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Design of Experiment: An Accelerating Tool for Product Discovery from Actinobacteria

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ABSTRACT

The conventional method of medium optimization is one factor at a time (OFAT); time consumption and absence of factors interaction are the main drawbacks. To address this problem, Box and colleagues developed software called Response Surface Methodology (RSM), which comprises different models of designs for medium optimization. Design of Experiment (DOE) consists of different optimization models, commonly used models are Box-Behnken Design (BBD), Central Composite Design (CCD), and Artificial Neuron Network (ANN). These models are founded on the domain, matrix, variables, and responses. Each stage of design can be evaluated by different tools to find out the most influenced factors for the significant scale up of production. Many microbial species are sources of a wide spectrum of bioactive molecule production with considerable applications. Amongst the diverse microbial groups, the Phylum Actinobacteria shares common properties with both bacteria and fungi and is reported to have the bulk share of commercial biomolecules. The commercially available microbial products within Actinomycetes are predominantly from Streptomyces sp. The optimization studies using RSM gained much attention in fermentation using Actinobacteria for reducing time and enhancing production. This review paper explores the application of Design of Experiment (DOE) for the discovery of different metabolites from Actinobacteria, its applications and medium optimization for enhanced production of these biomolecules using different models.

Key words: Actinobacteria, Metabolites, Design of Experiment, One Factor at a Time, Box-Behnken Design, Central Composite Design, Artificial Neuron Network

INTRODUCTION

Microbial growth and metabolite production are economically influenced by the culture medium's composition. This context has a significant impact on the downstream process's cost and target metabolite production, making it essential to optimize the medium composition and other physical parameters.Optimizing one aspect at a time is the conventional approach, however this strategy excludes the impacts of other factors and makes attaining the desired result challenging. Response surface methodology (RSM), which enables the understanding of interaction at the combined level for multivariable systems using various optimization strategies, is a very helpful tool to address the drawbacks associated with multiple parameter optimization (Aghaie –Khouzani et al., 2012).In the past few decades, RSM optimization adopted as a successful method. The two main RSM techniques for the rapid analysis of medium components that can improve production are Box-Behnken and Placket-Burman approach (Banik et al., 2007;Deepak et al., 2008). These approaches concentrate on creating models, formulating assortments for research, and assessing how different variables interact to increase metabolite synthesis (Majumder et al.,2009). Implementing optimal models is the first step towards formulating a medium composition for enhanced yeild output (Managamuri et al.,2016). As part of the RSM approach, distinct phases of experiment design, including screening, interactions, analysis, and optimization of independent variables, can be addressed to eliminate ambiguity.The optimization process, that utilizes statistical techniques, allows for swift assessment and control of individual components affecting the optimal output.Box et al. (2009) devised a novel method to enhance the medium's performance compared to conventionalstrategies through the surface methodology approach (Tarley et al.,2009)A polynomial equation between controllable variables can be created by applying statistical approaches to optimize the nutritional requirements of microorganisms for maximum yield generation. Such optimization can lead to the development or discovery of previously untapped factors and conditions that can improve the yield output with fewer cost-efficient experiments (Elibol, 2004). In RSM, the several key terms used to describe the process and technical aspects are mentioned below.

a. Experimental domain - Maximum and minimum limits of the variable.
b. Experimental design - Matrix of experimental combination.
c. Factors or variables - Different variables selected for the experiment such as pH, temperature, concentration, flow rate, time, etc.
d. Level of variables - Different values of variables considered for the production (+, 0, -).
e. Responses - The outcome of the experiment.
f. Residuals - Difference between observed and predicted data, for good result residual values are always negligible.

DIFFERENT STAGES OF OPTIMIZATION USING DESIGN OF EXPERIMENT (DOE)

The successful design of the fermentation medium with significant factors is influenced by various parameters. The primary objective is to examine for particular factors that have significant influence over the expected output.The selection of design models depends on several variables that can be assessed using ANOVA, fitness, regression, and mathematical or statistical description for fermentation, behavior, and model effectiveness.The direction and interaction between the variables must be examined visually in addition to using these linear models. The relevance of such model containing factors and their interaction are often described through contour plots, Pareto charts, and 3D models.Based on its form, graphs can be classified into maximum, plateau, maximum outside the experimental region, minimum and saddle surface. These represent the design success under optimal conditions and the interplay of variables for specific parameters (Managamuri et al.,2016;Tarley et al., 2009)

APPLICATION OF MULTIPLE TOOLS OF RESPONSE SURFACE METHODOLOGY (RSM)

Full three-level factorial design

The model's applicability in RSM are limited sincethe variables are more than two, it is challenging to predict the design's conditions accurately using this method (Elibol,2004), Itcan be calculated by

$$N = 3^k$$

Wherein, N- Experiment number, K- number of factors.

Central Composite Design (CCD)

Introduced by Box and Wilson to screen a broad spectrum of variables and to find out is influence on each other within the medium [7]. Well defined with characteristic features as follows

a. Full factorial or fractional factorial design.
b. Center point and experimental points are equally distributed from the center point.
c. Experimental numbers can be calculated by the following two different equations,

$x_1 = X_1 - X_1 / \Delta X_1$

Where,

x_1Coded level

X_1means for the natural level of the independent variable

ΔX_1 Step change value

Second-order polynomial model

$y = \beta_0 + \Sigma i^4 1 \beta_i X_i + \Sigma i^4 = 1 \beta ij X_i X_j + \Sigma 1^4 = 1 \beta_{ii} X_i^2$

Where y represents the independent response, β_0, β_i, β_{ij}, and β_{ii} represent the regression coefficient.

d. α value can be calculated by $\alpha = 2^{(k-p)/4}$.

e. Factors assigned at -α, -1, 0, +1, +α.

f. Each variable is studied at different levels.

g. Adjacent points are used for the displacement of one experimental matrix.

