ISSN Online: 2349-7785

Optimization of Biodegradation Process of Pharmaceutical Industries Waste Water (PIWW) Using Response Surface Methodology by *Xanthomonas campestreis* (*X. campestreis*)

Vikas Kandari [1,2], Rajender S Rana [2], Prashant Singh [2] and Sanjay Gupta [1]*

[1] Department of Biochemistry and Biotechnology, SBSPGI, Balawala, Dehradun, India
[2] Department of Chemistry, DAV (PG) College, Dehradun, India

Received 11 September 2016; accepted in revised form 21 October 2016

Abstract: The biodegradation process was investigated using an isolated single bacterial culture of *X. campestreis*. The *X. campestreis* was able to reduced 75-80 % pollution load within 48 hrs under aerobic condition in term of Chemical Oxygen Demand (COD) in Pharmaceutical Industry Wastewater (PIWW). Obtained data showed that *X. campestreis* was potent bacterial culture for PIWW treatment. The influence of three process parameters such as temperature (20-50^{o}C), pH (4-9) and agitation speed (30-150 rpm) influenced the rate of biodegradation extent. Central composite design (CCD), an experimental design for response surface methodology (RSM), was used to create a set of 36 experimental runs needed for optimizing operating conditions. The optimal degradation conditions were determined by response surface methodology based on three-variable central composite design to obtain maximum reduction and to determine the significance and interaction effect of the variables on degradation. The optimal conditions of response were found to be 35^{o}C, pH 8.05 and 120 rpm agitation speed, respectively, giving an experimental degradation value of 80.07 %. Very high regression coefficient between the variables and the response (R2 = 0.6447) indicated a good evaluation of experimental data by polynomial regression model.

Key words: *Xanthomonas campestreis*.

Introduction

Pharmaceutical industry wastewaters are considered as an emerging environmental problem due to their toxicity and chemical persistence in the environment. They can remain in the environment for a long time and their residence time is considered as dangerous at both low and high concentrations. It has been estimated that up to half of the pharmaceutical wastewater produced worldwide is released without any treatment and the components are difficult to destroy or decompose by common treatment in conventional wastewater treatment plants [1,2]. Hence, the treatment strategies for PIWW is under considerable pressure to efficiently and effectively treat the effluents discharged to the environment to comply with environmental legislation restricting the discharge of wastewater and to prevent deterioration of ecosystems [3,4]. However, these techniques have shown limited success for the treatment of pharmaceutical wastewater due to the nature and composition of pharmaceutical effluents. Therefore, other technologies have been explored with the aim to further reduce the concentration of pharmaceutical contaminants in wastewaters. These technologies include membrane separation, advanced oxidation technology, and electrochemical techniques such as electro-flotation and

*Corresponding author (Sanjay Gupta)
E-mail: < sanjay_gupta9999@rediffmail.com >

electro-coagulation. Electro-coagulation has shown the potential to largely overcome the drawbacks of conventional processing techniques [5,6,7].

Until now, the effects of environmental factors on microbial degradation of complex PIWW are usually examined with the conventional single-factor optimization [8,9] in which experiments were conducted by varying systematically the studied parameter while keeping other parameters constant[10]. This is usually repeated for all the parameters influencing degradation thus [11,12], resulting in an unreliable number of experiments. In addition, the combined effect of the effective influence parameters cannot be determined using this exhaustive procedure. Hence, a novel experimental design method such as the response surface methodology (RSM) [13,14,15] which can estimate quadratic effects and linear interaction of the factors and predict a model for the response with a minimum number of experiments could be a useful tool for optimization of effective parameters of PIWW degradation. Here, we report the isolation and identification of a PIWW degrading bacterial culture. *X. campestries,* capable of degradation of generated high pollution load in a aerobic process. There are almost no reports on the biodegradation of PIWW by *X. campestries* and hence, it was significant to develop this new microbial procees in bioremediation for PIWW. The effect of process parameters for degradation was determined by an optimized condition using the Response surface methodology (RSM) based on central composite design (CCD).

Materials and methods

Sample collection area and its preservation

Pharmacity, Selaqui of Dehradun district was chosen for study area located 25 Km from Dehradun which is capital of Uttarakhand State, India and 225 Km from National Capital Delhi. Total area of Pharmacity is 50 Acres.

