

OPTIMIZATION OF BIOLOGICAL TREATMENT OF DAIRY EFFLUENT USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

The current and unexpected growth of the world's population warrants an increased production of high quality animal protein. With this increasing demand for dairy, there is growing pressure on natural resources, including freshwater and soil. Dairy industry generates strong wastewater characterized by high biological oxygen demand (BOD) and chemical oxygen demand (COD) due to their high organic content. With increasing social awareness about the environment, the dairy industry is forced to treat its effluent effectively and efficiently before disposal into the public drainage. Large amount of water used in dairy industry results in significant generation of effluent volume; hence the challenge of its disposal cannot be ignored. The present study was conducted to analyze the microbial properties of the effluent from Dairy Plant and biological treatment of dairy effluent using *Kluyveromyces marxianus* and optimization of various conditions using response surface methodology (RSM). It was observed that bacteria, yeast and fungal count was 3.9×10^6 cfu/ml, 5.3×10^5 cfu/ml and 4.5×10^4 cfu/ml respectively and also the RSM model was significant and four factors interact with each other positively. Comparison between actual and experimental value indicated there was conformation between predicted and experimental data.

INTRODUCTION

Milk production takes place all around the world. Global demand for dairy continues to increase in large part due to population growth, rising incomes, urbanization and westernization of diets in countries such as India and China. With this increasing demand for dairy there is a growing pressure on freshwater resources. WWF works with dairy farmers, industry groups and other stakeholders in various countries to conserve and protect natural resources. Dairy industries have shown tremendous growth in size and number in most countries of the world. Large quantity of wastewater produced from dairy industrial operations. The organic substance in wastes comes directly in same form or in degraded form. Sources of dairy wastewater are from receiving stations, boiling plant, cheese plant, butter and dried milk plant as well as from can washing plant. Dairy wastewater is organic in nature so when dairy wastewater directly discharged into river or stream then deficiency in oxygen level may get occur. Dairy

effluent has high levels of chemical oxygen demand, biological oxygen demand, oil and grease, nitrogen and phosphorous content (Braio and Taveres, 2007).

Disposal of untreated water is rapidly becoming a major economic and societal problem faced by dairy industry in many respects. In India dairy industry is one of the leading food industry. Total milk production in India was 146.3 million tones (2014-2015) and per capita availability of milk was 322 gm/day (Anonymous, 2014). The liquid waste influences the choice of the wastewater treatment, as specific biological systems have difficulties dealing with wastewater of varying organic loads. Majority of the wastewater treatment processes are multi-variable and optimization through the classical method is inflexible, unreliable and time-consuming. Optimization plays a key role in environmental engineering parameters to optimize the system's performance. Thus, an alternative method which will be more effective and can be adapted for parameter optimization of various wastewater

treatment processes is favored. Response surface methodology (RSM) is one of the most efficient and widely used mathematical and statistical tools for system performance optimization.

RSM was introduced in year 1951 by Box and Wilson using a second-degree polynomial model (Box and Wilson, 1951). RSM can be employed to optimize and analyze the effects of several independent factors on a treatment process to obtain the maximum output. The appraisal indicated that the usage of RSM in wastewater treatment process optimization, ultimately contributes to removal efficiency enhancement and operation cost reduction. Keeping all these points in view present study focused on to analyze the microbial properties and biological treatment of the effluent using *Kluyveromyces marxianus* and then optimization of various conditions using response surface methodology (RSM).

MATERIALS AND METHODS

Collection of sample

The dairy effluent sample required for the experimental purpose was collected from Milk Plant. Before filling, sample bottle was rinsed two or three times with the water being collected and then transported immediately to the laboratory for analysis.

Microbiological analysis

The effluent sample was quantitatively and qualitatively analyzed for their micro flora following serial dilution technique. 1 ml of diluted sample was plated and incubated for taking bacterial, fungal and yeast counts. The bacterial count was taken using Nutrient Agar (NA) having composition Beef extract (3.0 g/l), Peptone (5.0 g/l), Sodium chloride (5.0 g/l), Agar (1.5 g/l) and pH 7.0 and fungal count was taken by plating the samples on Czapek Dox's media having composition Sucrose (30.0 g/l), Sodium nitrate (3.0 g/l), Magnesium sulphate (0.5 g/l), Potassium chloride (0.5 g/l), Ferrous sulphate (0.01 g/l), Potassium hydrogen phosphate (1.0 g/l), Agar (13.0 g/l), pH 7.3 and Yeast count was taken by plating the samples on Malt Extract Agar media having composition Malt extract (3.0 g/l), Yeast extract (3.0 g/l), Peptone (5.0 g/l), Glucose (10.0 g/l), Agar (20.0 g/l) and pH 7.0.

