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A Review on Response Surface Methodology Optimization in Microbial Biotransformation

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ABSTRACT

Response Surface Methodology (RSM) is an optimisation technique used by research studies in biology and analytical chemistry for optimisation. Several environmental conditions impact microbial biotransformation, both directly and indirectly. The optimisation of these parameters is essential for successful biotransformation by selected microorganisms. Furthermore, the optimisation process can reduce operational time and costs. This review discusses how to improve the biotransformation process by employing the response surface methodology. The level of accuracy held by the developed model was evaluated using analysis of variance (ANOVA) based on various responses. RSM assists in selecting the most suitable experimental design for evaluating the relationship between parameters.

Keywords: *Response surface methodology, Optimisation, Biotransformation*

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INTRODUCTION

The term "optimisation" refers to selecting the best element from independent parameters [1]. The most conventional approach to determining the best design parameters is to evaluate the effect of each parameter on the response separately at one time [2]. In order to determine the output-input relationship, it is crucial to understand the interaction effect between parameters [3]. Therefore, most conventional approaches are seldom used to determine interactions [4]. Response surface methodology (RSM) is a tool for optimising and modelling a process that includes significant calculations [5]. Using mathematical model approaches to optimise parameters is the preferred way of analysing response variables since it is efficient, time-saving, and cost-effective [3]. This method creates an appropriate experimental design that incorporates all of the independent parameters and uses the required data from the experiment to generate a set of equations that can calculate the theoretical value of a result [2].

In a typical RSM study, the experimenter will develop an empirical model for each response, such as a second-order model, and use these models to evaluate the combination of the design parameters that provide optimal or at least suitable response values [6]. The significance of the resulting model equation was evaluated using analysis of variance (ANOVA), which analyses the goodness of fit of the regression model and the importance of each parameter that affects the model [7]. Finally, the outcome validation is just as significant as the ANOVA since it compares the predicted value to the actual experimental value [8]. The RSM model has been used for optimisation in the disciplines of biology, such as fermentation, biotransformation, extraction, and analytical chemistry [9]. RSM is now widely and successfully used to improve the performance of microbial biotransformation [10].

This article reviewed how RSM may be used to model and optimise the biodegradation of organic compounds by selected microorganisms. Most recently published RSM experiments have been analysed in this chapter to broaden the scope and access RSM's applicability in microbial biotransformation.

Design of Experiment (DoE)

The Design of Experiments (DOE) is a mathematical framework for designing and performing experiments and examining and interpreting the results. It is a multifunctional tool that may be utilised in various settings, including comparative design, variable screening, transfer function selection, optimisation, and resilient design [11]. DOE can be designed and analysed using appropriate statistical software [7]. Design-Expert is software that allows the user to create experiments, do statistical analysis, model data, and optimise results [2]. Design-Expert software provides several programs such as full factorial, fractional factorial design, D-optimal designs, and surface response [5]. This type of applied statistics is used to conduct scientific studies of a process in which input parameters are manipulated to investigate the effects on response parameters [11].

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The Principle of Response Surface Methodology (RSM)

Design of experiment (DoE) is a key component of Response Surface Methodology (RSM) [9]. The statistical design of an experiment in planning an experiment so that adequate data can be analysed using statistical methods, leading to valid and accurate results [10]. Box and Wilson created RSM to model experimental response before proceeding to numerical experiment modelling [11]. RSM can be thought of as a method for solving the optimisation problem in a systematic way [12]. This technique provided an adequate experimental procedure that combines all independent parameters and uses the experiment's data to build a set of equations that can provide the theoretical value [13]. Furthermore, this is an effective statistical method for modelling and optimising several parameters with few experiments to predict the best performance parameters [14].

RSM uses many optimisation phases and can be completed in three simple steps, including experiments for determining the criteria to be considered, followed by the greatest either ascent or descent path. The quadratic polynomial model is eventually fitted and optimised [13]. The presentation of the yield as a surface plot is one of RSM's most crucial inputs. It can give many responses simultaneously by considering the interactions between parameters, which is crucial for process design and optimisation [15]. Since the theoretical correlations between the dependent and independent parameters are not very well understood, mainly based on a second-order equation, a multiple regression model can be used to determine dependent parameters [14].

