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Optimisation of biological wastewater treatment for yeast processing effluent using cultured bacteria: Application of response surface methodology

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In the present study, the interactive effects of temperature and cultured bacteria on the performance of a biological treatment system of wastewater from a local yeast producing plant were investigated. The main objective of this study was to optimize the operating parameters that reduce organic load and colour. Biological treatment was conducted using a Central Composite Design (CCD) and optimised using Response Surface Methodology (RSM) on Design Expert 7 software. Two dependent variables namely, Chemical Oxygen Demand (COD) removal and colour reduction were studied. COD removal efficiency of 26% and decolourization efficiency of 44% were recorded for the wastewater treatment. The optimised conditions for the biological treatment were found to be at 16.37 g/l of bacterial powder at 25°C for COD removal and colour reduction. The optimised parameters can be used for biological treatment of yeast plant effluent for removal of organic load and colour.

Key words: Cultured bacteria, wastewater, treatment, response surface methodology.

INTRODUCTION

The large quantity of aqueous waste generated by domestic activities and industries has become a significant environmental problem worldwide, due to its harmful nature (Thirugnanasambandham and Sivakumar, 2015). The escalating pollution levels in water bodies has been attributed to massive industrialization and booming population densities coupled by escalating urbanization (Chiuta et al., 2002; Moyo and Mtetwa, 2002; Chakona, 2005; Noukeu et al., 2016). Water bodies serve as recipients of untreated or partially untreated industrial wastewater which in turn alters the physical structures of such water sources. To address surface water pollution and protect eco-systems, wastewater needs to be treated in order to contribute to a cleaner environment (Noukeu et al., 2016). Effluent originating from baker's yeast production process leads to extensive soil and water pollution. The yeast processing industries produce very large amounts of wastewater characterized by high

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biological oxygen demand (BOD_5), Chemical Oxygen Demand (COD) and a dark brown color (Christoforakos and Lazaridis, 2018). Wastewater from baker's yeasts industries contain dissolved organic substances, namely dextrins, organic acids, resins, gums, trimethylglycine, coloured melanoidins and phenol substances. It is also characterised by insoluble organic substances or materials in suspension, high concentrations of total nitrogen, variable phosphorus content and sulphate (Sirbu and Begea, 2011). Some of these coloured compounds are melanoidins formed through a process known as Maillard reaction (Peña et al., 2003). Melanoidins impart a characteristic dark brown colour to water which blocks sunlight penetration thereby impairing the ability of aquatic flora to photosynthesize. In combination with other compounds, this quality of wastewater reduces soil alkalinity, with strong and objectionable odour that presents significant disposal or treatment problem (Agarwal et al., 2010). Elimination of pollutants and colour from the effluent is becoming increasingly important from environmental and aesthetic point of view.

Food-processing industries in Zimbabwe are under increasing pressure to reduce the impact of their wastewater discharge on the environment. With the advent of the Statutory Instrument 6 of 2007 in the Environmental Management Act of 2007 (the basic legislation requirement on pollution control in Zimbabwe), companies have been forced to establish pre-treatment facilities on site. Treatment systems are developed to reduce toxicity of effluent and minimize chances of pollution. Biological treatment systems involve the use of activated sludge, commercial seed granules or more recently mixed culture bacterial formulations for wastewater treatment. Since environmental protection has become a global issue, cleaner and greener technologies are warranted to abate industrial pollution (Zeinu and Sahu, 2015). Readily Fermenting Mixed (RFM) cultured bacteria is a ready-to-use bacterial powder formula made of wheat bran grain-like substance as a substrate, and used for accelerating organic wastewater degradation by commercial industries to reduce pollution of well, underground water sources, and sewage pits. However, the efficacy of these bacterial formulations has not been fully optimised in high strength wastewater treatment.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects on the response of several independent variables (Hadavifar et al., 2010). Optimisation aims at enhancing effectiveness in any stage of response in an experiment for long term implementation of change. Optimisation as a tool enables the increase of efficiency in a process while costs of operation are kept low. A suitable value for the concerned variable has to be determined in order to obtain the maximum yield in an experiment. Myers and Montgomery (2012) suggest that RSM can be employed for optimisation of chemical reactions and in other industrial processes. One of the main objectives of RSM is the determination of the optimum settings of the control variables that result in a maximum (or a minimum) response over a certain region of interest (Korbahti and Tanyolac, 2008). Optimisation of biological wastewater treatment by the classical method involves changing any one of the independent variables while maintaining all others at a fixed level which is extremely time consuming and expensive for a large number of variables. To overcome this difficulty, experimental factorial design and response methodology can be employed to optimize the process.