Wastewater samples were collected in Tarson bottles previously rinsed with non-ionic detergent, rinsed with tap water and later leached with 10 % nitric acid for 24 hours and finally rinsed with deionised water prior to use. This process has importance because certain metal ions like chromium, cadmium, lead are subject to loss or adsorption or ion exchange with walls of container. Before sample collection these bottles were rinsed with the sample. All the samples after maintaining in cold chain were brought to the laboratory to store in sampling box at 4°C.

Culture media, strain isolation and characterization

Nutrient Agar Media and Tris Minimal Salt Media were used for the isolation. Further, the shake flask studies were conducted using wastewater supplemented with minimal mineral media. The soil samples for the isolation of the microorganisms were collected in the sterilized zip lock polybags to prevent environmental contamination. The samples were immediately transported to the lab and maintained below 4^{o}C, till their utilization. For isolation of microbes serial dilution method was adopted; 1 g of contaminated soil sample was inoculated in a 250 mL Erlenmeyer flask containing 100 mL of nutrient broth medium and spread in Nutrient Agar Media at 37^{o}C in 24 hrs. the isolate were identified by biochemical characterization and bacterial growth kinetics. The organism was used for subsequent treatment and optimization studies.

Treatment of pharmaceutical waste water by *Xanthomonas campestreis* (*X. campestreis*)

The treatment capability of *X. Campestreis* was determined using PIWW (100 ml/l) in minimal salt media. Bacterial culture was inoculated in pre-autoclaved minimal salt media (10^7 cfu/ml approximate cell density, pH-7.2) and incubated 35^{o}C for 48 hours under shaking condition. Using the above performance parameters aerobic bacteria was encourage to decrease the high pollution load in 48 hours incubation time and determine the equilibrium time required for maximum treatment of PIWW. Control consists of PIWW and minimal salt media without bacterial culture. Further experiment were performed to determine the effect of incubation temperature, pH and agitation speed condition on PIWW treatment by varying the incubation temperature (20-45^{o}C), medium pH (5-9) and shaking condition (50-150 rpm), while keeping other conditions constant. The pH of the MSM solution was adjusted using 0.1 M HCl or 0.1 M NaOH. All the experiments were performed in triplicates.

Optimization of treatment conditions by response surface methodology

RSM is a collection of mathematical and numerical techniques that are useful for modelling and analysis of the processes having numerous variables influencing the response and the objective is to optimize process settings in an efficient use of the resources [16]. It can be used for predicting the functional relationship between a set of experimental design variables and a response variable [17,18].

The RSM approach was applied to determine the optimal levels of three input variables, namely temperature (factor A), pH (factor B), incubation time (factor C) and agitation speed (factor D) and to identify the relationship between the response functions and process variables. Design Expert 8.0.2 software was used to analyze the obtained results. The values used were based on results of preliminary experiments carried out to determine range of values of parameters for effective treatment.

For four variables (n = 3) and five levels [low (-) and high (+)], the total number of experiments was 36 determined by the expression: 2n (2^3 = 8 factorial points in triplicates) + 2n (2×3 = 6 axial points) + 6 (center points: six replications) as given in Table 1. PIWW treatment was selected as the response for the combination of the independent variables. Randomised experimental runs were performed to reduce to the barest minimum the effects of unexpected variability in the observed responses [17]. Design Expert 8.0.7.1 software was used to analyze the obtained results.

For statistical analysis, a quadratic polynomial equation by central composite design was developed to predict the response as a function of independent variables and their interaction. In general, the response for the quadratic polynomials is described below:

$$Y = \beta_0 + £\beta ixi + £\beta iixi_2 + ££\ \beta ijxijxj \quad (1)$$

where Y is the response (COD reduction); β_0 is the intercept coefficient, bi is the linear terms, *bii* is the squared terms and β*ij* is the interaction terms, and *xi* and *xj* are the uncoded independent variables [18]. Data from the central composite experimental design were subjected to regression analysis using least square regression methodology to obtain the parameters of the mathematical models. The F test was then used to evaluate the significance of the model equation and model terms. The statistical significance of the model was evaluated using the analysis of variance (ANOVA) while, the optimal values were obtained by solving the regression equation and analyzing the response surface plot.