Central Composite Design (CCD)

The Central Composite Design (CCD) is a fundamental response surface experiment that involves the combination of fractional and factorial designs using axial points and centre points to measure curvature [7]. According to Ait-Amir [15], Central composite designs could be used to build orthogonal blocks, providing model terms and block impacts to be analysed independently and decreasing the coefficient of determination. Furthermore, rotatable designs provide constant prediction variance through all equidistant sites from the design centre [7]. The CCD comprises the three elements in general:

1. Center points
2. 2k factorial
3. 2k axial

Where k is the total number of components. Figure 1 shows the design points for the CCD experiment with K = 2 components.

According to Ranade and Thiagarajan [6]. The set of experiments in the CCD is computed using the formula N = k2 +2k+cp, where the number of components is K, and the number of replicates for the centre point is cp. The formula α = 2(k-p)/4 calculates the α value. When constructing the design, the major component of CCD includes five elements which are (-α, -1, 0, +1, +α) [6].

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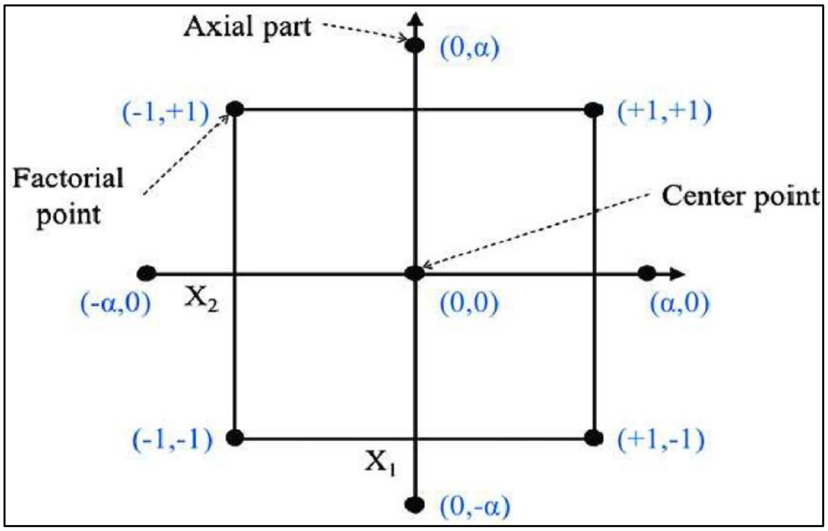

Figure 1: CCD Design Points for Number of Components k = 2. Adopted from [16]

Box Behnken Design (BBD)

The Box–Behnken Design (BBD) method proposed a method for identifying points from a three-level parameter model to assess the statistical model's first and second-level coefficients [2]. As a result, these designs are more practical and cost-effective than respective 3k design models, especially for multiple variables [6]. BBD requires at least three numerical elements that vary throughout three levels [5]. The experimental endpoints in Box–Behnken models are evenly separated from the centre point on a hypersphere [2]. The requirement of an experimental number based on formula N = 2 k (k - 1) + cp is one of BBD's fundamental properties, which is acceptable to adjust all factor levels at three levels only (-1, 0, + 1) with intervals equally distributed, where k is the number of components and (cp) is the number of central point's [17]. BBD has been effectively employed in various optimisation techniques as a physical and chemical approach. More RSM design strategies are accessible, including optimal design, one factor, historical data designs, miscellaneous design, and user-defined [5].

Inspiration for the Chapter

RSM by microbial biotransformation researchers is one of the most successful techniques since it has proven to be very useful in the design and precision of experiments due to difficulties facing the conventional approach [14]. Mathematical modelling and optimisation of the numerous parameters that affect the biotransformation performance output help understand what influences many variables [17]. This experimental approach creates a mathematical equation that aids in the clarification procedures that require fewer experimental runs [6]. Thus, RSM is important for biology researchers.

