agriculture farm of Banaras Hindu University Varanasi, Uttar Pradesh, India (25°19'N; 83°3'E, 129 m above mean sea level) where atrazine used as herbicides for many years for Maize crop. Bacterial isolation technique used in this study has been covered elsewhere[30]. The enrichment of isolated bacteria was carried out in MSM using atrazine as the only carbon source at 30°C by increasing the concentration of substrate from the soil.

Bacterial Identification

The potential bacteria that we obtained were sent to Triyat scientific Private Limited for characterization which is located in Nagpur, Maharashtra, India. For DNA amplification, Polymerase chain reaction (PCR) was used with universal reverse (11492R-5′CGGTGTGTACA AGGCCCGG) and forward (27F-5′GGATGAGCC CGCGGCCTA) primers. The operating conditions for PCR were mentioned as: 1 min Annealing at 50°C, 1 min Denaturation at 94°C, 2 min Extension at 72°C, 7 min final extension at 72°C and Initial denaturation for 3 min at 94°C. For sequencing purpose of the PCR sample, ABI 3130×l sequencer was used. Using similarity analysis, the resulting 16S rRNA were uploaded for identification of bacterial species in the NCBI database. Obtained bacterial species have accession number as NR 157734.1. The obtained bacterial was named *Bacillus*

paramycoides Strain, and the phylogenetic tree (Fig. 1) was drawn using MEGA7 software.

Residual atrazine Analysis and Optical Density Measurement

The Residual concentration of atrazine is determined by HPLC (High-performance liquid Chromatography) model (UFLC Shimadzu, Japan) equipped with C-18 column (4.6mm×250mm× 5mm, reverse phase, Inert Sustain) at 30 □C. At a wavelength of 210 nm, atrazine and it's degraded by products were measured using the mobile phase (acetonitrile: water; 45:55) for 15 minutes at a flow rate of 1.0 ml⊙ min^{-1} [31]. The samples for the atrazine assay were extracted twice with Dichloromethane having the same volume, the contaminant was then removed by centrifugation for 10 minutes at 10,000 rpm. The collected sample was filtered using 0.20 µm disposable syringe filter and supernatant was collected. Optical density (OD) for growth kinetics was measured by absorbance at 600 nm with the help of a UV spectrophotometer (PerkinElmer Lambda 25UV/VIS spectrophotometer)[32,33]. All the analyses were performed in triplicate manner and the final result was taken as a mean of three values.

The final degradation of atrazine was calculated using the following equation:

$$\% \text{ Degradation} = \frac{(A_f - A_o)}{A_o} \times 100 \quad \ldots (1)$$

Where A_o - atrazine Initial concentration of mg/L

And A_f - atrazine Final concentration in mg/L after biodegradation

Kinetics growth study for Biodegradation of atrazine

By measuring bacterial growth rate in a batch process at various initial atrazine concentration, various kinetic growth variables were derived. One of the most important aspects to consider when assessing the growth kinetics of microorganisms is an equation showing the link between the Substrate concentration and the specific growth rate.

$$\mu = \frac{1}{X}\frac{dX}{dt} \quad \ldots (2)$$

on integrating equation (1) from time range between 0 to t

$$\mu = \frac{\ln(X_t) - \ln(X_o)}{t - t_o} \quad \ldots (3)$$

Where X_t and X_o are biomass concentrations at time $t=t$ and $t=0$ respectively. The cell-specific growth rate in the log-phase was calculated using Eq. (1). We obtain the value of the specific growth rate (µ) as the slope of the drawing line while producing the Δt vs ln $(\frac{X}{X_o})$ graph. The concentration of cell (X) was measured at a wavelength of 600 nm with UV-VIS spectrophotometer.

Microbes used the pollutant as their substrate and their degradation study has been studied by many researchers aiming detoxification of the pollutant. In this biodegradation mechanism, several models were proposed which are used for the biodegradation process[34,35]. These models can be used for determining different kinetics parameters. Table 1 shows the different models used for degradation of atrazine for kinetics study.