Biological treatment

Kluyveromyces marxianus MTCC 3772 used for the biological treatment of dairy effluent was procured from Institute of microbial technology (IMTECH) Chandigarh. The culture was revived in Malt yeast

agar and incubates for 48 h at 25°C. For further use Malt yeast agar slants were made and stored at 4°C. Slants were sub cultured after every 30 days.

To optimize the biological conditions RSM DESIGN EXPERT 8.0.2 was used. RSM is the response surface methodology, explores the relationships between several explanatory variables and one or more response variables. RSM can be used to evaluate the relative significance of several affecting factors even in the presence of complex interactions (Hounsa, *et al.*, 1996).

Statistical analysis was performed by design of experiments (DOE) with Design-Expert software. Design Expert is a program for design of experiments, statistical analysis, modeling and optimization. It offers a range of programs including full factorial and fractional factorial designs, response surface method, mixing and D-optimal designs. The Design-Expert was used to develop the experimental plan for RSM (Anonymous, 2006). The same software was also used to analyze the data collected. Design-Expert provided rotatable 3D plots to visualize the response surface. The design was made using four variables namely:

Factor 1 (A): pH

Factor 2 (B): Cell count (cells/ml)

Factor 3 (C): Aeration (rpm)

Factor 4 (D): Time period (h)

Three responses were observed namely:

Response 1: COD (mg/ml)

Response 2: Cell count (cells/ml)

Response 3: PH

RSM was used to determine the interactive effect of these variables on the response. Level of each variable was chosen and a Central Composite Experimental Design and Quadratic mode was used for the four selected factors believed to influence the process and analysis was done by polynomial equation. Design expert software provided a list of 30 experiments given in Table 1.

RESULTS AND DISCUSSION

Microbiological analysis

The effluent sample analyzed for the micro flora included bacteria, yeast and fungi and the result obtained is given in Table 2. The bacteria, yeast and fungal count was 3.9×10^6 cfu/ml, 5.3×10^5 cfu/ml and 4.5×10^4 cfu/ml respectively. Count of bacteria was higher than yeast and fungi. This is due to the

Table 1. List of experiments designed by design expert 8.0.2

Std.	Run	Factor 1 pH	Factor 2 Cell count cells/ml	Factor 3 Aeration (rpm)	Factor 4 (Time per h)	Response 1 COD (mg/l)	Response 2 Cell count (cells/ml)	Response 3 pH
21	1	6.00	5.5×10^6	100	72.00	--	--	--
25	2	6.00	5.5×10^6	100	72.00	--	--	--
3	3	2.00	10×10^6	0	24.00	--	--	--
10	4	10.00	1×10^6	0	120.00	--	--	--
17	5	2.00	5.5×10^6	100	72.00	--	--	--
7	6	2.00	10×10^6	200	24.00	--	--	--
29	7	6.00	5.5×10^6	100	72.00	--	--	--
1	8	2.00	1×10^6	0	24.00	--	--	--
6	9	10.00	1×10^6	200	24.00	--	--	--
14	10	10.00	1×10^6	200	120.00	--	--	--
2	11	10.00	1×10^6	0	24.00	--	--	--
30	12	6.00	5.5×10^6	100	72.00	--	--	--
20	13	6.00	14.5×10^6	100	72.00	--	--	--
4	14	10.00	10×10^6	0	24.00	--	--	--
19	15	6.00	3.5×10^6	100	72.00	--	--	--
18	16	14.00	5.5×10^6	100	72.00	--	--	--
15	17	2.00	10×10^6	200	120.00	--	--	--
26	18	6.00	5.5×10^6	100	72.00	--	--	--
23	19	6.00	5.5×10^6	100	24.00	--	--	--
24	20	6.00	5.5×10^6	100	168.00	--	--	--
11	21	2.00	10×10^6	0	120.00	--	--	--
27	22	6.00	5.5×10^6	100	72.00	--	--	--
8	23	10.00	10×10^6	200	24.00	--	--	--
9	24	2.00	1×10^6	0	120.00	--	--	--
12	25	10.00	10×10^6	0	120.00	--	--	--
5	26	2.00	1×10^6	200	24.00	--	--	--
22	27	6.00	5.5×10^6	300	72.00	--	--	--
13	28	2.00	1×10^6	200	120.00	--	--	--
16	29	10.00	10×10^6	200	120.00	--	--	--
28	30	6.00	5.5×10^6	100	72.00	--	--	--

