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**BIODEGRADATION OF TEREPHTHALIC ACID BY *ASPERGILLUS* SP.  
DS03 ISOLATED FROM SYNTHETIC TEXTILE EFFLUENT IN  
SURAT**

**JAMWAL S\* AND SHAH GS**

Department of Biotechnology, Veer Narmad South Gujarat University, Udna Magdalla Road,  
Surat, 395007, India

\*Corresponding Author: Subika Jamwal: E Mail: [subikajamwal@gmail.com](mailto:subikajamwal@gmail.com)

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**ABSTRACT**

Terephthalic acid, a major pollutant in synthetic textile effluents, is hazardous to human health and the environment. Microorganisms isolated from synthetic textile effluent and dumping soil from GIDC, Surat were identified to have TA biodegradation potential based on their ability to use TA as the sole carbon source in MSM media. Isolate DS03 showed the highest TA degrading capacity and was identified as *Aspergillus* sp. DS03 by ITS screening and homology analysis. The effect of pH, temperature, RPM, inoculum volume, and initial TA concentration on degradation potential was investigated in the present study. Orthogonal array (L9) and RSM (central composite design) were used to identify significant parameters and their optimum values. Temperature and pH were identified as significant parameters and optimum values of pH= 8.2 and Temperature = 27.6°C were predicted to give an estimated 98.2 % TA biodegradation. Experimental analysis using predicted values showed more than 95 % biodegradation within 72 hrs for TA concentrations up to 5 %. This study implies that *Aspergillus* sp. DS03 has application in TA bioremediation.

**Keywords:** Terephthalic acid, Biodegradation, *Aspergillus* sp., Synthetic textiles, RSM

**I. INTRODUCTION**

Surat is the hub of synthetic textile processing in India accounting for nearly 25 million meters of processed fabric daily. It is the ideal place to isolate microorganisms

with the potential to reduce the toxicity of textile effluents. Synthetic textile industries are characterized as high water-intensive industries and consume an excessive amount of water for the pre-treatment (de-sizing, scouring-bleaching) and dyeing processes [1]. Wastewater produced after Alkali peeling or caustic treatment used for processing and pre-treatment of polyester fabric contains high concentrations of Terephthalic acid (TA) and its derivatives like sodium terephthalate [2]. TA is on the hazardous chemical list and its discharge is closely monitored by the US EPA [3]. It is a known pollutant with toxic properties that cause bladder cancer and impair renal, liver, and testicular functions [4, 5].

Wastewater treatment in most synthetic textile units involves the precipitation of TA by acidification and then burning the TA releasing large amounts of CO<sub>2</sub> into the atmosphere, which is both cost-intensive and harmful for the environment. Releasing untreated or wastewater with chemically precipitated TA into water streams is an ecological and health hazard [6].

Microbial degradation is a more cost-effective and eco-friendly method of removal of TA from textile industry wastewater. Different microbial genera like *Arthrobacter*, *Bacillus*, *Comamonas*, *Delftia*, *Pseudomonas*, *Rhodococcus*, *Sphingomonas* have shown promising TA biodegradation activity [3, 7]. In the current

study, a fungal species with high TA biodegradation potential was isolated from synthetic textile industry wastewater in Surat. The scope of the work was to optimize parameters to achieve the highest biodegradation potential using statistical methods.

## II. MATERIALS AND METHODS

### a. Media

Bushnell Haas (BH) media was used for isolation and primary screening. Minimal salt (MS) media used for degradation studies was prepared by adding 0.20 g/L Magnesium Sulphate, 0.02 g/L Calcium Chloride, 1 g/L Potassium Dihydrogenphosphate, 1 g/L Dipotassium Hydrogen Phosphate, 1 g/L Ammonium Nitrate, and 0.05 g/L Ferric Chloride to BH media. Analytical-grade chemicals were used. All media were autoclaved at 121°C for 15 min. All experiments were carried out in triplicates.