Analytical method for chemical oxygen demand determination

Original and treated samples were measured by monitoring as per APHA guidelines for waste water. COD vials and caps was rinsed with 20 % H_2SO_4 to prevent contamination. 2.5 ml of the sample was added to the COD vial and for blank 2.5 ml distilled water was added to the COD vial and then 1.5 ml of digestion solution was added to each COD vials. 3.5 ml sulphuric acid reagent was added in the same manner. At this time caution should be taken because COD vials are hot now. COD vials was then placed in COD digester and heated at 150°C for 2 hrs. After digestion the vials was allowed to cool at the room temperature in test tube rack. After cooling, the content of the vials was transferred to conical flask. 1 to 2 drops ferroin indicator was then added in conical flask containing sample. The solution was titrated against the Mohr's salt and the titer volume required for the color change from blue-green to reddish brown was noted. The procedure was repeated for blank titration.

Calculation

$$\text{COD (mg/L)} = [(B-A) \times N \times 8000] / V \quad (2)$$

Where: B = volume of titrant used against blank, A = volume of titrant used against sample. N = normality of FAS, V= volume of the sample.

Result and discussion

Reduction capabilities and growth kinetic study of bacterial strains for pharmaceutical industrial wastewater

The six bacterial cultures showing maximum growth capability in pharmaceutical industry wastewater was selected. Time course study was done for the isolated strains among them, *Xanthomonas campestris* showed the maximum degradation capability. Further, to determine the growth and multiplication of bacterial cells, the highest cell numbers were observed after 24h of incubation and this corresponds to the late log phase/early stationary phase of *Xanthomonas campestris* growth cycle. It has been observed that maximum degradation capability occurs in the initial stage of the stationary phase which corresponds to the maximum numbers of the viable cells of bacteria in growth media. Fig. 1 showed the adaptation period of 15 hours for the bacterial strain *Xanthomonas campestris* with the wastewater supplemented media, followed by a rapid growth period of 15-22 hours and consequently showed the decline phase after 35 hours of culture growth. The results showed a typical bacterial growth curve corresponding to the physiology of the bacterial growth kinetics. The *Xanthomonas campestris* along with the other strains procured from MTCC was further subjected to the optimization studies under different nutritional parameters to observe the enhanced reduction capability of the strains.

Optimization of process parameter for reduction of pharmaceutical industrial wastewater by Xanthomonas campestris

Data obtained on effect of three process parameters on degradation potential of *Xanthomonas campestris* are as presented in Fig. 2

Effect of pH

In order to find out the optimum pH for the effective degradation of wastewater by bacterial strain, experiment was performed at six different pH and the results obtained are shown in figure 2. After 24 hours of culture incubation the percentage reduction of pollution load at pH 4 was 60.02 %, while at pH 5 and pH 6 the percentage reduction was 60 and 74.2 % respectively and at pH 7 maximum reduction (85 %) capability was observed. As the treatment process was drifting towards the alkali conditions at pH 8.0 and 9.0, there observed the sharp decrease in the reduction capabilities of the *Xanthomonas campestris* as shown in figure 2. The result obtained showed that pH 7.0 was desirable for the maximum degradation capabilities of the above tested strain. Therefore, the further studies were carried out at neutral pH of 7.0 unless stated otherwise.

Effect of temperature

The culture transfer technique selected for microbial communities were adapted to a specific temperature as well as the feast-famine growth conditions imposed in culture techniques. The impact of temperature on high pollution load during the biological treatment of the pharmaceutical wastewater was determined for a culture transfers. The efficiency of pollution load removal were best at 35°C (75.2 %), however pollution removal declined as temperature was incrementally in-