This research was conducted to reduce COD and colour of the wastewater from a yeast producing plant using the RSM. This involved the optimisation of biological treatment of yeast processing wastewater using a cultured bacterial formulation.

METHODOLOGY

Experimental design

A sample of wastewater was collected from a local yeast processing plant. The wastewater was refrigerated at 4°C to minimize auto-decomposition. RSM in this study was done using Design Expert 7 software. A two-factor Central Composite Design (CCD) was used to design the experiments for determining the effect of variables on color and COD. A set of 13 experiments were produced employing CCD on Design Expert 7. Optimisation was used to assess the outcome of two variables; temperature and bacterial concentration on reduction of COD and colour of yeast processing wastewater.

Wastewater treatment

Batch experiments for biological wastewater treatment were performed in 500-ml serum bottles. Serum bottles were seeded with Readily Fermenting Mixed (RFM) cultured bacteria. The powdered bacterial culture was thoroughly mixed and filtered through a screen of pore size 1 mm before use. The selected parameters were adjusted as per the experimental design and run in a reciprocating water bath shaker incubator (ZWY-110X50, Zhicheng, China) at a prescribed temperature range shown in Table 1.

Analyses

COD was analyzed as per the closed reflux colorimetric method (APHA, 1998). In the COD method, the water sample is oxidized by digesting in a sealed reaction tube with sulphuric acid and potassium dichromate in the presence of a silver sulphate catalyst. Digestion of the samples is carried out in a COD reactor (Model HI 839800, HANNA Instruments, USA). COD measurement was carried out on a Multiparameter Bench photometer (Model 83214, HANNA Instruments, USA). The colour of the sample was measured in terms of the absorbance at $\lambda = 475$ nm using a UV–vis spectrophotometer (Sahu, 2017). The percent colour reduction was calculated using Equation 1:

$$\text{Percent decolourisation} = [A_b - A_a / (A_b)] \times 100\% \quad (1)$$

Table 1. Summary of the experimental design in the biological treatment of yeast processing effluent.

Study type:	Response surface
Initial design:	Central composite
Design model:	Quadratic
Experiments:	13

Factor	Low actual (Code)	High Actual (Code)
A: (RFM) bacterial formulation (g/l)	**4 (-1)**	**20 (+1)**
B: Temperature (%)	**25(-1)**	**45 (+1)**