### b. Isolation of microorganism

Wastewater and soil from textile dumping grounds were collected from a textile processing unit in Gujarat Industrial Development Corporation, Sachin, Surat. The effluent and soil suspension of 1mg/mL were serially diluted (10<sup>-1</sup> to 10<sup>-6</sup>) with Sterile Distilled water (SDW) and the three highest dilutions (10<sup>-4</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup>) were inoculated in flasks containing Bushnell Haas (BH) broth with 1 g/L TA as sole carbon source. All the flasks were incubated

at 27°C for 7 days at 120 rpm. An alkaline pH of 8-8.5 was maintained for all media as TA precipitates at neutral and acidic pH and synthetic textile wastewater is alkaline.

### **c. Screening and Identification of the best isolate**

Microbial broth, from the flasks showing growth, was inoculated in BH agar plates containing 1 g/L TA as the sole carbon source. Colony Forming Units (CFUs) were obtained by the streak plate method. BH agar plates containing TA have a thick haze appearance and clear zones around CFUs indicate high TA biodegradation potential. Isolates forming clear zones were further inoculated in BH broth with 1 % TA as the sole carbon source and incubated at 27°C for 72 hrs under shaking conditions at 120 rpm. TA biodegradation was analyzed by growth, signifying utilization of sole carbon source, and Spectrophotometric analysis.

The isolates showing the highest TA degradation were selected for further studies. The isolate chosen for this study was identified as *Aspergillus* sp. based on morphological characteristics and microbial identification using the ITS gene.

### **d. Effect of pH, Temperature, RPM, inoculum volume, and initial concentration**

MS broth, containing different TA concentrations (1 %, 2 %, 5 %, 10 %, and 15 %) as the sole carbon source and pH 8 was

inoculated with 1 % inoculum and incubated at 27°C and 120 rpm for 120 hrs. All the flasks were analyzed for growth and TA biodegradation. Further, the effects of inoculum volume (1 %, 2 %, 5 %, and 10 %), pH levels (6, 7, 8, 9, and 10), Temperature (25°C, 27°C, 30°C, and 37°C), and Rotations per minute (RPM) (static, 50, 120, 150 and 200) were observed by the classical method of varying one parameter and keeping the others constant. 1% of seed culture was inoculated in MS broth with 1 g/L TA, pH 8 at 27°C and 120 rpm for all flasks other than the ones where the effect of the parameter was observed. All the flasks were analyzed for growth and TA biodegradation by spectrophotometric analysis. The experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL broth. Readings for the effect of pH, RPM, Temperature, initial TA concentration, and inoculum volume were taken after 72 hrs. All the experiments were carried out in triplicates.

### **e. Parameter Optimization by statistical analysis**

Optimization of parameters was done using two statistical approaches by the Orthogonal test design (L<sub>9</sub>) and Response Surface Methods (RSM) using the MINITAB software. For orthogonal design, three variables with three levels were analyzed and nine experiments were performed to identify significant variables

and their effect on the result. **Table 1** shows the list of variables with low and high level values and the array design can be seen in **Table 2**. All of the experiments were

performed in triplicates in MS media containing 1 % TA and TA biodegradation was analysed after 72 hrs.

**Table 1: Factors and Levels for Orthogonal Design**

Factor	Level		
	1	2	3
pH	6	8	9
Temp.	25	27	30
RPM	50	100	120

**Table 2: Orthogonal array design**

Run	pH	Temp. (°C)	RPM	% TA Degradation
1	6	25	50	72.1
2	6	27	100	77.6
3	6	30	120	71.3
4	8	25	100	95.4
5	8	27	120	89.4
6	8	30	50	78.3
7	9	25	120	95.1
8	9	27	50	82.2
9	9	30	100	84.3

Optimization of pH, RPM and Temperature and their interactions and effect on TA biodegradation were investigated by RSM (Central Composite Design) using MINITAB. RSM helps to identify the optimum values of the significant parameters and effect of their interaction on TA biodegradation. The CCD was a two

level full factorial and consisted of 13 base runs, 4 cube points, 5 centre points in cube, 4 axial points and  $\alpha = 1.41421$ . **Table 3** shows the design of the experiment. All of the experiments were performed in triplicates in MS media containing 1 % TA and TA biodegradation was analysed after 72 hrs.