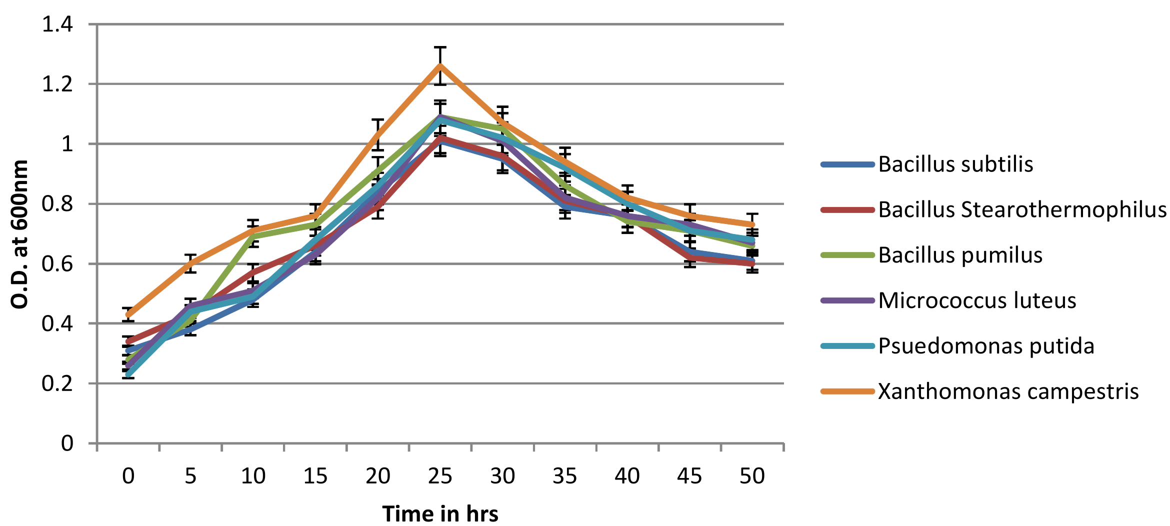

Fig 1. Time interval study of growth kinetics of different isolated bacterial culture in Minimal salt media (MSM)

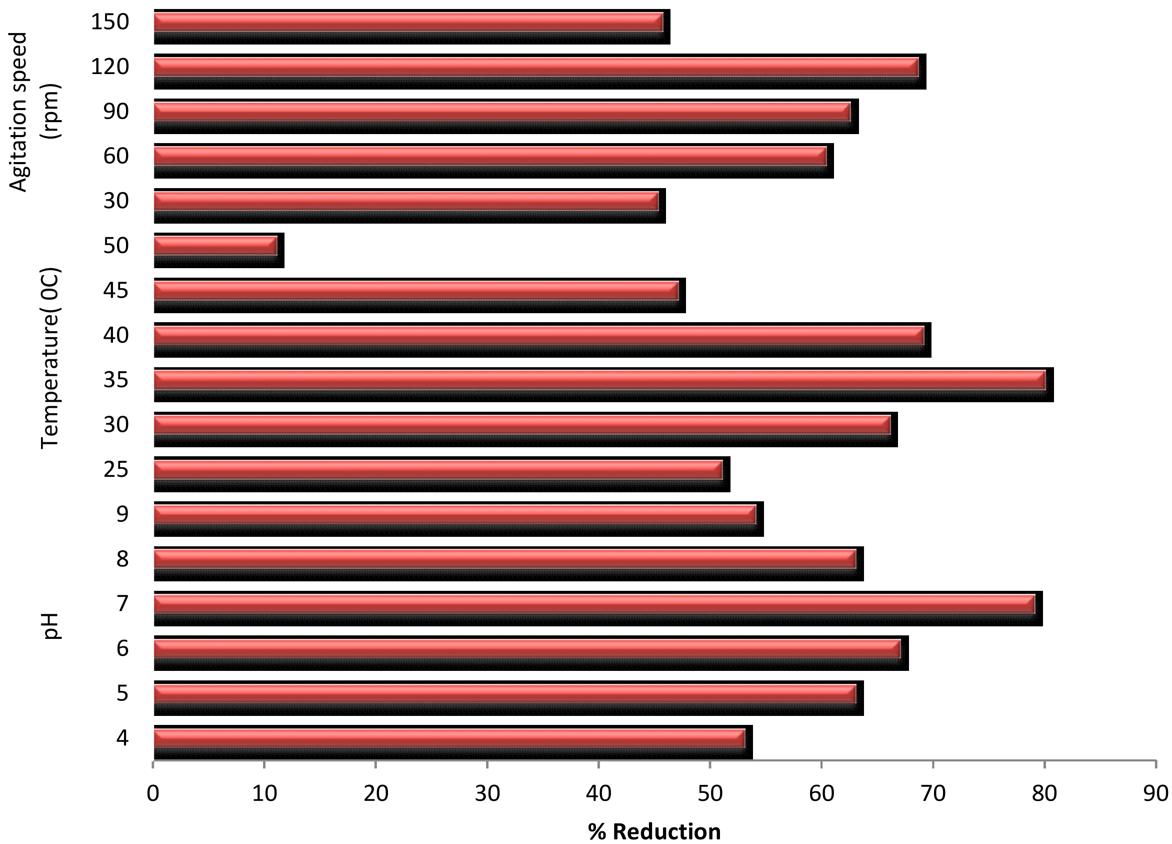

Figure 2. Effect of process parameters (pH, incubation temperature and agitation speed) on percentage reduction of PIWW by X. campestries under 48 h incubation time

creased to 45°C (38 %). No bacterial growth, and thus no pollution removal, occurred at 50°C or 55°C. The influence of incubation temperature on the time required for the complete degradation by bacterial culture was studied by different temperature from 30 to 55°C.