Response	Observations
Y1: COD reduction (%)	**13**
Y2: Colour (%)	**13**

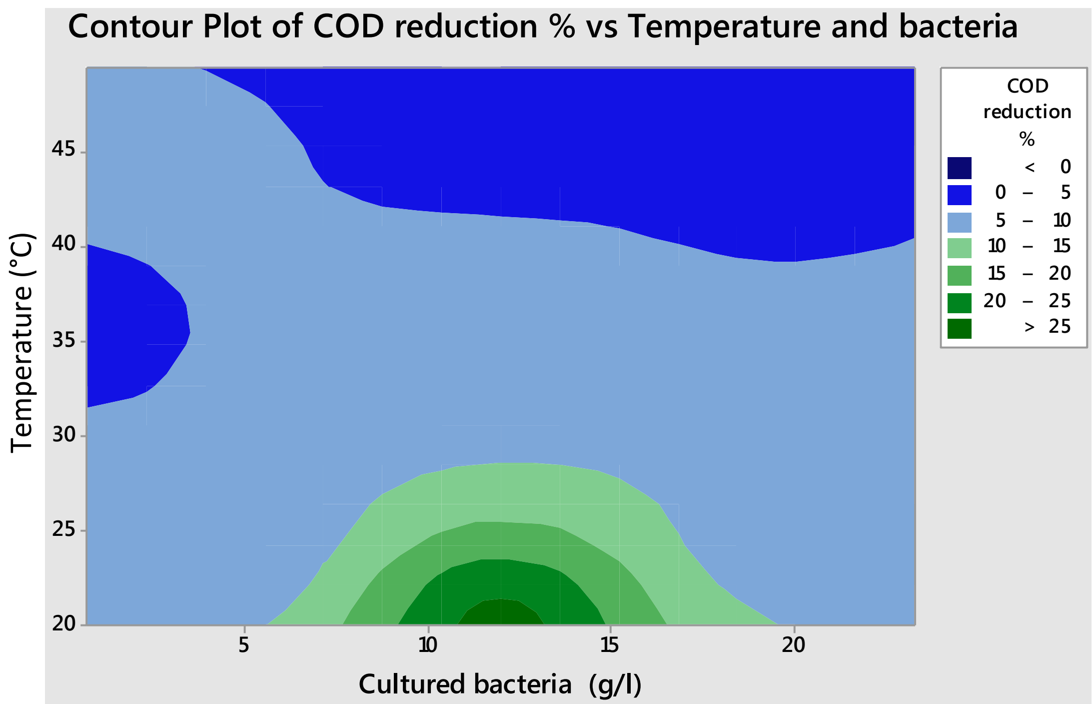

Figure 1. Contour plot of the % COD reduction at prescribed concentrations of RFM cultured bacteria (g/l) and temperature (°C) using Minitab 17.

Where A_b is absorbance of wastewater at 475 nm before degradation and A_a is absorbance at same wavelength after degradation.

Statistical analysis

Using the Design Expert Software 7, the responses from the data were analysed. To evaluate the goodness-of-fit of the mathematical models, analysis of variance (ANOVA) was used in a confidence interval of 95% (Veli et al., 2016). The ANOVA results for all responses were summarized. Contour plots were done using Minitab 17.

RESULTS

COD reduction

Figure 1 shows blue regions indicating lowest percentage of COD reduction while dark green regions indicate highest percentage of COD removal. The maximum COD reduction recorded in this study was 26.2%. The highest percent removal (26.2%) was obtained using cultured bacteria concentration of 12 g/l and temperature 20°C). The lowest activity of the cultured bacteria was noted at