**Table 3: Experimental Plan for TA Biodegradation using Response Surface Methodology**

Runs	pH	Temp	% TA Biodegradation
1	7.00000	31.0355	78.3
2	7.00000	27.5000	92.4
3	7.00000	27.5000	93.1
4	8.41421	27.5000	95.6
5	5.58579	27.5000	76.3
6	6.00000	25.0000	73.4
7	7.00000	27.5000	92.2
8	8.00000	30.0000	92.6
9	7.00000	27.5000	96.8
10	7.00000	27.5000	94.6
11	6.00000	30.0000	74.1
12	8.00000	25.0000	92.3
13	7.00000	23.9645	68.3

**f. Analytical methods:**

Supernatant obtained after centrifugation of broth at 10,000 rpm for 10 min was analyzed for TA concentration by measuring absorbance at 240 nm using UV 1800 Shimadzu UV-Vis spectrophotometer and plotting against a standard calibration curve [2]. Growth was analyzed by cell concentration and dry weight.

**III. RESULTS AND DISCUSSION**

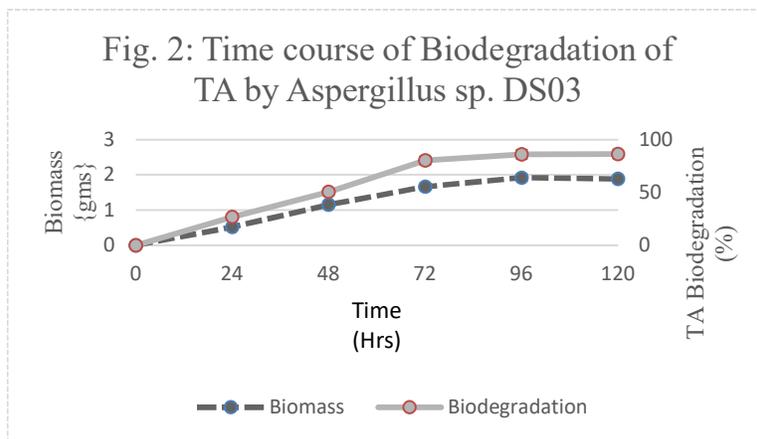
**a. Isolation and Identification**

2 isolates (EF02, EF03) from effluent and 1 (DS03) from dumping ground soil showed presence of clear zones in BHA medium containing 1 % TA. DS03 showed the highest degradation potential (85.3 % in

72 hrs.) and was selected for the purpose of this study. DS03 showed high similarity with *Aspergillus sp.* based on nucleotide homology and phylogenetic analysis (**Figure 1**). *Aspergillus sp.* is novel for biodegradation of Terephthalic acid. Copolymers of Terephthalic acid and Phthalate esters have been reported to be degraded by *Aspergillus sp.* previously [10, 11]. The strain (*Aspergillus sp.* DS03) was selected for further investigation. **Figure 2** shows the time course of Biodegradation of Terephthalic acid by *Aspergillus sp.* DS03. It can be clearly seen that the organisms uses TA as the primary carbon source and the biodegradation of TA is growth associated.