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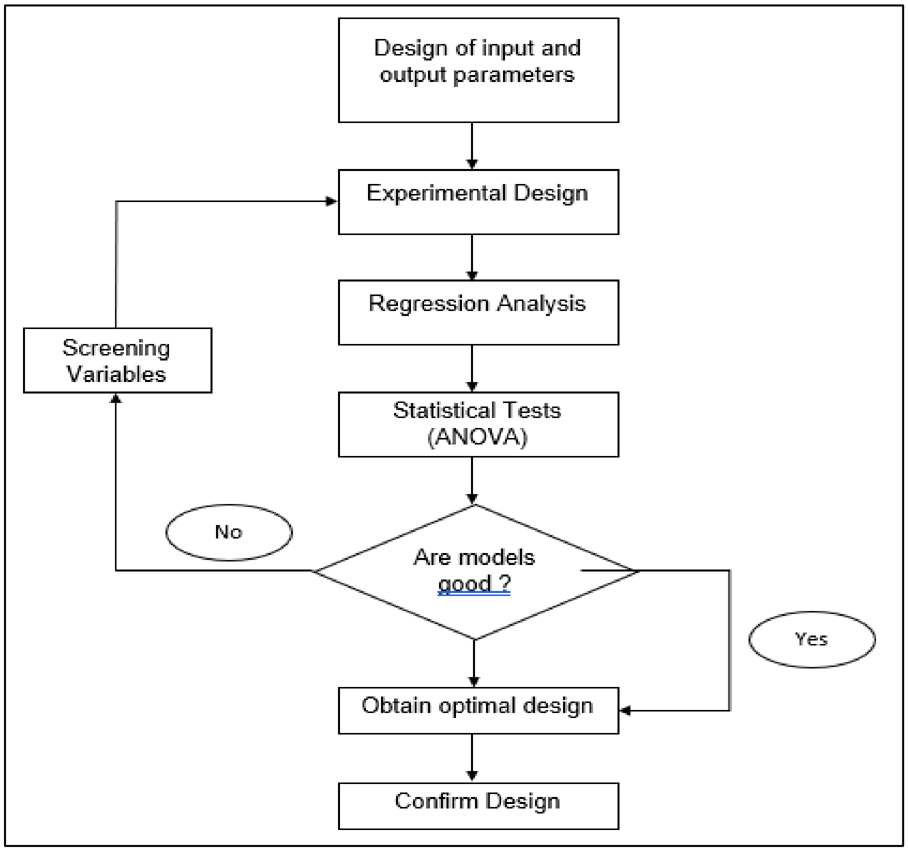

Figure 2: Basic RSM design flowchart. Adopted from [14]

Another important purpose of RSM is to determine the best parameters for control variables that result in the highest or lowest yield in each area of research [14]. The interpolation equations derived can be employed to obtain either the most or least predictable outcomes within the range of the research variables [5]. Figure 2 depicts the RSM statistical approach framework's general design flowchart.

Application of RSM in Microbial Biotransformation

Several RSM research has been published. However, this study focuses on using RSM to optimise the biodegradation of organic compounds by selected microorganisms based on previous literature findings. Farhan [8] recently published preliminary findings showing that the biodegradation of chlorpyrifos by isolated bacteria and fungus using the CCD model based on RSM revealed a maximum degradation of up to 75 %. Farhan [8] designed the experiments using the Central Composite Design (CCD) model. According to four significant parameters, there were 31 runs consisting of 7 centre points and eight axial points: concentration, temperature, carbon, and inoculum. Thus, the quadratic model obtained a P – value of less

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than 0.05, revealed the ANOVA results were significant. The RSM model has been proved to be simple, practical, and capable of determining the optimum parameters [18].

Liu [19] employed a CCD model based on RSM to perform a successful experiment on the biotransformation of diosgenin drugs through submerged fermentation by endophytic fungus. The experiment results showed that a fermentation time of 11.89 days could yield maximum diosgenin yields of 2.22 %, which agreed with the predicted value [19]. The researcher claimed that the experiments are easy to conduct using RSM compared to the conventional approach, which is time-consuming and costly [19]. Based on fundamental statistic principles, RSM improves process optimisation [20]. The CCD model is often used in RSM due to the experiment's precision [18].