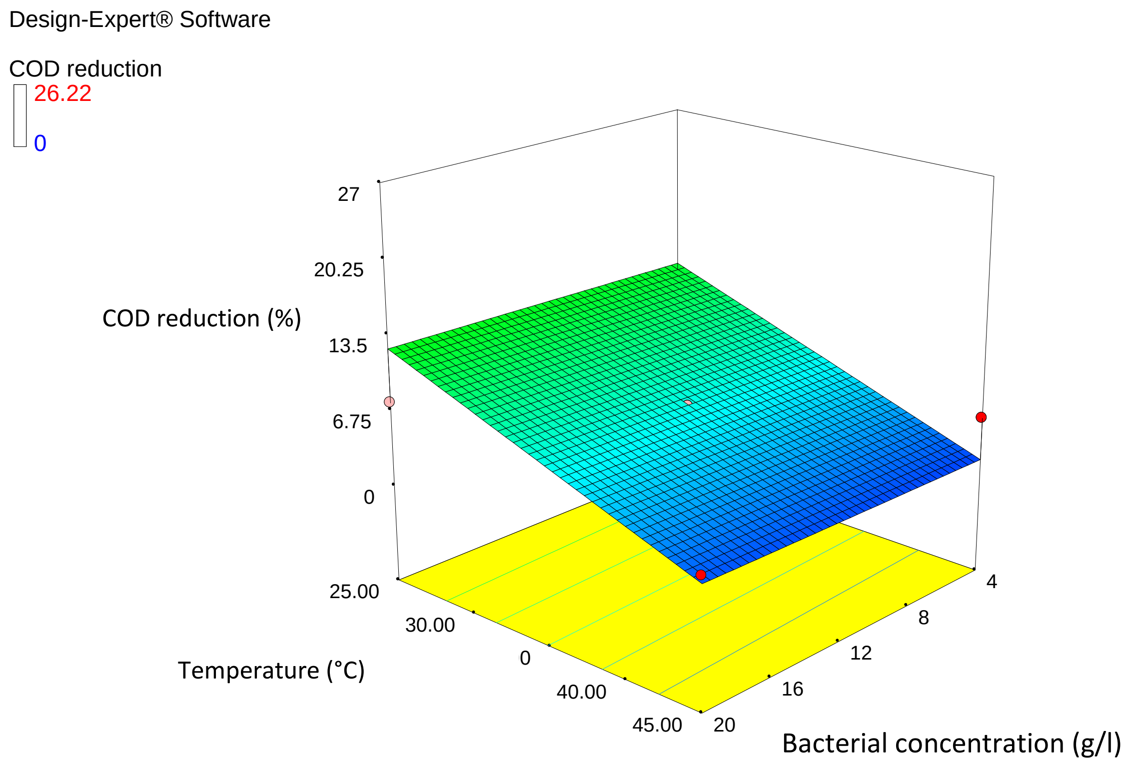

Figure 2. Three-dimensional surface plot showing the outcome in percent COD reduction of different bacterial culture concentration and temperature combinations using a linear model.

the combination of RFM concentration of 12 g/l and temperature 49.4°C where COD reduction was at its minimal.

RSM helps us to obtain graphical response surfaces and contour plots through computations from the developed model regression equations. A three-dimensional (3-D) representation of the outcome under various RFM culture concentrations and temperature effect is noted in Figure 2, with an increasingly higher reduction at lower temperatures and higher cultured bacteria concentrations.

For optimisation using Design Expert 7, the desired maximum COD reduction was set at a target of the maximum COD response. Desirabilities ranged from 0 to 1 for any given response. The program combines individual desirabilities into a single number and then searches for the greatest overall desirability. A value of 1 represents the ideal case. A zero indicates that one or more responses fall outside of the desirable limits. The highest desirability value as shown in Figure 3 was 0.634 at 16.37 g/l of cultured bacteria and 25°C with the lowest desirability value of 0.106.

The Model F-Value of 4.66 implies the model is significant. There is only a 3.71% chance that a "Model F-Value" this large could occur due to noise. Concluding from the analysis in Tables 2 and 3, values of "Prob > F" less than 0.0500 indicate model terms are significant.

Colour reduction

The main purpose of using RFM bacteria for treating industrial yeast processing wastewater was to reduce COD and to decolorize the wastewater. Baker's yeast production industry is one of the most polluting industries, which generates large volumes of high strength, bad smell, and dark brown color wastewater (Pirsaheb et al., 2015). Coincidentally, maximum experimental decolourization of 44% was obtained at 12 g/l cultured bacteria concentration and 20°C temperature as shown in Figure 4, which was similar to the parameter levels observed for the COD reduction. However, reduction of wastewater decolourisation was noted at temperature above 45°C. This might be attributed to heat effect rather than the RFM bacteria concentration and their enzymes. The enzymes as biological systems are denatured by heat. Therefore, complete removal of melanoidins that impart a dark colour to the wastewater was made difficult.

Figure 5 is the 3-D surface plot showing the relationship between bacteria culture formulation and temperature in colour reduction. Evidently, there is low colour reduction at extreme temperatures used in this study, that is, 45 and 25°C. The response surface plot has a clear peak which suggested that the optimum condition fell well inside the design boundary (Han et al., 2012). At optimum temperatures in the range of 35°C,