**Sample-S – Isolate DS03**  
**Figure 1: A neighbour joining analysis tree of the Sample S = isolate DS03**



**Figure 2: Time course of Biodegradation of TA by *Aspergillus sp.* DS03**

**b. Effect of pH, Temperature, RPM, inoculum volume and initial concentration on TA biodegradation.**

The effect of initial TA concentration on the biodegradation capacity of *Aspergillus* sp. DS03 is shown in **Figure 3**. 80.4 % and 82 % of TA is biodegraded within 72 hrs with 1 % and 2 % initial concentration respectively. The biodegradation rate slows down after that. The degradation rates do not show marked difference between 1 % and 2 % initial concentrations. Decrease in degradation rate is seen with increase in initial TA concentration with rates decreasing by half at 10 % initial concentration. Concentrations higher than 10 % were inhibitory to growth as well as biodegradation. A high initial TA concentration has been reported to have inhibitory effects on growth and biodegradation of TA [8, 9]. *Aspergillus* sp. DS03 shows higher tolerance to increased initial TA concentration and a good rate of degradation till 5 % and even at higher concentrations like 10 % appreciable degradation is seen.

**Figure 4** highlights the effect of temperature on the degradation potential of *Aspergillus* sp. DS03. Marginal difference is observed in degradation potential at 25<sup>0</sup>C and 27<sup>0</sup>C with gradual decrease from 30<sup>0</sup>C to 37<sup>0</sup>C. Highest potential is seen at 27<sup>0</sup>C.

Increase in biodegradation of TA is observed with increasing RPM as seen in **Figure 5**. *Aspergillus* sp. DS03 is an aerobic strain and shows highest TA biodegradation of 81 % at 150 rpm which decreases at 200 rpm. Very low degradation is seen at static and low rpm suggesting that oxygen availability plays an important role in degradation and it is growth associated.

**Figure 6** shows that Inoculum size has a positive correlation with TA biodegradation by *Aspergillus* sp. DS03 and highest degradation is seen with maximum inoculum of 10 %. As TA is the sole carbon source and its biodegradation is growth associated this observation is along expected lines.

The effect of pH on TA biodegradation by *Aspergillus* sp. DS03 can be seen in **Figure 7**. Neutral to alkaline pH are optimum for TA biodegradation and the highest degradation values are seen at pH 8. *Aspergillus* is known to be tolerant towards alkalophilic environments and synthetic textile effluents are alkaline. Degradation levels drop at both ends of the pH spectrum and are highest at pH 8. *Arthrobacter* sp., *Pseudomonas* sp., and *Rhodococcus biphenylivorans* have been reported to have optimum pH values between 6 to 8 for degradation of TA [3, 8, 9].

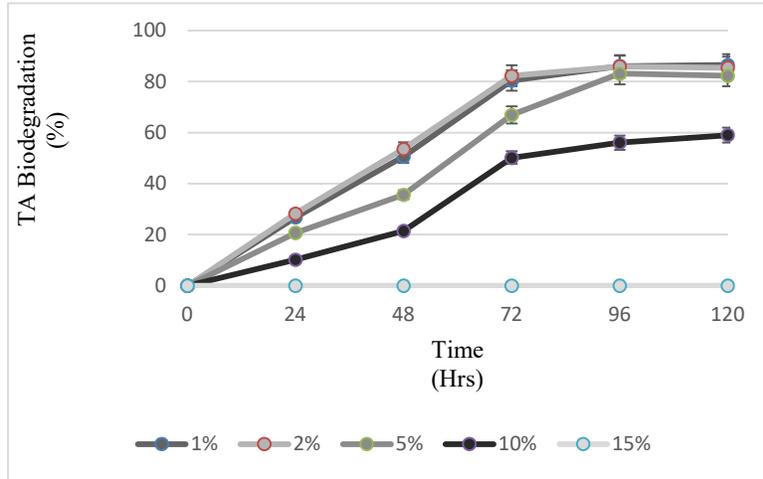


Figure 3: Time Course of TA Biodegradation by *Aspergillus* sp. DS03 for different initial concentrations