Table 2. Microbiological analysis of dairy effluent

S. no	Micro Flora	Count (Cfu/MI)
1.	Bacteria	3.9×10^6
2.	Yeast	5.3×10^5
3.	Fungi	4.5×10^4

aerobic conditions of the effluent that favor the growth of bacterial micro flora. However, yeast and fungal micro flora also grow in dairy effluent due to its acidic pH that ranged between 6.2-7.2 which favor their growth.

Milk contains all the ingredients that most bacteria need for their growth some of the diseases such as dysentery, diarrhea, tuberculosis, typhoid, food poisoning and cholera caused by these bacteria (Hashmi, *et al.*, 2004).

Optimization of conditions using RSM

This design was constructed for four variables as pH, cell count, aeration and time and three responses as

COD, cell count and pH and the result obtained by RSM is given in Table 3.

Analysis of Variance (ANOVA)

A wide range of statistical and diagnostic studies are obtainable from RSM. The Analysis of Variance (ANOVA) including sequential F-test, lack-of-fit test and other adequacy measures. ANOVA could also verify the efficiency of the developed model. ANOVA for response surface reduced quadratic model and polynomial equation is summarized in Table 4 for response 1 (COD), the analysis implies that model is significant and the four variables interact with each other positively. Value of Prob > F

Table 3. RSM design expert 8.0.2 to optimize treatment conditions

Std.	Run	Factor 1 (pH)	Factor 2 Cell count (cells/ml)	Factor 3 Aeration (rpm)	Factor 4 (Time per h)	Response 1 COD (mg/l)	Response 2 Cell count (cells/ml)	Response 3 (pH)
21	1	6.00	5.5×10^6	100	72.00	4000	4×10^6	4.45
25	2	6.00	5.5×10^6	100	72.00	2400	4×10^6	7.44
3	3	2.00	10×10^6	0	24.00	6000	0	1.92
10	4	10.00	1×10^6	0	120.00	2400	0	8.12
17	5	2.00	5.5×10^6	100	72.00	4800	4×10^6	2.00
7	6	2.00	10×10^6	200	24.00	4000	0	3.36
29	7	6.00	5.5×10^6	100	72.00	2400	4×10^6	7.44
1	8	2.00	1×10^6	0	24.00	6200	0	1.90
6	9	10.00	1×10^6	200	24.00	3200	0	5.78
14	10	10.00	1×10^6	200	120.00	2800	0	9.32
2	11	10.00	1×10^6	0	24.00	6000	0	4.43
30	12	6.00	5.5×10^6	100	72.00	2400	4×10^6	7.44
20	13	6.00	14.5×10^6	100	72.00	1600	12×10^6	7.88
4	14	10.00	10×10^6	0	24.00	5600	8×10^6	4.33
19	15	6.00	3.5×10^6	100	72.00	3200	4×10^6	7.86
18	16	14.00	5.5×10^6	100	72.00	4000	0	9.05
15	17	2.00	10×10^6	200	120.00	3600	0	3.45
26	18	6.00	5.5×10^6	100	72.00	2400	4×10^6	7.44
23	19	6.00	5.5×10^6	100	24.00	3600	4×10^6	7.82
24	20	6.00	5.5×10^6	100	168.00	1600	0	9.00
11	21	2.00	10×10^6	0	120.00	5600	0	1.82
27	22	6.00	5.5×10^6	100	72.00	2400	4×10^6	7.44
8	23	10.00	10×10^6	200	24.00	2400	8×10^6	6.67
9	24	2.00	1×10^6	0	120.00	5600	0	1.74
12	25	10.00	10×10^6	0	120.00	1760	4×10^6	8.29
5	26	2.00	1×10^6	200	24.00	4800	0	3.40
22	27	6.00	5.5×10^6	300	72.00	3200	4×10^6	8.49
13	28	2.00	1×10^6	200	120.00	3600	0	3.48
16	29	10.00	10×10^6	200	120.00	1600	4×10^6	9.24
28	30	6.00	5.5×10^6	100	72.00	2400	4×10^6	7.44