Effect of agitation speed condition

Incubation period of 48 hours and aeration conditions ranging from 30, 60, 90, 120 and 150 rpm was used to measure its effect on reduction capabilities of the tested strain for all selective parameter. It was observed that 120 rpm after 40 hours of culture incubation showed maximum degradation efficiency of 68-70 %. At the higher agitation speed condition, the lower reduction capability of the strains was observed.

Optimization of PIWW treatment process using response surface methodology

A total of 36 experiments with different combinations of temperature (factor A, 20-45°C), pH (factor B, 7.5-10) and (factor C, 90-150 rpm) were performed within 48 h using the CCD method. PIWW treatment was used as the response (R, %) and the results obtained from the experiments (observed and predicted) are summarized in Table 1. By applying multiple regression analysis, the following second-order polynomial equation (in coded units) that could relate degradation of PIWW to the parameters studied was obtained as Eq. (3).

$$R1 = +76.58 +5.29 A -0.30B -3.38 C +3.21 AB -6.88 AC - 4.49 BC -14.24 A2 -14.44 B2 -2.49 C2 \quad (3)$$

From the Eq. (3) above, the second-order response functions are represented by: R, the response for PIWW treatment; A, the coded value of variable incubation temperature; B, the coded value of variable pH and C, the coded value of variable agitation speed (rpm). Data obtained on

Table 1. Observed and predicted values of PIWW treatment by *X. campestries* using of RSM

Run	temperature	pH	Agitation speed	% response
1	32.5	7.5	90	69.88
2	32.5	7.5	222.8	60.08
3	45	10	30	76.09
4	45	10	150	45.05
5	20	10	150	32.02
6	45	10	150	34.06
7	45	10	150	34.03
8	20	10	30	15.05
9	32.5	7.5	90	68.32
10	45	5	30	45.76
11	20	10	150	17.03
12	20	5	150	17.04
13	20	5	30	16.23
14	20	5	30	16.5
15	32.5	13.0	90	11.03
16	4.8	7.5	90	12.03
17	32.5	7.5	90	80.02
18	45	5	150	55.97
19	45	10	30	63.07
20	45	5	30	55.06
21	20	5	30	66.04
22	32.5	7.5	90	82.09
23	45	10	30	70.03
24	20	5	150	55.06
25	45	5	150	33.04
26	20	10	30	35.05
27	20	10	150	50.09
28	45	5	150	44.04
29	20	10	30	44.07
30	60.2	7.5	90	11.03
31	32.5	7.5	90	88.04
32	32.5	1.97	90	10.04
33	45	5	30	56.02
34	32.5	7.5	-42.8	78.05
35	32.5	7.5	90	89
36	20	5	150	67.08

predicted values and observed values show the empirical models actually fit the actual data with R^2 of 0.9624. When R^2 is closer to unity as obtained in this study, the empirical models fit the actual data better whereas, the relevance of the dependent variables in the model in explaining the behaviour of variations cannot be ascertained by a smaller value [13] of R^2. Table 2 shows the data obtained from the analysis of variance (ANOVA) for the response surface reduced quadratic model. The Model F-value of 8.06 implies the model is significant. There is only a 0.01 % chance that a “Model F-Value” this large could occur due to noise Values of “Prob > F” less than 0.0500 indi-

Table 2. ANOVA analyses for the response surface quadratic model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	15731.38	9	1747.931	8.05652	< 0.0001	significant
A-temperature	945.334	1	945.334	4.357209	0.0468	
B-pH	2.96395	1	2.96395	0.013661	0.9079	
C-agitation spped	386.1014	1	386.1014	1.779609	0.1938	
AB	247.5553	1	247.5553	1.141025	0.2953	
AC	1137.402	1	1137.402	5.242484	0.0304	
BC	483.3038	1	483.3038	2.227631	0.1476	
A^2	7419.884	1	7419.884	34.19954	< 0.0001	
B^2	7633.066	1	7633.066	35.18213	< 0.0001	
C^2	227.7704	1	227.7704	1.049834	0.3150	
Residual	5640.924	26	216.9586			
Lack of Fit	761.3377	5	152.2675	0.655305	0.6609	not significant
Pure Error	4879.586	21	232.3612			
Cor Total	21372.31	35				

cate In Values greater than 0.1000 indicate the model terms are not significant this case A, AC, A^2, B^2 are significant model terms If model reduction may improve your model. there are many insignificant model terms (not counting those required to support hierarchy), model terms are significant The "Lack of Fit F-value" of 0.66 implies the Lack of Fit is not significant relative to the pure error. There is a 66.09 % chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -we want the model to fit. The "Pred R-Squared" of 0.4767 is in reasonable agreement with the "Adj R-Squared" of 0.6447. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 10.494 indicates an adequate signal. This model can be used to navigate the design space [19,20,21].