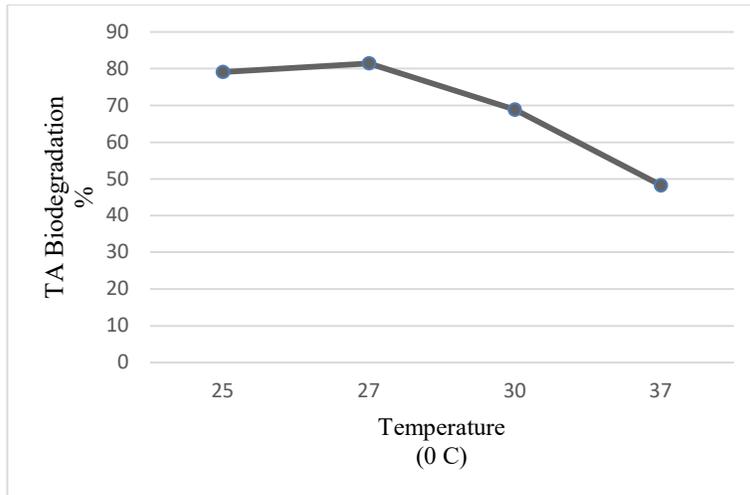


Figure 4: Effect of Temperature on TA Biodegradation by *Aspergillus* sp. DS03

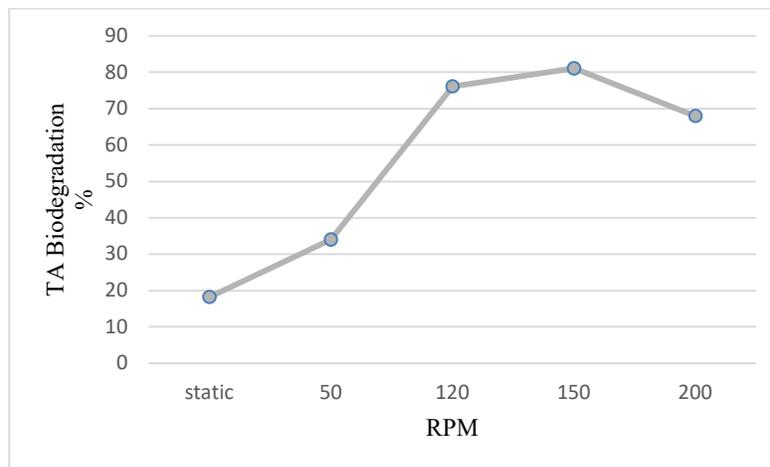


Figure 5: Effect of RPM on TA Biodegradation by *Aspergillus* sp. DS03

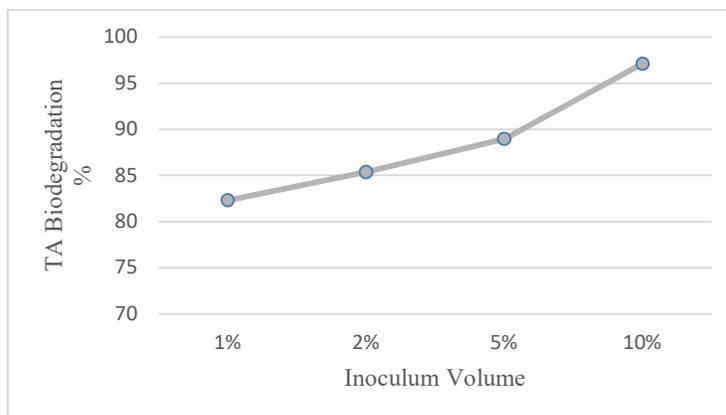


Figure 6: Effect of Inoculum Volume on TA Biodegradation by *Aspergillus* sp. DS03