Table 4. ANOVA for response surface reduced quadratic model for COD

Source	Sum of squares	df	Mean Square	F Value	P value Prob > F
Model	5.532E+007	8	6.915E+006	20.49	0.0001
A-pH	1.497E+007	1	1.497E+007	44.37	0.0001
B-Cell count	2.041E+006	1	2.041E+006	6.05	0.227
C-Aeration	1.181E+007	1	1.181E+007	35.00	0.0001
D-Time period	9.745E+006	1	9.745E+006	28.87	0.0001
AD	2.280E+006	1	2.280E+006	6.76	0.0167
CD	1.988E+006	1	1.988E+006	5.89	0.0243
A ²	1.044E+007	1	1.044E+007	30.93	0.0001
C ²	4.885E+006	1	4.885E+006	14.47	0.0010
Residual	7.088E+006	21	3.375E+005	--	--
Lack of Fit	4.588E+006	15	3.059E+005	0.73	0.7083
Pure Error	2.499E+006	6	4.165E+005	--	--
C or Total	6.241E+007	29	--	--	--

less than 0.0500 indicate model terms are significant. In this case A-pH, C-Aeration and D-Time period are significant model terms. Value greater than 0.0500 indicate the model terms are not significant. For response 1, B- Cell count is non-significant model

term having value 0.227. The Model F-value of 20.49 implies the model is significant. Values of Prob > F less than 0.0500 indicate model terms are significant. In this case A, B, C, D, AD, CD, A², C² are significant model terms. Lack of fit of 0.73 implies it is not

significant. Non-significant lack of fit is good because we want the model to be fit. Final equation in terms of coded factors: $COD = +2693.64 - 896.18 * A - 319.46 * B - 828.88 * C - 682.55 * D - 377.50 * A * D + 352.50 * C * D + 812.31 * A^2 + 553.79 * C^2$

For response 2 (cell count) (Table 5), significant model terms are A-pH and B-Cell count. The Prob > F value for model term D-Time period is 0.6985, implies that it is non-significant model term for response 2. The Model F-value of 57.82 implies the model is significant. Values of Prob > F less than 0.0500 indicate model terms are significant. In this case A, B, AB, A², B² and D² are significant model terms. Lack of fit of 633.51 implies it is significant. Significant lack of fit is bad because we want the model to be fit. Final equation in terms of coded factors: $Log_{10}(\text{Cell count} + 0.10) = +7.09 + 1.65 * A + 2.00 * B - 0.092 * D + 1.94 * A * B - 2.77 * A^2 - 1.16 * B^2 - 2.05 * D^2$

For response 3 (pH) (Table 6), the model value 0.0001 implies that model is significant and the significant model terms are A-pH, C-Aeration and D-Time period. The Model F-value of 26.25 implies the model is significant. Values of Prob > F less than 0.0500 indicate model terms are significant. In this case A, C, D, AD, A² are significant model terms. Lack of fit of 0.91 implies it is non-significant. Non-significant

lack of fit is good because we want the model to be fit. Final equation in terms of coded factors: $pH = +6.70 + 2.56 * A + 0.79 * C + 0.82 * D + 0.87 * A * D - 1.31 * A^2$

Response surface plots for three responses based on final model

The fitted response surfaces plots for COD, pH and cell count based on the final model were obtained by holding two variables at their optimum level while varying the other two within their experimental range. The graphical representation provides a method to visualize the relation between the response and experimental levels of each variable. The effect of significant variables pH, Time period and Aeration on Response 1 (COD) shown in (Fig. 1 and 2) by keeping two variables at constant value and altering other two variables and observed that for response 1, interaction between Time period and pH is more significant than interaction between Time period and aeration.

Response surface graph for Response 2 (cell count) shown in (Fig. 3) and observed that at constant aeration and time period, the interaction between two factors pH and cell count is more for response 2.