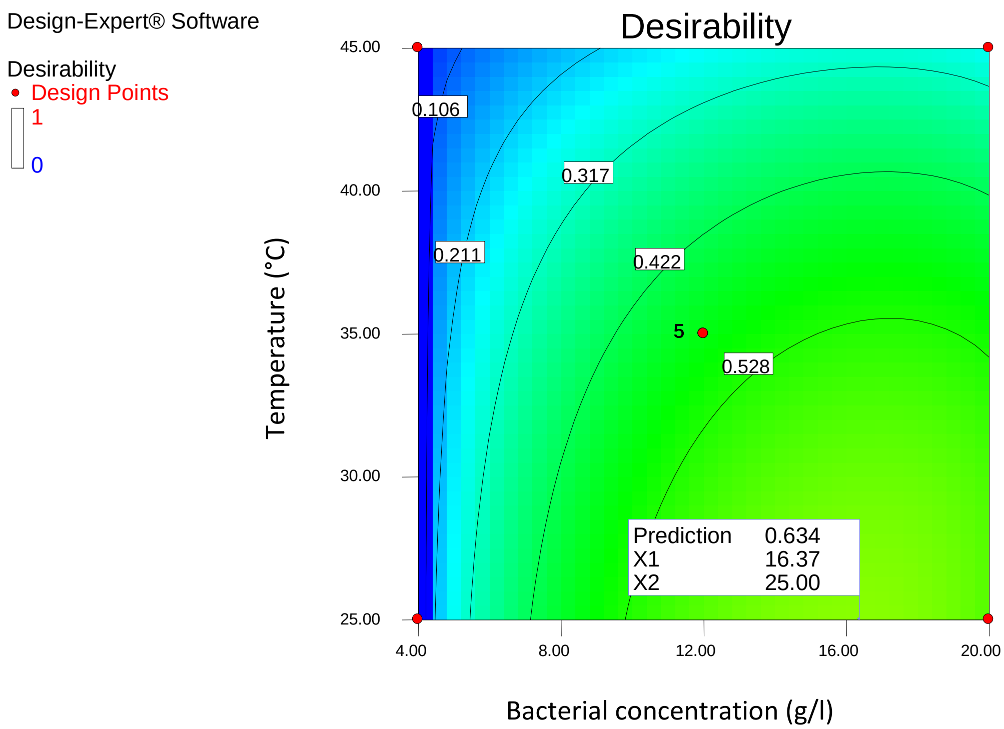

Figure 3. A summary of desirability showing the optimum temperature and RFM culture concentration for COD reduction.

Table 2. ANOVA table for linear model using design expert 7 for COD reduction.

Source	Sum of squares	df	Mean square	F Value	p-value Prob > F
Model	218.68	2	109.34	4.66	0.0371 sig.
A-Bacterial formulation	0.48	1	0.48	0.021	0.8889
B-Temperature	218.20	1	218.20	9.30	0.0122
Residual	234.51	10	23.45		
Lack of Fit	234.51	6	39.09		
Pure Error	0.000	4	0.000		
Corrected Total	453.19	12			

Table 3. ANOVA for Response Surface Quadratic Model using Design Expert 7 for colour reduction.

Source	Sum of squares	df	Mean Square	F Value	p-value Prob > F
Model	1987.42	5	397.48	4.95	0.0295 sig.
A-Bacterial formulation	31.36	1	31.36	*0.39*	*0.5518*
B-Temperature	484.00	1	484.00	*6.03*	*0.0438*
AB	64.00	1	64.00	*0.80*	*0.4016*
A^2	1216.70	1	1216.70	*15.16*	*0.0060*
B^2	333.60	1	333.60	*4.16*	*0.0809*
Residual	561.94	7	80.28		

maximum reduction in colour is obtained, giving a typical concave 3-D graph shown in Figure 5.

Desirability values range from values of 0 to 1 since it is a probability of occurrence. The aim is to select values