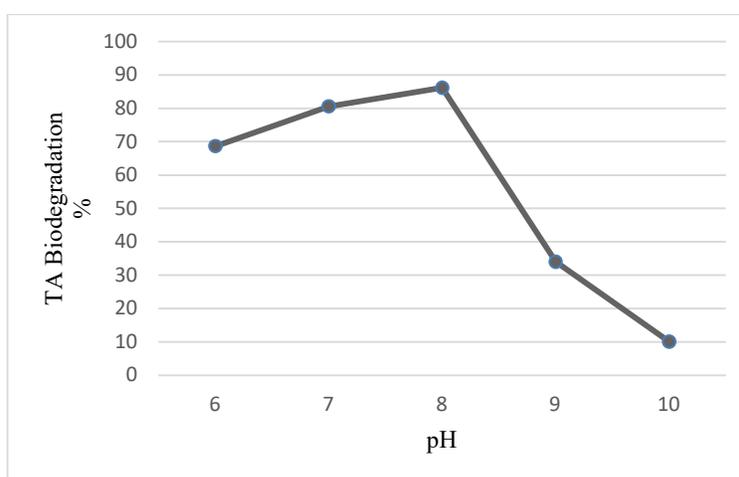


Figure 7: Effect of pH on TA Biodegradation by *Aspergillus* sp. DS03

### c. Parameter Optimization by Statistical analysis

The Orthogonal Test (L9) was performed to identify the significant variables for TA biodegradation using three parameters (pH, temperature, and rpm) at three levels. Various analyses were done to determine the effect of factors on TA biodegradation. **Table 4** shows the Response table of the Orthogonal Test (L9) of the strain on TA biodegradation after 72 hrs. A high delta value implies that the factor has a strong effect on the result. The ranking

of order in which the three parameters affect TA biodegradability was pH > Temp > RPM > as shown in **Table 4**. Regression analysis was done to identify the relation between dependent (TA degradation) and independent variables (pH, Temperature, RPM) and their significance is shown in **Table 5**. The higher the F value and the lower the P value, the greater the significance of the parameter. pH is the most significant parameter for TA biodegradation by the selected strain in a medium containing 1 % TA after 72 hrs. The

regression equation after the orthogonal test correlating factors and response is TA biodegradation = 86.6 + 4.862 a - 1.896 b + 0.1209 c, where a = pH, b = Temperature, and c = RPM. R squared and R adjusted are in reasonable agreement with each other implying that there is a significant correlation between variables and response.

Central Composite design was used to optimize the significant parameters at two levels (pH = 6-8 and Temperature = 25<sup>0</sup>C – 30<sup>0</sup>C). The statistical significance of the results of CCD was evaluated using variance analysis as shown in **Figure 6**. The model is significant as the p- value is ≤ α (1.41421). As R squared is ≥ 90, the model fits more than 90 % of the variables. The R squared and adjusted R squared values are also in reasonable agreement implying the

significance of the model. The 3D response surface and 2D contour plots are shown in **Figures 8 and 9** respectively. The elliptical nature of the 3D response surface shows mutual interactions of the variables and its effect on the response (TA biodegradation). Each contour curve in **Figure 9** represents various combinations of values of pH and Temperature, with the highest result being predicted for the values lying in the smallest ellipse in the center. The optimum values predicted by the method are pH= 8.2 and Temperature = 27.6 °C to give more than 98 % TA biodegradation as shown in **Table 7**. Experimental analysis carried out using predicted values showed more than 95 % biodegradation within 72 hrs for TA concentrations up to 5 %.

**Table 4: Response table of means Orthogonal Test (L<sub>9</sub>) of the strain on TA biodegradation after 72 hrs**

Level	R(pH)	R(Temp)	R(RPM)
1	73.70	87.57	77.57
2	87.77	83.10	85.80
3	87.20	78.00	85.30
Delta	14.07	9.57	8.23
Rank	1	2	3

**Table 5: Analysis of variance of Orthogonal Test (L<sub>9</sub>) of the strain on TA biodegradation after 72 hrs**

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Regression	3	581.61	193.87	14.11	0.007	Significant
pH	1	330.93	330.93	24.08	0.004	
Temp	1	136.67	136.67	9.94	0.025	
RPM	1	114.01	114.01	8.30	0.035	
Error	5	68.72	13.74			
Total	8	650.33				
<b>Model Summary</b>						
	S	R-sq	R-sq (adj)	R-sq (pred)		
	3.70716	89.43%	83.09%	62.98%		