Different cell growth and substrate inhibition models like Yano model, Monod model, Webb model, Aiba-Edwards model, Andrew-Haldane model and Tessier model were fitted to the experimental results of the variation of the specific growth rates

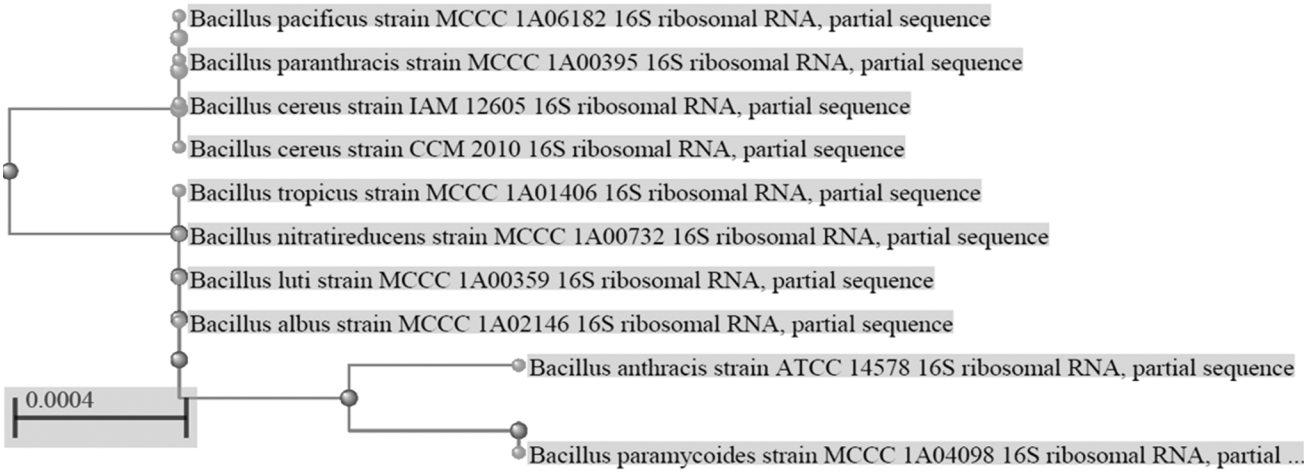

Fig. 1 — Phylogenetic tree of *Bacillus paramycoides* strain after the characterization by the 16S rRNA technique.

Table 1 — Kinetic growth model's parameters

Kinetic model	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)	K (mg/L)	R^2
Andrew-Haldane model: $\mu = \frac{\mu_{max}S}{S+K_s+\frac{S^2}{K_i}}$	0.18	15	5.5	-	0.97
Monod-Model: $\mu = \frac{\mu_{max}S}{S+K_s}$	0.08	8	-	-	0.75
Webb-Model: $\mu = \frac{(\mu_{max}*S)(1+\frac{S}{K_1})}{S+K_s+\frac{S^2}{K_i}}$	0.1	3	7	24	0.72
Aiba-Edwards model: $\mu = \frac{\mu_{max}S}{S+K_s} * \exp(-\frac{S}{K_i})$	0.14	5	20	-	0.74
Tessier model: $\mu = \mu_{max} * [\exp(-\frac{S}{K_i}) - \exp(-\frac{S}{K_s})]$	0.15	6	18	-	0.72
Yano Model: $\mu = \frac{\mu_{max}*S}{(S+K_s+(\frac{S^2}{K_i})*(1+\frac{S}{K}))}$	0.15	8	10	50	0.77

Table 2 — Independent factors for biodegradation of atrazine

Factor	Name	Units	Min.	Max.	Mean
A	Atrazine conc.	ppm	1	25	13
B	pH		5	9	7
C	Inoculum conc.	%	5	10	7.5

with initial concentration of atrazine[36-40]. The best-fit model is chosen as the one with the highest correlation coefficient (R^2) value. The value of growth kinetic parameters like μ_{max} (maximum specific growth rate, h^{-1}), K_I (Substrate-inhibition constant, mg. L^{-1}), K (Yano and Webb model constant) and K_s (substrate-affinity constant mg. L^{-1}) were determined with the help of trial-and-error method.