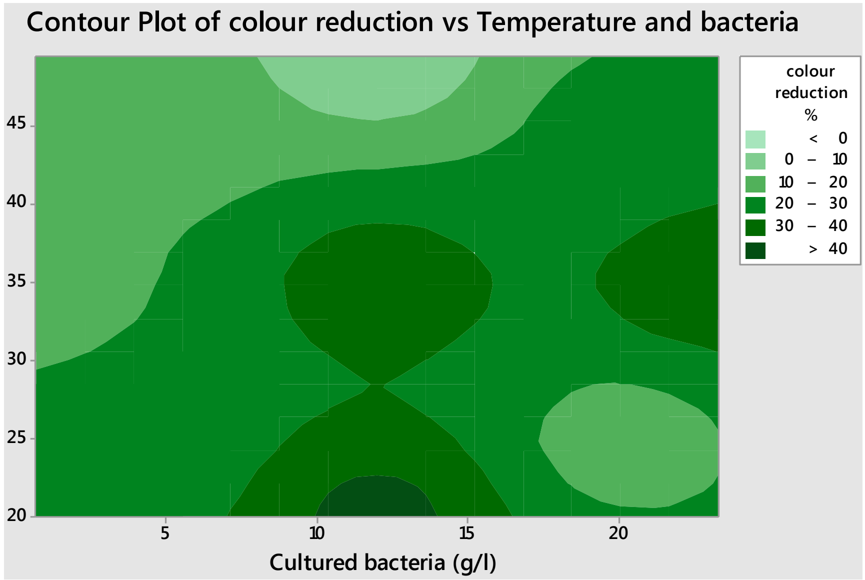

Figure 4. Contour plot showing colour variations to highlight difference in colour reduction at different RFM concentration (g/l), temperature (°C) combinations.

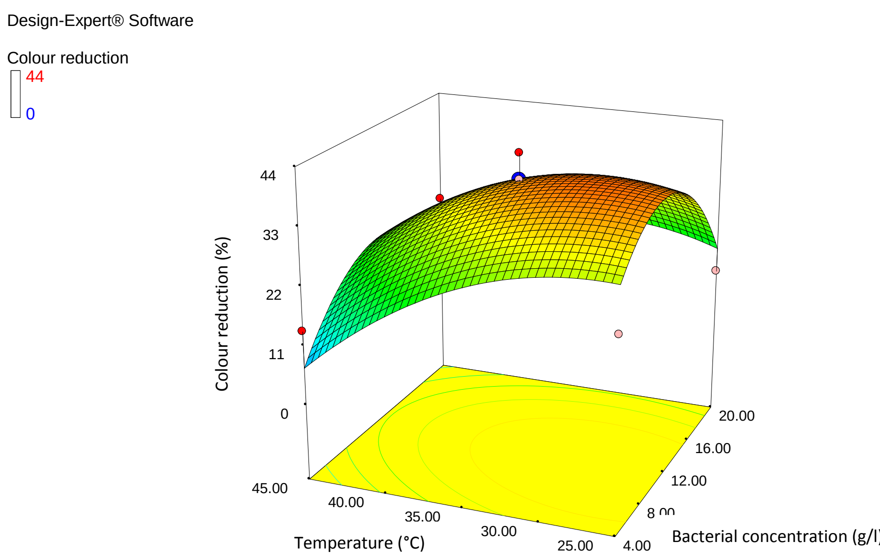

Figure 5. Three-dimensional surface plot showing the outcome in percentage colour reduction at different bacterial concentrations (g/l) and temperature (˚C) combinations.

with the highest desirability for optimisation. Design-Expert 7 software sorts the results from most desirable to least. Therefore, in Figure 6 the highest prediction value of desired colour reduction that can be obtained is 0.634 at 16.37 g/l and 25°C.

The Model F-Value of 4.95 implies the model is significant. There is only a 2.95% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, B and A^2 are significant model terms.

DISCUSSION

COD reduction after treatment of yeast processing wastewater

The maximum COD reduction recorded in this study was 26% (Figure 1). This was very minimal reduction, showing that biological treatment cannot be used as a sole wastewater treatment system. Elsewhere, it has been recorded that conventional anaerobic and aerobic treatment could accomplish degradation of the