Response surface plot for Response 3 (pH) shown in

Table 5. ANOVA for response surface reduced quadratic model for cell count

Source	Sum of squares	df	Mean Square	F Value	P value Prob > F
Model	416.06	7	59.44	57.82	0.0001
A-pH	50.80	1	50.80	49.42	0.0001
B-Cell count	68.72	1	68.72	66.84	0.0001
D-Time period	0.16	1	0.16	0.15	0.6985
AB	60.10	1	60.10	58.46	0.0001
A ²	121.59	1	121.59	118.27	0.0001
B ²	20.78	1	20.78	20.21	0.0002
D ²	66.67	1	66.67	64.86	0.0001
Residual	22.62	22	1.03	--	--
Lack of Fit	22.60	16	1.41	633.51	0.0001
Pure Error	0.013	6	2.230E-003	--	--
C or Total	438.67	29	--	--	--

Table 6. ANOVA for response surface reduced quadratic model for pH

Source	Sum of squares	df	Mean Square	F Value	P value Prob > F
Model	168.32	5	33.66	26.25	0.0001
A-pH	122.74	1	122.74	95.69	0.0001
C-Aeration	12.20	1	12.20	9.51	0.0051
D-Time period	13.96	1	13.96	10.88	0.0030
AD	11.99	1	11.99	9.35	0.0054
A ²	27.95	1	27.95	21.79	0.0001
Residual	30.78	24	1.28	--	--
Lack of Fit	22.51	18	1.25	0.91	0.6011
Pure Error	8.27	6	1.38	--	--
C or Total	199.11	29	--	--	--

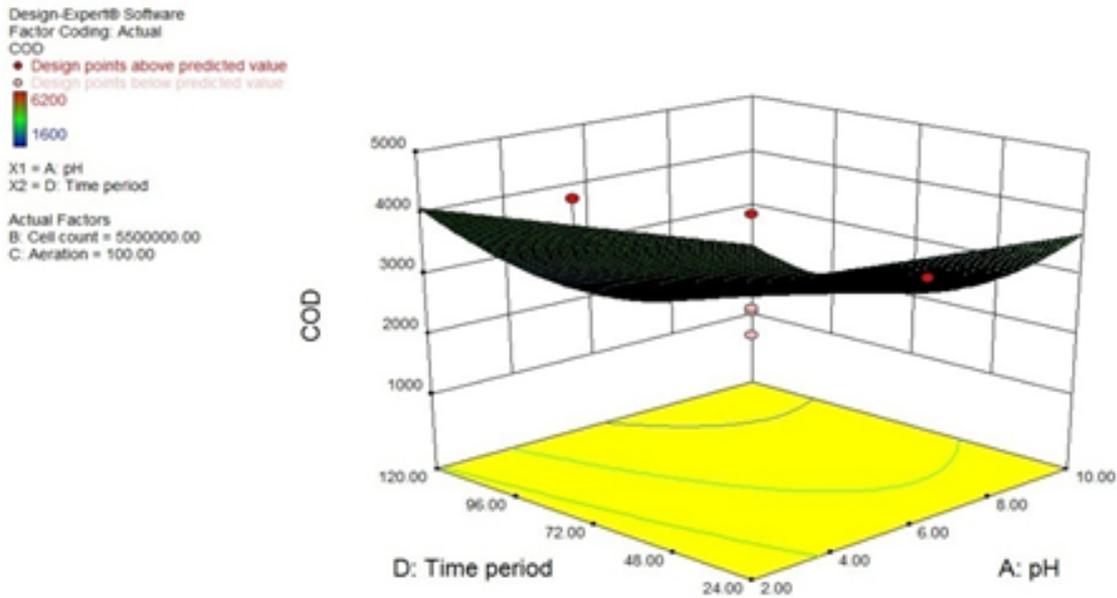


Fig. 1 Response surface plot showing the effect of time period and pH on response 1 COD.