Process variable optimization by response surface methodology

CCD in RSM is a useful method for investigating the relationship between the outcomes and several process variables. The RSM approach has studied process variables like Inoculum concentration, Temperature and substrate concentration[41-44]. The impact of independent process variables was assessed using the Design-Expert software (Version13, Stat-Ease Inc., Minneapolis, USA) system. Plotting contour graphs and 3D surface allowed researchers to examine how various factors influence the response. On the basis of the F (Fisher's) values and P (probability), ideal circumstances were predicted. 20 experiments using the RSM and CCD were conducted to optimize the operating conditions. A quadratic model presented in prior study provided the significance between the operating parameters and the removal efficiency (%RE) of atrazine. To test the effectiveness of the model ANOVA was used with trial and error was used to gather the regression data.

Table 3 — Conditions in experiment of central composite design for degradation of atrazine (Atz)

Run	Factor A Atz conc.(mg/L)	Factor B pH	Factor 3 Inoculum dose (%)	Response % Degradation
1	25	5	10	50.2
2	13	7	7.5	85.8
3	25	9	10	58.4
4	13	7	7.5	87.8
5	13	7	10	93.4
6	1	9	10	68.5
7	13	5	7.5	56.2
8	1	9	5	68.4
9	1	7	7.5	90.1
10	13	7	7.5	87.5
11	25	7	7.5	78.5
12	13	7	7.5	87.2
13	25	5	5	41.2
14	25	9	5	56.5
15	13	9	7.5	64.3
16	13	7	7.5	86.2
17	1	5	5	51.9
18	13	7	10	93.2
19	1	5	10	60.4
20	13	7	7.5	86.8

Table 2 depicts the variety of levels and independent variables. According to central composite design, as shown in Table 3, experiments were conducted. For treatments purpose three levels were used for Inoculum concentration, atrazine concentration and pH in accordance with the central composite design's specifications. The ability of bacteria to degrade atrazine was then examined with the help of inoculating the potential bacterial culture into different 250 mL Erlenmeyer flasks filled with MSM and substrate under the aforementioned optimal conditions, and then placed the flasks inside an incubator. The amounts of atrazine residues were then measured by HPLC at intervals ranging from 0 to 10 days after biodegradation.

Polynomial equation of second order having linear, quadratic and interaction effects were used for further analysed the experimental data, is given below:

$$Y=\alpha_o+\alpha_1A+\alpha_2B+\alpha_3C+\alpha_1\alpha_2AB+\alpha_2\alpha_3BC+\alpha_1\alpha_3AC+\alpha_{11}A^2+\alpha_{22}B^2+\alpha_{33}C^2 \quad \ldots (4)$$

where Y= Response variable (dependent variable), A= Atrazine concentration, B= pH, C= Inoculum concentration, respectively.

α_o= offset term, α_1, α_2, α_3=linear function's regression coefficients, $\alpha_1\alpha_2$, $\alpha_2\alpha_3$, $\alpha_1\alpha_3$=Interaction effect's regression coefficients, α_{11}, α_{22}, α_{33}=quadratic function's regression coefficient. After conducting trials, atrazine elimination efficiency was determined

using the response plot, anticipated and modified regression coefficient value and variance analysis.

Evaluation of Chlorophyll content

Under ideal circumstances, chlorophyll is a vital pigment that perform photosynthesis for raising cell biomass by absorbing light energy[45]. Chlorophylls a and b are the primary substances involved in photosynthesis in green algae and land plants[46]. Chlorophyll b is an additional pigment which receives energy and sends it for chlorophyll a[47]. The primary light-harvesting chlorophyll-binding proteins require chlorophyll b to remain stable. Dimethyl sulfoxide, an amphiphilic solvent, was employed to extract chlorophyll from *Vigna radiata* leaves. It instantly diffused over the membrane and damaged the protein by displacing the surrounding water. Small pieces of 0.1 g of leaves were broken up and soaked in 10 mL solvent of DMSO (Dimethyl sulfoxide) before being heated to 60-70°C for an hour and then cooled at room temperature (27 ± 5 °C) for 30 min. The acquired material underwent centrifugation and a 0.20 µm filter paper filtering process. Using DMSO as a blank, absorbance was determined using an SL 159 UV-VIS spectrophotometer. Total chlorophyll concentrations and chlorophyll a and b concentrations were estimated in terms of mg/g fresh weight (F.W.)[48]. It was determined using the formula proposed by[49].