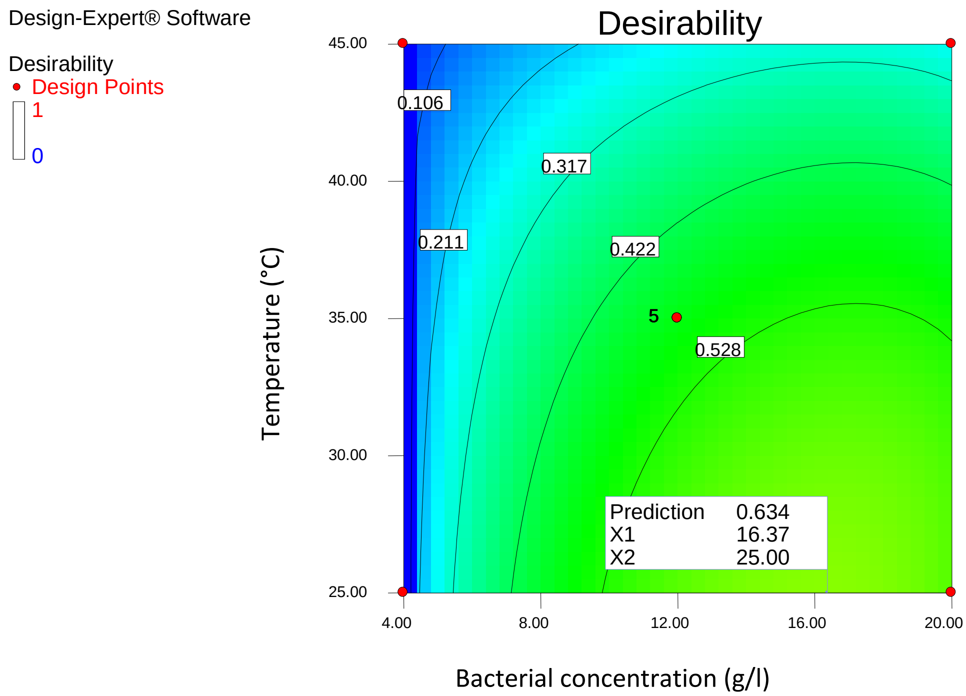

Figure 6. Desirability values for optimising the bacterial concentration (g/l) and temperature (°C) and their effect on percentage colour reduction.

melanoidins up to only about 6-7% (Agarwal et al., 2010; Onyango et al., 2012). Yeast processing wastewater contains melanoidins which are known to be lethal to many microorganisms because of their antioxidant properties (Kumar, 1997). Absence of COD reduction at 49.4°C with RFM culture of 12 g/l can be a result of recalcitrant melanoidins which limit growth of bacteria. As shown in Figure 2, COD removal efficiency increases with increase in RFM dose, since metabolic activities leading to pollutant removal are increased. However, high temperatures are known to encourage repolymerization of melanoidins and in the process inactivating bacteria. Generally, biotreatment of melanoidins containing wastewater has not given impressive results and post treatment is necessary. In a related study, post-treatment using coagulation step and ozonation impacted on the biologically quality of the wastewater effluent, resulting in the reduction of COD by 30–49% (Zub, 2007).

Colour reduction after treatment of yeast processing wastewater

The decolourization pattern indicated that the degradation of melanoidins was highly temperature dependent. In Figure 4, a contour plot showed the highest colour reduction of 44%. Melanoidins removal by micro-organisms can occur through enzymatic degradation, utilizing the pigments as carbon and nitrogen sources as noted elsewhere (Bezuneh, 2016). Various forms of intracellular and extracellular enzymes such as laccases, manganese peroxidases, lignin peroxidase, sugar oxidases such as sorbose oxidase have been reported to show melanoidins degradation activity (Couto et al., 2005; Freitas et al., 2009).

There was a notable increase in intensity of the wastewater colour when COD removal was at its lowest. The reduction in decolourization might be due to the fact that melanoidins stability varies with pH and temperature as additional parameters. This might be due to the fact that at higher temperatures some bacterial proteins are denatured; therefore removal of melanoidins which are responsible for dark colour in yeast processing wastewater is made difficult. After conventional biological treatment, most of the organic load is removed but nevertheless, the dark brown colour still persists and it can even increase due to repolymerization of coloured compounds (Jiranuntipona et al., 2009).

Process optimisation for COD reduction

In an effort to optimize this process, the Design Expert 7 software provided selection of the desired goal for each factor and response from the menu with 5 possible goals (maximizing, minimizing, target within range, none (for responses only) and set to an exact value (factors only). A minimum and maximum level is to be provided for each parameter included in the Optimisation. The maximum COD reduction was obtained at a temperature of 25°C and 16.37 g/l of bacterial culture. The obtained desirability value of 0.634 demonstrates that the estimated regression function may represent the experimental model and desired conditions satisfactorily.

Conclusion

It has been shown that biological treatment of wastewater from a yeast producing plant is a suitable process for the removal of organic load and colour from wastewater, especially when the operating parameters are optimized as confirmed. However, due to the recalcitrant nature of the complex compounds in the effluent the treatment efficiencies are generally low.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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