**Table 6: Analysis of Variance for %TA biodegradation for central composite design**

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Model	5	1168.19	233.638	23.33	0.000	Significant
Linear	2	538.58	269.292	26.88	0.001	
pH	1	509.92	509.923	50.91	0.000	
Temp	1	28.66	28.661	2.86	0.135	
Square	2	629.36	314.679	31.42	0.000	

pH*pH	1	65.78	65.778	6.57	0.037
Temp*Temp	1	604.91	604.909	60.39	0.000
2-Way Interaction	1	0.25	0.250	0.02	0.879
pH*Temp	1	0.25	0.250	0.02	0.879
Error	7	70.12	10.017		
Lack-of-Fit	3	58.92	19.639	7.01	0.045
Pure Error	4	11.20	2.800		
Total	12	1238.31			
<b>Model Summary</b>					
	S	R sq	R-sq (adj)	R-sq (pred)	
	3.16491	94.34%	90.29%	64.75%	

Table 7: Solution predicted by Central composite method for optimum TA biodegradation

Solution	pH	Temp	TA Biodegradation Fit	Composite Desirability
1	8.29	27.67	98.6465	1

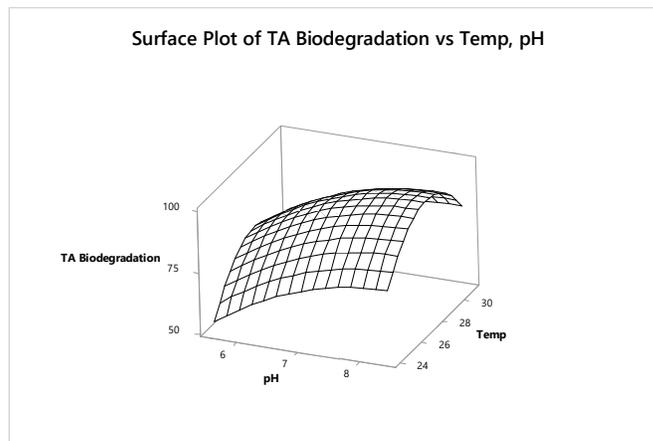


Figure 8: 3D RSM plots for TA biodegradation for optimization of pH and Temp.

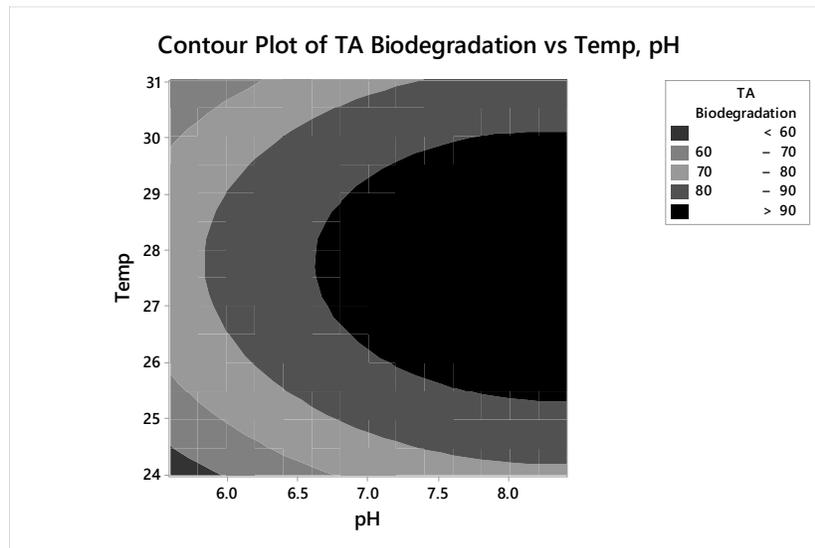


Figure 9: 2D contour plots for TA biodegradation for optimization of pH and Temp.