The choices for level combinations of the three variables such as, temperature, pH and agitation speed were made into contour plots (Fig. 3 a-c) which indicated the percentages of reduction within 48 h of incubation. The behavior of percentage reduction with respect to changes in temperature and pH is shown in Fig. 3a. These two parameters showed positive influence on PIWW treatment. The percentage reduction increased with increase in temperature and pH until a certain level where further increases in both parameters led to a decrease in percentage reduction. In Fig. 3b, the variation of percentage degredation as specified by the variables, pH and temperature shows that with the increase in pH, the percentage reduction decreased. The result shows that optimal percentage reduction of PIWW was between 35 and 40^{o}C and slightly above basicity of pH. Three dimensional and contour plots for interaction effect of reaction pH and agitation speed toward PIWW reduction are shown in Fig. 3c. PIWW reduction increased as the pH increased to its medium level (pH 8) and agitation speed increased to its central level (100 rpm) [22,23,24]. Optimal reduction of PIWW was obtained in culture broth of pH 8 and 100 rpm agitation speed. Generally, a stronger influence of pH and agitation speed occurred when both parameters were at their median level. The decreasing the reduction of PIWW at higher agitation and pH were probably as a result of increasing toxicity of the PIWW to the microbial cells inactivation at such high concentrations [25,26,27]. In each contour plot, the other one variable was held constant. Generally, increase in the three process parameters to a certain threshold value resulted in increase in percentage reduction of PIWW by *X. Campetreis*.