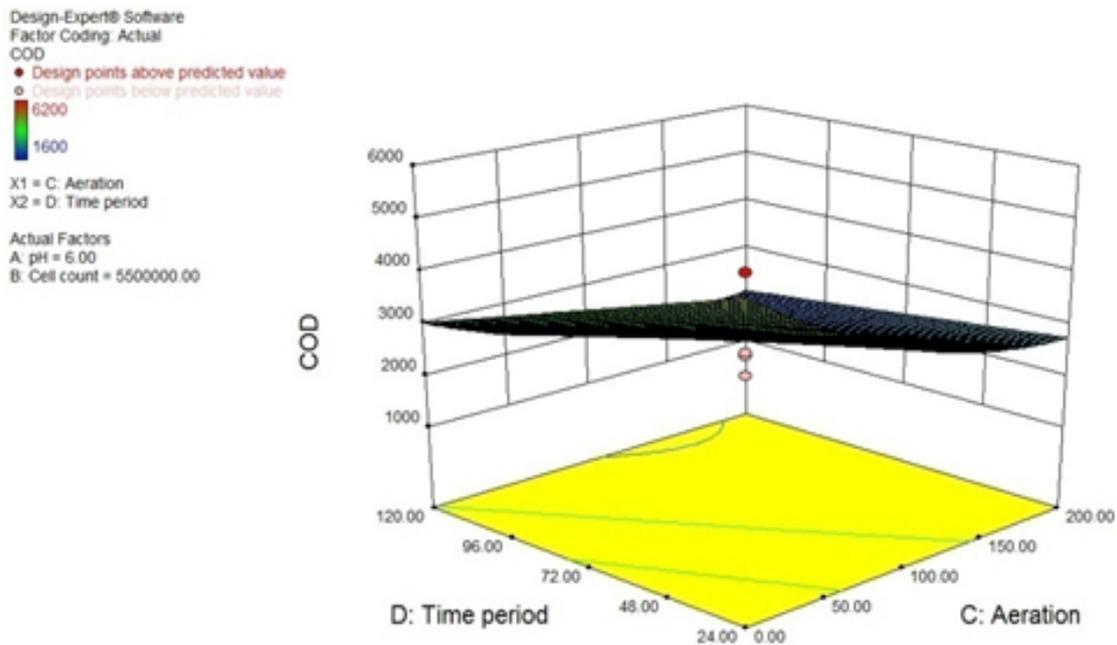


Fig. 2 Response surface plot showing the effect of time period and aeration on response 1 COD.

(Fig. 4) and observed that at constant cell count and aeration, the two factors time period and pH interact with each other.

Validation of the optimized conditions for dairy effluent

RSM gives a large amount of knowledge from a small number of experimental runs. The interaction effect of the independent parameters on the

response can be observed investigated via RSM. The model equation easily clarifies these effects for binary combination of the independent parameters. Moreover, the empirical model that related the response to the independent variables is utilized to obtain information about the process. Optimized conditions for the treatment of dairy effluent as predicted by the final quadratic model along with the corresponding observed values are given in Table 7.

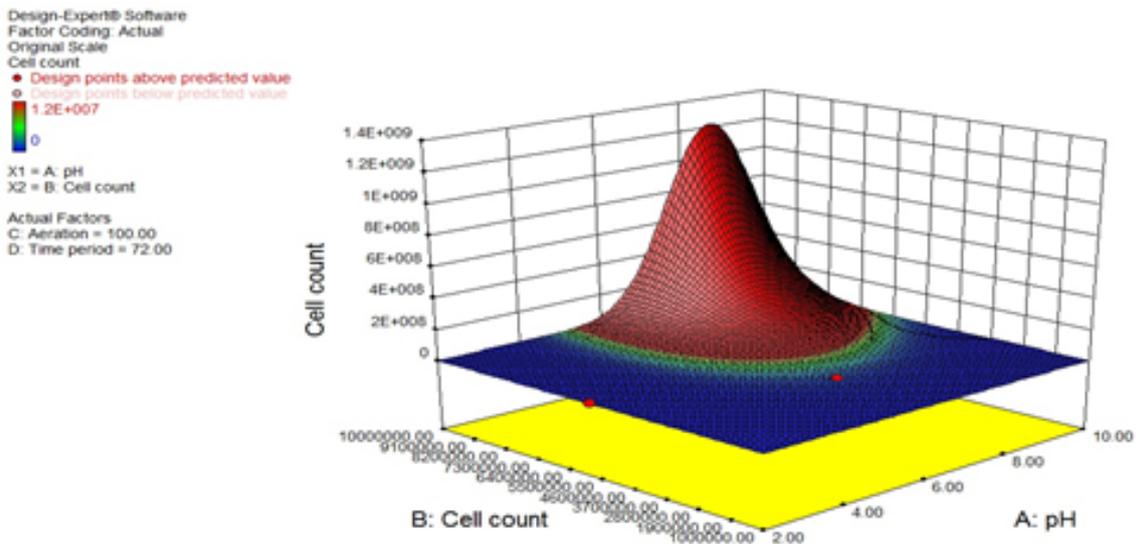


Fig. 3 Response surface plot showing the effect of cell count and pH on response 2 cell count.

