Mathematical Methods and Models in Biosciences
June 18-23, 2023, Pomorie, Bulgaria
https://biomath.math.bas.bg/biomath/index.php/bmcs

Response surface designs and R package based modelling for predictive capabilities, sensitivity and validation of model for improving the bacterial growth, laccase production, and textile dye decolorization alongside a detoxification study

Pravin Dudhagara, Sunil Bhavsar

Department of Biosciences (UGC-SAP-DRS-II & DST-FIST-I),
Veer Narmad South Gujarat University, Surat-395007, India
dudhagarapr@gmail.com

The thermophilic bacterium, *Bacillus licheniformis* U1 is used for the optimization of bacterial growth (R1), laccase production (R2) and synthetic disperse blue DBR textile dye decolorization (R3) in the present study. Preliminary optimization has been performed by one variable at time (OVAT) approach using four media components viz., dye concentration, copper sulphate concentration, pH, and inoculum size. Based on OVAT result further statistical optimization of R1, R2 and R3 performed by Box–Behnken design (BBD) using response surface methodology (RSM) in DOE Design-Expert (Stat-Ease) and R software with R Commander package. The total 29 experimental runs conducted in the experimental design study towards the construction of a quadratic model. The model indicated that dye concentration 110 ppm, copper sulphate 0.2 mM, pH 7.5 and inoculum size 6% v/v were found to be optimum to maximize the laccase production and bacterial growth. Whereas, maximum dye decolorization achieved in media containing dye concentration 110 ppm, copper sulphate 0.6 mM, pH 6 and inoculum size 6% v/v. R package predicted R2 of R1, R2 and R3 were 0.9917, 0.9831 and 0.9703 respectively; likened to DOE predicted R2 of R1, R2, and R3 were 0.9893, 0.9822 and 0.8442 respectively. The values obtained by R software were more precise, reliable and reproducible, compared to the DOE model. The laccase production was 1.80 fold increased, and 2.24 fold enhancements in dye decolorization were achieved using optimized medium than initial experiments. Moreover, the laccase-treated sample demonstrated the less cytotoxic effect on L132 and MCF-7 cell lines compared to untreated sample using MTT assay. Higher cell viability and lower cytotoxicity observed in

a laccase-treated sample suggest the impending application of bacterial laccase in the reduction of toxicity of dye to design rapid biodegradation process.

In the present research, DOE and R package compared mainly for modelling perspective. All three responses i.e. R1, R2 and R3 were checked using DOE and R package for predictive capabilities, Sensitivity and Validation of model. ANOVA and regression analysis for all three responses were superior in R package. Similarly, the predictive values of R1, R2, and R3 by R software are more suitable and justifiable than the predictive responses values of DOE. In contrast to DOE data, R package data showed the lower standard error of the regression line which represents a quick approximation of 95% prediction interval. To the best of our knowledge, there are no reports on the use of statistical design of R package for optimization of three responses simultaneously. We also emphasized R software package's sensitivity analysis and its usefulness in the optimization process through the validation approach.

This study aims to optimize the medium composition mainly required for growth of isolate, the laccase production and dye decolorization by a *Bacillus licheniformis* U1 strain using R open source software with R Commander (Rcmdr) package.