Ungureanu also used the CCD model for experimental design in the bioremediation of clofibric acid by a *Trametes pubescens* fungal strain and yielding up to 60% biodegradation according to four significant parameters [21]. After 14 days of submerged fermentation at 25 C and 135 rpm, a high biotransformation level was achieved in a growing medium including 3 g. L $^{-1}$ yeast extract, 15 g. L $^{-1}$ peptone, 5 g. L $^{-1}$ glucose, at pH 5.5, and 2 % inoculum concentration. The most significant experimental response for clofibric acid biotransformation rate was 60 %, whereas the predicted value was 58.21 %, demonstrating that the mathematical model and the natural biotechnological process are compatible [21]. The CCD model and RSM method, including regression analysis and factorial design, assist in preparing experiments and constructing models to prove the interactions between independent parameters and responses [22].

The role of redox mediators and factors on the performance parameters includes pH, temperature, concentration, and dry weight in choosing the optimum conditions for *Aspergillus aculeatus* to degrade olsalazine using Box Behnken Design (BBD) model based on RSM [23]. This results in a high biotransformation level of 89.43 % on day seven which showed a great significance compared to the one factor at a time (OFAT) conventional approach [23]. Thus, kinetic modelling in the fermentation process is essential for biotransformation development [24]. The application of RSM demonstrated that experimental data combined with mathematical modelling allowed quantitative and qualitative interpretation [25].

Besides that, the application of RSM in microbial biotransformation at an industrial scale has been promoted to be the most time-saving and cost-effective technology for pollutant contaminated site remediation [26]. This is because the process in industrial reactors revealed good performance to achieve high turnover rates and bulk productivity [27]. Sales [28] aimed to optimise limonene biotransformation by *Colletotrichum nymphaea* in industry scale bioreactors. The researcher claimed that the bioreactors produced up to three times the maximum productivity compared to shaking flasks, thus, resulting in a high biotransformation level up to 70 % by the CCD model based on RSM [28].

Liang [29] used a bubble column reactor to transform β-alanine production by *Rhodococcus* sp. The researcher performed a successful experiment by using the CCD model to predict optimum conditions for the productivity of β-alanine [29]. According to the study, the time of β-aminopropionitrile transformation was short. There was no concern of microbial contamination due to a shortage of culture medium when using a bubble column reactor [29]. This finding will assist researchers in figuring out the optimum parameters for biotransformation at an industrial scale.

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Xiong [19] used a BBD model based on RSM to conduct a successful experiment on the biodegradation of ochratoxin by *the Aspergillus oryzae* strain. The maximum degradation rate achieved 94% after three days of fermentation. Due to the parameter's adjustment, the time required for ochratoxin degradation has decreased by 57 % by RSM application [19]. Traditional "one-factor-at-a-time (OFAT) strategy is typically applied in optimization experiment [25]. This technique, however, is not only time consuming but analysis of a linear and quadratic model for OFAT is not simultaneous. However, the conventional OFAT does not statistically analyse the effect of correlation between parameters. As a result, predictions of optimum conditions are relatively poor [25]. Therefore, the application of BBD is well anticipated in optimisation at an industrial scale due to its robustness and requiring a fewer number of runs.

CONCLUSION

The importance of selecting the most suitable optimisation techniques, such as response surface methodology (RSM), has been demonstrated in this discussion. The elimination of experimental runs for the same objective, identifying the optimal parameter's value for the highest response, is an excellent achievement of the response surface approach compared to conventional methods. Furthermore, before the experimentation phase, the proposed framework can be utilised to estimate the response. Therefore, this phase might assist researchers in focusing on specific factors that have the most significant impact on process output. Using Box Behnken design (BBD) or central composite design (CCD) for microbial biotransformation, particularly with high cost, is a cost-effective alternative to the conventional one-factor optimisation strategy. Model prediction can be improved significantly by combining microbial biotransformation with RSM.

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AUTHOR'S CONTRIBUTION

Nurul Tasha Zulkifle carried out the literature search and wrote and revised the manuscript. Zaidah Zainal Ariffin conceptualised the central research idea, provided the theoretical framework, anchored the review and revisions and approved the article submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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