This is due to the positive quadratic model as

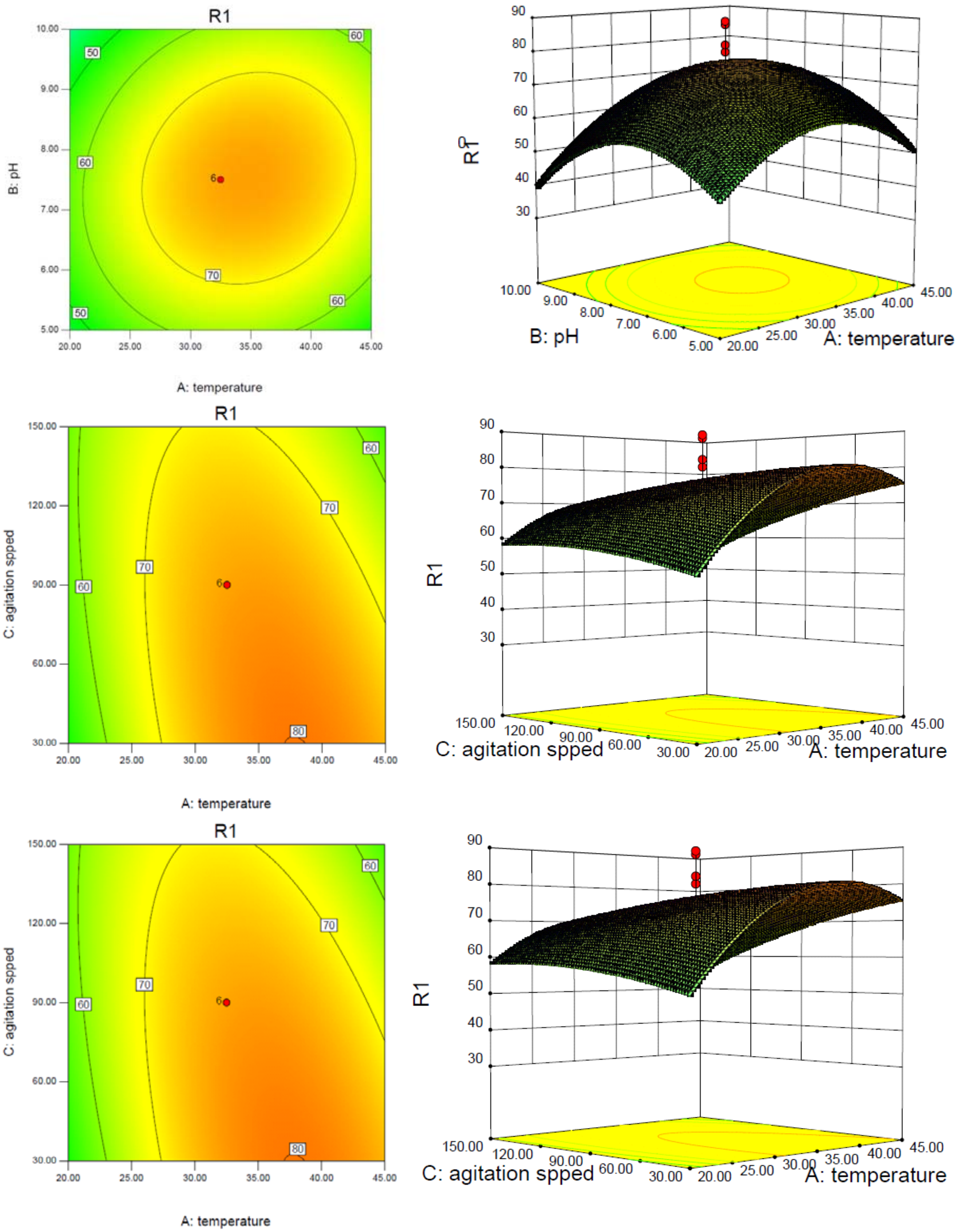

Fig 3. a Contour plot and 3-D surface showing degerdation extent as a function of temperature and pH (Actual factor B: pH = 8.02). **b** Contour plot and 3-D surface showing degerdation extent as a function of temperature and agitation speed (Actual factor C: agitation speed = 120 rpm). **c** Contour plot and 3-D surface showing degerdation of PIWW extent as a function of temperatare and agitation speed (Actual factor A: temperature = 35.05)

shown in Eq. 2. It also indicates that the experimental value must consider the running effect of these significant factors at the stipulated levels to maximize reduction of PIWW by *X. Campetreis* [14,15].

Conclusion

In this research work, treatment of PIWW for *X. campestris* was investigated by CCD using RSM by varying three control factor such as temperature, pH and agitation speed. The function of these factors in terms of optimization of process parameter and its treatment operation was well explained by the results obtained from RSM. By applying the RSM design to the optimization experiments, the process variables were well studied and reduction effciency extent up to 88 % was achieved in a shorter time (24 h). The experimental and the predicted values were very similar which reflected the accuracy and the applicability of RSM. Efficient PIWW treatment by *X. campestris* suggests its potential for real industrial applications in treatment of PIWW.

Acknowledgements

The authors duly acknowledge the financial support received from WTI-DST, Ministry of S&T. Govt. of India, New Delhi.

References

1. **Baughman, G.L., Weber, E.J. (1994).** Transformation of dyes and related compounds in anoxic sediment: kinetics and products. Environ. Sci. Technol. 28: 267-276.
2. **O'Neill, C., Lopez, A., Esteves, S., Hawkes, F.R., Hawkes, D.L., Wilcox, S. (2000).** Azo-dye degradation in an anaerobic-aerobic treatment system operating on simulated textile effluent. Appl. Environ. Microbiol. 53: 249-254
3. **Amin, A., Naik, A.T.R., Azhar, M., Nayak, H. (2013).** Bioremediation of different waste waters - a review. Cont. J. Fish Aquat. Sci. 7(2): 7-17.
4. **Choudhary, S., Parmar, N. (2013).** Hazard assessment of liquid effluent treatment plant in pharmaceutical industry. VSRD Int. J. Technol. NonTechnol Res. 4(9): 209-214.
5. **Damodhar, U., Reddy, M.V. (2013).** Impact of pharmaceutical industry treated effluents on the water quality of river Uppanar, South east coast of India: a case study. Appl. Water Sci. 3(2): 501-514.
6. **Das, M.P., Bashwant, M., Kumar, K., Das, J. (2012).** Control of pharmaceutical effluent parameters through bioremediation. J. Chem. Pharm. Res. 4(2): 1061-1065.
7. **Deegan, A.M., Shaik, B., Nolan, K., Urell, K., Oelgemöller, M., Tobin, J., Morrissey, A. (2011).** Treatment options for wastewater effluents from pharmaceutical companies. Int. J. Environ. Sci. Technol. 8(3): 649-666.
8. **Parshetti, G., Kalme, S., Saratale, G., Govindwar, S. (2006).** Biodegradation of Malachite Green by Kocuria rosea MTCC 1532. Acta Chim. Slov. 53: 492-498
9. **Khataee, A.R., Ayazloo, M., Pourhassan, M. (2009).** Biological decolorization of C.I. Basic Green 4 solution by Chlorella sp.: effect of operational parameters. Chin. J. Appl. Environ. Biol. 15: 110-114.
10. **Sedighi, M., Karimi, A., Vahabzadeh, F. (2009).** Involvement of Ligninolytic Enzymes of Phanerochaete chrysosporium in treating the textile effluent containing Astrazon Red FBL in a packed-bed bioreactor. J. Hazard Mater. 169: 88-93.
11. **Dixit, D., Parmar, N. (2013).** Treatment of pharmaceutical waste water by electro-coagulation and natural coagulation process: review. VSRD Int. J. Technol. NonTechnol Res. 4(5): 79-88.
12. **El-Gohary, F.A., Abou-Elela, S.I., Aly, H.I. (1995).** Evaluation of biological technologies for wastewater treatment in the pharmaceutical industry Water Sci. Technol. 32(11):13-20.
13. **Cao, W., Zhang, C., Hong, P., Ji, H. (2008).** Response surface methodology for autolysis parameters optimization of shrimp head and amino acids released during autolysis. Food Chem. 109: 176-183.