$$\text{Chlorophyll a (mg/g F.W.)} = (14.85\ ab_{665} - 5.14\ ab_{648}) \quad \ldots (5)$$

$$\text{Chlorophyll b (mg/g F.W.)} = (25.48\ ab_{665} - 7.36\ ab_{648}) \quad \ldots (6)$$

$$\text{Total chlorophyll (mg/g F.W.)} = (7.49\ ab_{665} + 20.34\ ab_{648}) \quad \ldots (7)$$

Phytotoxicity

Evaluation of phytotoxicity demonstrated how the contaminant negatively impacted plant growth and metabolism. Disruptions in physiological processes, like water and nutrient accumulation, photosynthesis, seed germination, have an impact on plant metabolism and growth. *Vigna radiata* (mung bean) seed was used in the phytotoxicity analysis. To prevent any fungus, *Vigna radiata* was rinsed thrice in double distilled water after being steeped in 1% NaClO for five minutes[50] A double layer, a cotton layer with a 5 mm thickness at the bottom, and Whatman filter paper at the top and bottom of three petri dishes were used. Between these two layers, seven *Vigna radiata* seeds were planted, and they were given 5 mL of treated samples, control water (double-distilled water), and untreated samples, and after that they were irrigated with the sufficient amount of water at regular time. At 30°C, each plate was placed for germination. A seed would be considered germinated when the radicle length was greater than or equal to 2 mm[51], Percentage relative root growth (% RRG), percentage relative seed germination (% RSG), and percentage germination index (% GI) and phytotoxicity index (PI), were determined based on analysis over 2 and 5 days, respectively.

$$\%RRG = \frac{Sample's\ root\ length}{Control\ sample's\ root\ length} \times 100 \quad \ldots (8)$$

$$\%RSG = \frac{Seed'sgermination\ in\ sample}{Seed's\ germinatiion\ in\ control\ sample} \times 100 \quad \ldots (9)$$

$$PI = 1 - \frac{Sample's\ root\ length}{Control\ sample's\ root\ length} \times 100 \quad \ldots (10)$$

$$\%GI = \frac{RSG \times RRG}{100} \quad \ldots (11)$$

Toxicity for Fluorescens bacteria

Based on previously published articles assessment for bacterial toxicity was performed[52]. For Bioluminescence toxic study, luminescent bacteria were obtained from the NCMR (National centre for Microbial Resource) Pune, India and grown in Luria Bertani (LB) broth. For the assessment of bacterial toxicity, optical density-based bacterial cultures were used. We have three different types of samples, first one is distilled water as control, second one is Biodegraded Atrazine and third one is Atrazine solution without any degradation. From each sample five mL solution is taken and mixed with 1 mL of *Pseudomonas fluorescens* bacterial solution separately. For acute toxicity, the sample was taken out after 30 minutes while for chronic toxicity it was taken out after 24 h, after inoculation were made with *P. fluorescens* bacteria.

$$\%\ \text{Bioluminescence Inhibition} = \frac{control\ samle\ intensity - sample\ intensity}{Control\ sample\ intensity} \times 100 \quad \ldots (12)$$

Intensities of bioluminescence were expressed as counts per second.

Results and Discussion

Using *Bacillus paramycoides* strain, experiments were conducted to determine the combined impact of three different process variables on the breakdown of atrazine. The Design Expert was used to perform