**IV. CONCLUSION**

*Aspergillus* sp. DS03 was identified as a potentially viable fungal strain for

microbial biodegradation of TA up-to 10 % TA concentrations. The effect of different physical parameters like pH, temperature,

RPM and inoculum volume and initial TA concentrations was investigated and two types of statistical analysis were used to arrive at the optimum values of the significant parameters (pH=8.2 and Temp.=27.6 °C). TA is a pollutant and *Aspergillus* sp. DS03 can be used to significantly lower its concentrations in textile effluents. Biodegradation potential of *Aspergillus* sp. DS03 on other pollutants found in synthetic textile effluents can be further investigated.

#### V. ACKNOWLEDGEMENT

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#### VI. REFERENCES

- [1] Sivaraj, R., Dorthy, C. A. M., & Venckatesh, R., Isolation, characterization and growth kinetics of bacteria metabolizing textile effluent. *Journal of Bioscience and Technology*, 2(4), 2011, 324–330.
- [2] Kimura, T., & Ito, Y., Two bacterial mixed culture systems suitable for degrading terephthalate in wastewater. *Journal of Bioscience and Bioengineering*, 91(4), 2001, 2001416–418.
- [3] Suwanawat, N., Parakulsuksatid, P., Nitayapat, N., Sanpamongkolchai, W., Biodegradation of Terephthalic Acid by *Rhodococcus biphenylivorans* isolated from Soil. *International Journal of Environmental Science and Development*, 10 (1), 2019, 30-33
- [4] Dai, G., Cui, L., Song, L., Gong, N., Chen, J., Zhao, R., Wang, S., Chang, H. C., & Wang, X, Terephthalic acid occupational exposure and its effect on organ functions in fiber workers. *Environmental Toxicology and Pharmacology*, 20(1), 2005, 209–214.
- [5] Zhang, Z., Ma, L., Zhang, X.-X., Li, W., Zhang, Y., Wu, B., Yang, L., & Cheng, S., Genomic expression profiles in liver of mice exposed to purified terephthalic acid manufacturing wastewater. *Journal of Hazardous Materials*, 181(1–3), 2020, 1121–1126.
- [6] Sugimori, D., Dake, T., & Nakamura., Microbial degradation of disodium terephthalate by alkaliphilic *Dietzia* sp. Strain GS-1. *Bioscience, Biotechnology, and Biochemistry*, 64(12), 2000, 2709–2711.
- [7] Ordaz-Cortés, A., Thalasso, F., Salgado-Manjarrez, E., & Garibay-Orijel, C., Treatment of wastewater containing high concentrations of terephthalic acid by *Comamonas* sp.

- And *Rhodococcus sp.*: Kinetic and stoichiometric characterization. *Water and Environment Journal*, 28(3), 2014, 393–400.
- [8] Wang, Z.-J., Teng, L., Zhang, J., Huang, X.-L., & Zhang, J.-F., Study on optimal biodegradation of terephthalic acid by an isolated *Pseudomonas sp.* *African Journal of Biotechnology*, 10(16), 2011, 3143–3148.
- [9] Zhang, Y.-M., Sun, Y.-Q., Wang, Z.-J., & Zhang, J., Degradation of terephthalic acid by a newly isolated strain of *Arthrobacter sp.* 0574. *South African Journal of Science*, 109(7), 2013, 1–4.
- [10] Nimchua, T., Punnapayak, H., & Zimmermann, W. (2007). Comparison of the hydrolysis of polyethylene terephthalate fibers by a hydrolase from *Fusarium oxysporum* LCH I and *Fusarium solani f. Sp. Pisi*. *Biotechnology Journal: Healthcare Nutrition Technology*, 2(3), 2007, 361–364.
- [11] Soni, R. K., Soam, S., & Dutt, K. Studies on biodegradability of copolymers of lactic acid, terephthalic acid and ethylene glycol. *Polymer Degradation and Stability*, 94(3), 2009, 432–437.