14. **Mohana, S., Shrivastava, S., Divecha, J., Madamwar, D. (2008).** Response surface methodology for optimization of medium for decolorization of textile dye Direct Black 22 by a novel bacterial consortium. Bioresour. Technol. 99: 562-569.
15. **Lau, W., Ismail, A. (2010).** Application of response surface methodology in pes/speek blend NF membrane for dyeing solution treatment. Membr. Water Treat. 1: 49-60
16. **Sharma, P., Singh, L., Dilbaghi, N. (2009).** Optimization of process variables for decolorization of Disperse Yellow 211 by Bacillus subtilis using Box-Behnken design. J. Hazard Mater. 164: 1024-1029.
17. **Montgomery, D.C. (2004).** Design and analysis of experiments, 5th edn. Wiley and Sons, Singapore.
18. **Ghadge, S.V., Raheman, H. (2006).** Process optimization for biodiesel production from mahua (Madhuca indica) oil using response surface methodology. Bioresour. Technol. 97: 379-384.
19. **Enright, A.M., McHugh, S., Collins, G., O'Flaherty, V. (2005).** Low-temperature anaerobic biological treatment of solvent containing pharmaceutical wastewater. Water Res. 39(19): 4587-96.
20. **Farhadi, S., Aminzadeh, B., Torabian, A., Khatibikamal, V., Fard, M.A. (2012).** Comparison of COD removal from pharmaceutical wastewater by electrocoagulation, photoelectrocoagulation, peroxi-electrocoagulation and peroxi-photoelectrocoagulation processes. J. Hazard Mater. 219-220: 35-42.
21. **Ghosh, M., Verma, S.C., Mengoni, A., Tripathi, A.K. (2004).** Enrichment and identification of bacteria capable of reducing chemical oxygen demand of anaerobically treated spent wash. J Appl. Microbiol. 6: 241-78.
22. **Gome, A., Upadhyay, K. (2013).** Biodegradability assessment of pharmaceutical wastewater treated by ozone. Int. Res. J. Environ. Sci. 2(4): 21-25.
23. **Heberer, T. (2002).** Occurrence, fate and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol. Lett. 131(1-2): 5-17.
24. **Idris, M.A., Kolo, B.G., Garba, S.T., Ismail, M.A. (2013).** Physico-chemical analysis of pharmaceutical effluent and surface water of river Gorax in Minna, Niger State, Nigeria. Bull. Env. Pharmacol. Life Sci. 2(3): 45-49.
25. **Ileri, R., Sengil, I.A., Kulac, S., Damar, Y. (2003).** Treatment of mixed pharmaceutical industry and domestic wastewater by sequencing batch reactor. J. Environ. Sci. Health A. 38(10): 2101-2111.
26. **Imran, H. (2005).** Wastewater monitoring of pharmaceutical industry: treatment and reuse options. Electron J. Environ. Agric. Food Chem. 4(4): 994-1004.
27. **Kaushik, K.A., Dalal, S.J., Panwar, S. (2012).** Impact of industrialization on culture of Uttarakhand and its role on career enhancement. VSRD Int. J. Bus. Manage. Res. 2(4):123-132. 103-110.