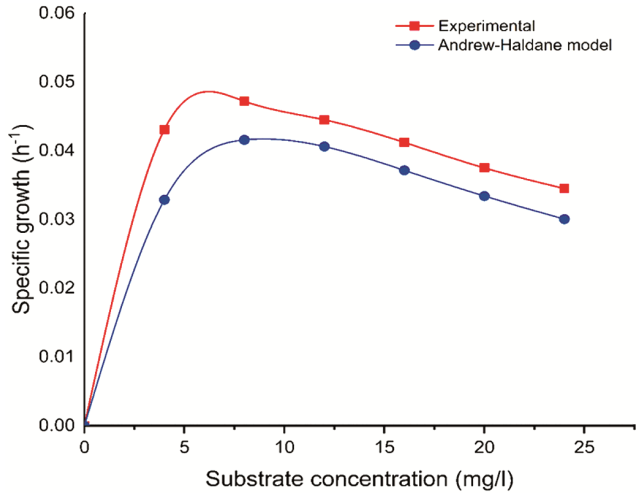

Fig. 2 — Figure represents specific growth rate and Andrew-Haldane model predicted growth rate of *Bacillus paramycoides* strain HL 1 at different atrazine concentration

multiple regression analysis in order to identify the coefficients for each term of the second order polynomial equation. Table 3 provides the experimental percentage of atrazine removal employing *B. paramycoi*des strain from agricultural soil.

Growth kinetic study

Numerous researchers have looked into how environmental pollutants are used by bacteria as a substrate. These are all efforts to cleanse the environment of contaminants. By drawing a graph between 't' *vs*. ln (X/X_o) t, it is possible to determine the Microorganism's specific growth at various concentrations of atrazine. Variation of atrazine substrate concentration with specific growth rate is shown in Fig. 2. Numerous models of the kinetics of microbial growth and destruction have been created. To analyse the experimental data, we have used six different type of substrate-inhibition kinetic models like Monod model, Tessier model, Andrew-Haldane model, Yano model, Aiba-Edwards model and Webb model. A variety of cell growth and above-mentioned substrate inhibition models were used to fit the experimental results of the specific growth rate fluctuation with starting atrazine concentration. The model with the highest correlation coefficient (R^2) selected as the best fit. In trial-and-error method, curve fitting was done by utilising nonlinear regression analysis to analyse the different models. These model's parameters were found and given in the Table 1 as illustrated in Fig. 2. The computed specific growth rate was plotted against the atrazine concentration. As we can see from the figure, with the increase in atrazine concentration the specific growth rate (μ) decreases. Consequently, it appears that the particular growth rate is influenced by the initial atrazine concentration. Models for substrate inhibition can be used to simulate the observation of substrate inhibition brought on by atrazine. Table 2 shows the overall bio-kinetics parameters and the coefficient of correlation (R^2). The Andrew-Haldane model matched the best data with an R^2 value of 0.97. The calculated values for the specific growth rate (μ), substrate affinity constant (K_S), and substrate inhibition constant (K_i) are 0.18 h^{-1}, 15 mg. L^{-1} and 5.5 mg. L^{-1}, respectively. Microorganisms grew at quantities up to 5.5 mg/L, but once that point was reached, substrate inhibition caused the growth to slow down (Fig. 2). The high concentration of atrazine has a negative impact on the metabolic activity of bacteria.

Table 4 — Analysis of variance for quadratic model

Source	Coeff. factor	Sum of squares	DOF	Mean square	F-value	*P*-value
Model	83.13	4639.84	9	515.54	172.96	<0.0001*
A-Atrazine conc.	6.41	297.02	1	297.02	99.65	<0.0001*
B-pH	315.84	315.84	1	315.84	105.97	<0.0001*
C-Inoculum conc.	5.77	38.93	1	38.93	13.60	0.0047*
AB	0.81	0.1512	1	0.1512	0.0507	0.8263*
AC	4.34	0.6613	1	0.6613	0.2219	0.6477*
BC	0.16	30.03	1	30.03	10.08	0.0099*
A^2	36.28	1.13	1	1.13	0.3797	0.5515*
B^2	39.13	1609.09	1	1609.09	539.85	<0.0001*
C^2	8.62	40.91	1	40.91	13.72	0.0041*
Residual		29.81	10	2.98		
Lack of fit		19.62	4	4.90	2.89	0.1188**
Pure error		10.19	6	1.70		
Cor Total		4669.65	19			

[*Significant **Not-Significant]