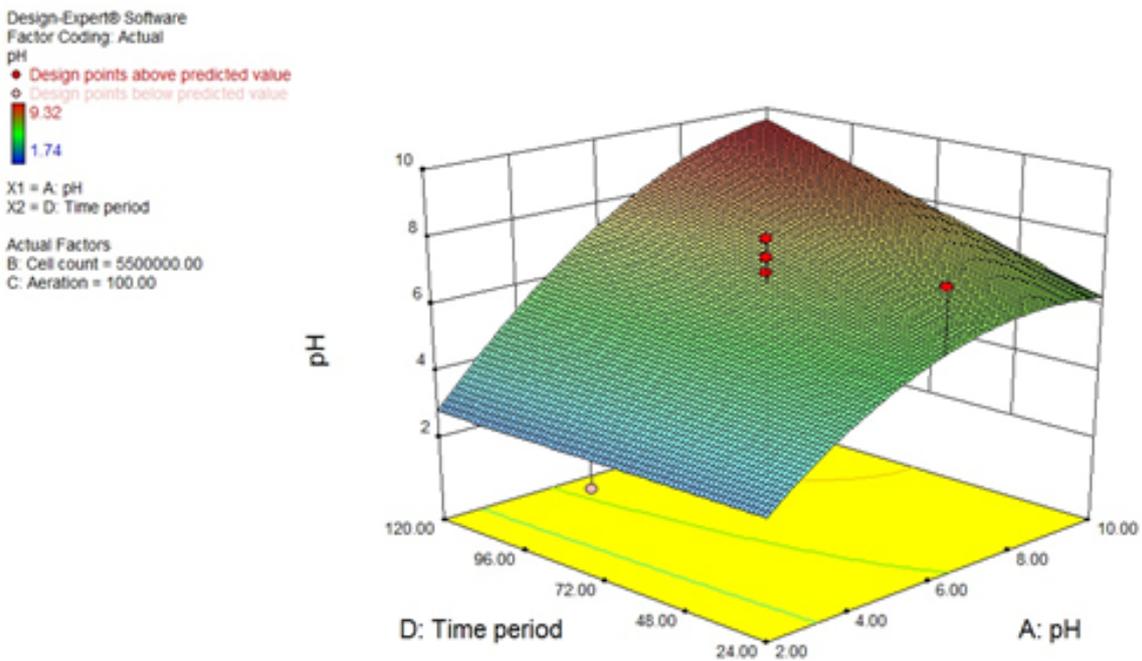


Fig. 4 Response surface plot showing the effect of pH and time period on response 3 pH.

Table 7. Comparison of predicted value and experimental value

S. no	Selected runs				Predicted value			Experimental value		
	pH	Count (cells/ml)	Aeration (rpm)	Time(h)	COD (mg/l)	Count (cells/ml)	pH	COD (mg/l)	Count cells/ml	pH
1.	7.00	10×10^6	199.0	113.18	1582.79	10×10^6	8.90	1600	8×10^6	8.92
2.	7.58	10×10^6	115.49	115.49	1458.23	8×10^6	9.20	1450	8×10^6	9.25

Comparison of experimental and predicted values indicated that there was a conformation between the predicted and experimental data.

CONCLUSION

Dairy waste contains large number of impurities, which contributes towards water pollution. We

analyzed for microbial characteristics and biological treatment was done using *Kluyveromyces marxianus* and then biological conditions was optimized using response surface methodology (RSM). Analysis of Variance (ANOVA) for response surface quadratic model implies that model was significant and four factors interact with each other positively. Validation of the optimized treatment conditions predicted by final quadratic model was done and comparison between actual and experimental value indicated there was conformation between predicted and experimental data. The present study was carried out with the aim to explore, standardize and adopt suitable management techniques to overcome and reuse the valuable natural resources. So, RSM is definitely a useful and accommodating tool for the reducing operation cost that associated with optimization of wastewater treatment processes.

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