Analysis of variance for response surface quadratic model

Analysis of Variance (ANOVA) is used to evaluate the quadratic response surface model's importance and suitability. The deterioration efficiency was predicted using a quadratic model, and the significance and interactions of the factors were assessed using an ANOVA. Analysis of variance results for all the observations for atrazine are shown in Table 4, where the determination coefficient (R^2) showed that the model effectively captured the majority of responses. Additionally, it was shown that the projected values and experimental values had a good degree of agreement. The p value (P <0.0001) indicates that the created model for the atrazine biodegradation by isolated bacteria was sufficient and believable in capturing the real interaction between factors and responses, according to the biodegradation

model for atrazine, which is highly noteworthy. The F-ratio is a useful indicator for illustrating how the variables in the data differ from their means. The ANOVA of the data's significant F-value (436.85) and non-significant lack of fit (0.1188) values indicate that it is extremely significant the quadratic model. The estimated factors effectively account for the differences in the data, according to the calculated F-value from the data (172.96), which also suggests that we got real estimated factors and the model is significant. The variables A, B, C, AB, BC, AC, AB, BC and AC were significant in terms of the model when their P values were P <0.0001. According to research, the fitted model is considered to be strongly connected if the regression equation's coefficient of determination (R^2) lies between 0.90 and 1.00. The model's R^2 in this investigation was 0.9936 which is shown in Table 5, meaning that it can account for 99.36% of the predicted and experimental data. Because the model the experimental data are superior, as indicated by the value of the coefficient of variation (CV = 2.41 %), smaller value of Coefficient of Variance signifies a higher precision degree. From Table 4 coefficient factors it is evident that the interaction among agitation speed and pH has an advantageous effect, similar to the vice observed between atrazine concentration and agitation speed. The relationship between the three variables and atrazine degradation was represented by the following Eq.:

$$\text{Atrazine degradation (\%)} = 86.11A-5.45A+5.62B+2.15C-0.1375AB+0.2875AC-1.94BC-0.6552\ A^2-24.71B^2-4.10C^2 \quad \ldots (13)$$

Process independent variables Effects on atrazine removal efficiency

Effect of pH and atrazine concentration

From the response curve Fig. 3, it can be seen that as the pH is increased from 5 to 7 the atrazine degradation is increased as we increased concentration from 1 ppm to 13 ppm. After that as we further increased the atrazine concentration from 13 ppm to 25 ppm and pH is increased from 7 to 9 the atrazine degradation is decreased it may be due to loss of microbial activity at slightly acidic and basic conditions. In the same way at higher atrazine concentration, there may be substrate inhibition at higher atrazine concentrations. At optimum pH 7 and 13 ppm atrazine we got the maximum degradation percentage of 93.4. Tolerance of pH is an important parameter of atrazine degradation. The result which is obtained is than previous results[53].

Table 5 — An overview of model fit statistics

Std. Dev.	Mean	C.V. %	R^2	Adjusted R^2	Predicted R^2	Adeq. Precision
1.73	71.59	2.41	0.9936	0.9879	0.9671	40.6006

[% C.V.: Coefficient of Variance]

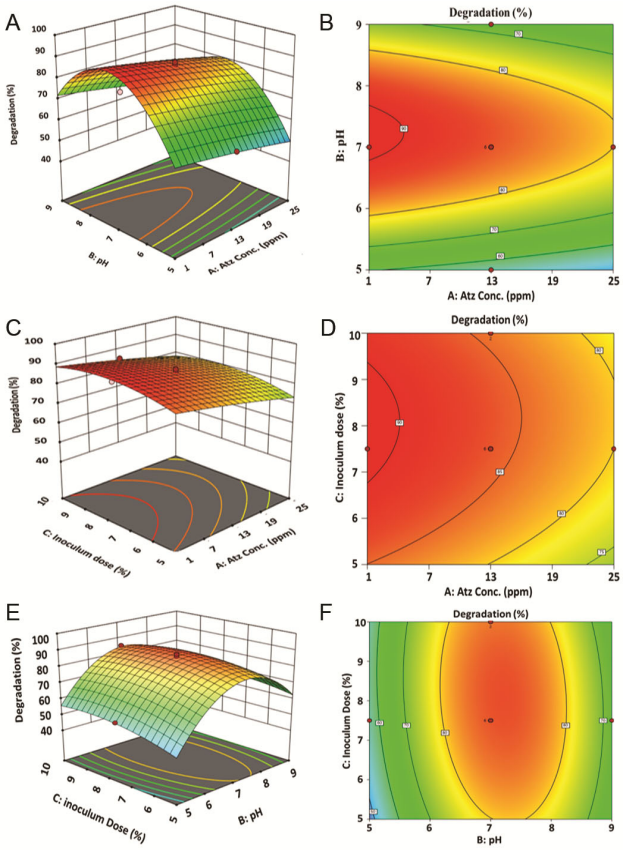

Fig. 3 — Contour and surface plot for atrazine degradation percentage. (A & B) pH effect and atrazine concentration effect; (C & D) pH effect and inoculum concentration effect; and (E & F) atrazine concentration effect and Inoculum concentration effect

Atrazine concentration and Inoculum concentration effect

In the contour plot Fig. 3, we can see as the concentration is increased from 1 ppm to 13 ppm and inoculum concentration is from 5 to 10% the atrazine degradation is increased and reached at its maximum percentage of 93.4 at a particular pH. After that, if we further increase inoculum percentage and atrazine concentration from 13 ppm then we got a decrease in atrazine degradation percentage. It may be possible that at a higher percentage of atrazine, we got the toxic effect of atrazine than the acceptable limit of Consortium. Such types of Inhibitory effects of atrazine were also reported in previous reports[54]. At the concentration of atrazine of 13 ppm and inoculum percentage of 10%, we got maximum degradation of 93.4 at a particular 7 pH.

Table 6 — Chlorophyll assessment in treated, control and untreated sample of *Vigna radiata* seeds

Sample	Chlorophyll a (mg/g F.W.)	Chlorophyll b (mg/g F.W.)	Total chlorophyll (mg/g F.W.)
Control	12.21±0.25	17.51±0.21	11.98±0.18
Treated	10.15±0.14	15.42±0.23	11.51±0.24
Untreated	6.21±0.51	7.42±0.35	6.52±0.36

Table 7 — Toxicity assessment of atrazine solution in treated, control and untreated sample using *Vigna radiata* seeds

Sample	Root length (cm)	Shoot length (cm)	% RRG	% RSG	PI	% GI
Control	2.52±0.5	14±1	100	100	0	100
Treated	2.50±0.2	13±1	92.86	90	0.07	50.0
Untreated	2.2±0.1	10±2	71.43	70	0.28	83.57

Effect of Inoculum concentration and pH

As shown in contour plot Fig. 3, if we increase pH from 5 to 7 the atrazine degradation percentage is increased on further increasing of pH from 7 to 9, we got decline in degradation percentage it may be due to loss of bacterial activity at slightly acidic and slightly basic condition. It is also reported in literature that most of the bacteria could degrade various aromatic compounds in the range of pH 6.0 to 6.8 such as *Pseudomonas Bacillus*[55] *Bacillus sp*. + *Pseudomonas* sp. In the same way if we increase the inoculum percentage from 5 to 10 % the Substrate degradation percentage is increased. At the optimum condition of pH 7, inoculum size and atrazine concentration of 13 ppm we got the maximum degradation percentage of 93.4. Similar of results were obtained in previous studies but we got better results as compared to those studies.

Determination of Chlorophyll a, b and total chlorophyll

The chlorophyll content of *Vigna radiata* was determined in 5 days of experiment and found out Chlorophyll a, b and total chlorophyll. The values which we obtained are shown in Table 6. From the obtained data on chlorophyll content, it can be observed that the Chlorophyll content of atrazine treated sample is significantly higher than the untreated sample. Since we got higher values of Chlorophyll contentment a, b, and total value in the treated sample so there would be more photosynthesis process hence more value of osmatic pressure.

Phytotoxicity analysis

For the phytotoxicity analysis of atrazine, we have used *Vigna radiata* in treated, untreated and control samples to germinate these seeds. In this analysis, we have observed how seed growth and seed germination are affected. The results which were obtained after 7 days of study are mentioned in Table 7. From table it can be easily observed that the soot length and root length in treated sample is significantly higher than the untreated sample but it is slightly less than the control sample and the treated sample's PI value is substantially better than the untreated sample. Higher the value of GI there will be more probability of germination rate. We may conclude that for irrigation purposes of the *Vigna radiata* seeds, we can use treated water sample after analysing several parameter values that are crucial for evaluating the growth of seeds.

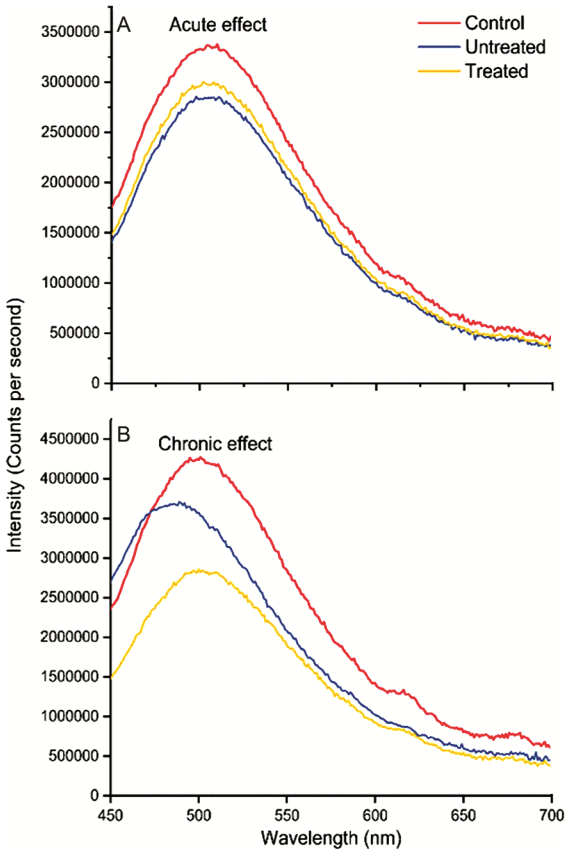

Fig. 4 — (A) Acute bioluminescence intensity; and (B) chronic bioluminescence intensity bioluminescence inhibition acute and chronic exposure of *pseudomonas fluorescence* bacteria in the presence of control (distilled water), degraded and without degraded sample

Bacterial toxicity assessment using *Pseudomonas fluorescens*

For the analysis of atrazine's bacterial toxicity for acute effect in a batch system, we measured bioluminescence intensity for *Pseudomonas fluorescens* in the atrazine solution both in biodegraded and without degraded samples. When compared to the control sample (distilled water) (3.9×10^6 counts per second), the *Pseudomonas fluorescens* bioluminescence intensity of the sample without degradation (2.5×10^6 counts per second) was found to be lower than the treated waste water (2.9×10^6 counts per second) due to biodegradation of atrazine as shown if Fig. 4.

For long-term exposure (chronic effects, 24 h), a similar mechanism was seen to breakdown atrazine in a batch method as shown in Fig. 4. When compared to the control sample (4.6×10^6 counts per second), the *Pseudomonas fluorescens* bioluminescence intensity of the sample without degradation (2.6×10^6 counts per second) was found to be lower than the treated waste water (4.3×10^6 counts per second) due to biodegradation of atrazine

Conclusion

In present study a potential atrazine degrading bacteria was isolated and a batch study was performed. The process parameters pH, atrazine concentration, and Inoculum percentage were optimized with the help of Central composite design of RSM, and we have found optimum conditions as pH 7.0 Inoculum Concentration 10% and atrazine concentration of 13 mg/L. Simultaneously we have also studied growth kinetic using different models and found that Andrew-Haldane models best-fitted Data with the value of correlation coefficient of 0.97. In the end, we have performed Bacterial and phytotoxicity analysis of degraded samples as well as chlorophyll content and found that Biodegraded samples shown less toxicity and higher chlorophyll content as compared to non-Biodegraded samples so it can be effectively used for irrigation purposes.

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