Box-Behnken design (BBD)

Introduced by Box and colleagues, it is one of the simple tools used for the medium design, that encompass optimization experiments with minimum variables and maximum production (Ahsan et al.,2017). According to Ranic*et al*., 2014, the feature that sets Box-Behnken apart from other designs is its ability to enter the run chart's variable midpoint without the need for a factorial point (Parmar et al.,2017). The number of runs in the model can be calculated by the equation

$$N = 2k\ (k - 1) + C_0$$

Where, N represents the number of runs, k represents the factorial number, and C_0 represents the replicate number of the central pointAll factors will be equally adjusted at three

levels(-1, 0, +1), and the central point aids in error detection and intermediate stage calculation. This approach was utilized by numerous researchers to optimize a variety of products such as emulsifiers, laccases, and enterocin (Colin et al., 2013;Hassan et al.,2017;Niladevi et al.,2009)

Artificial neuron network (ANN)

Since its introduction in the 1940s, the artificial neural network model has evolved to be one of the most effective and widely accepted tools for medium optimization. The network interaction is inspired and analyzed through the biological neurons pathway, based on the linear response experiment model.Structured layers comprise of the feed value and its resposnes, wherein, input layers are independent variables and output layers are dependent variables..Different researchershas used the model to design medium for citric acid, L-asparaginase, actinomycin V, scleroglucan lipase, and bio-surfactants (Singh et al.,2009).

Regression

At the end of every model, a regression equation is used to determine the relationship between one or more linear variables. This equation can be combined with an ANOVA to generate statistical output. The following polynomial equation can be used for determining the impact of factors on production.

$$y = \beta_0 + \Sigma\beta_i Xi^4 i{=}1 + \Sigma\Sigma\beta ij Xi^4 ji^4{=}1 X_j + \Sigma\beta ii Xi24i{=}1$$

Where, y is the predicted response quantified by an extraction recovery; Xi represents the independent variables (four variables); $\beta 0$ is model constant; βi is the linear coefficient; βii is the quadratic coefficient, and βij is the cross-product coefficient(Yadav et al.,2013)

ACTINOBACTERIA AS BIOMOLECULE'S ARRAY

Actinobacteria are aerobic, Gram-positive organisms that share characteristics with both fungi and bacteria.Streptomyces is the source of over 80% of known pharmaceutical compounds as they have a high G+C concentration that provides them with distinct characteristics.They are widely distributed in both aquatic and terrestrial habitats, and they develop mutualistic associations called endophytic actinobacteria within plants. Actinobacteria can thrive at any pH and can exist as autotrophic, heterotrophic, chemotrophic, or phototrophic(Koneman and Allen,2008).According to reports, the majority of actinobacteria are mesophilic, needing a temperature between 25 and 30 ˚C for optimal growth. Some belong to the category of

thermophilic species, which includes various types of Streptomyces, Thermomonospora, and Thermoactinomyces, when their temperature exceeds 55˚C (Vasconcellos et al.,2010). There are primarily four types of cell wall classifications: those that contain or lack diaminopimelic acid (DAP), LL-DAP, glycine, no sugar-I, meso-DAP, glycine, arabinose, xylose-II, meso-DAP, madurose (3-O-methyl-D-galactose)-III, meso-DAP, arabinose, galactose-IV, Deprived of DAP; possess lysine and ornithine-V, Deprived of DAP; variable presence of aspartic acid, galactose-VI, Deprived of DAP; diaminobutyric acid, glycine, with lysine variable-VII, Deprived of DAP; ornithine-VIII (Baraka et al.,2016).

Actinobacterial species are known to produce a variety of metabolites with a wide range of applications, and soil has been reported to be one of the most significant environments. Oilgotrophic soil, rocky niches, and mangrove soils are prominent locations for Actinobacteria isolation.Streptomyces, Micromonospora, and Nocardia proliferate in the mangrove rhizosphere soil as a result of tidal movement (Tan et al.,2009). Approximately 10% of the bacteria that inhabit sediments and aggregates in the marine environment are members Actinobacteria (Solanki et al., 2008). Bergey's classification manual divides the phylum Actinobacteria into six classes: Acidimicrobiia, Coriobacteria, Nitriliruptoria, Eubrobacteria, and Thermoleophilia.They exhibit an extensive array of morphological characteristics such as presence of aerial and substrate mycelium, mycelium colour, spore chain length and shape, diffusible pigment, and melanoid pigment synthesis.

Actino biomolecules fall under the general category of proteins, pigments, enzymes, antimicrobials, biosurfactants, and stimulants of plant development. Optimizing the fermentation conditions will be essential in achieving a higher product yield which can be attained through establishment of procedure that is both economical and time-efficient.The two primary strategies in the commercial production of microbial-based products are process improvement and optimization of production variables (Srivastava et al.,2018).A number of scientists have documented the highest amount of metabolites produced through the use of a variety of instruments that offer the ideal combination of real and potential components required for gene activationHenceforth, the present review documents the earlier reports that emphasized the design of experiments for bioactive components production from various Actinomycetes strains.

APPLICATION OF RESPONSE SURFACE METHODOLOGY: CENTRAL COMPOSITE DESIGN (CCD) AND BOX –BEHANKEN MODELS (BBD) FOR OPTIMIZING DIFFERENT METABOLITE FROM ACTINOBACTERIA

Enzymes from Actinobacteria using response surface methodology

Enzymes are biocatalysts that can accelerate many processes as well as slow down the same if required. Considerable investigation was conducted in the nineteenth century to extract, characterize, and use enzymes. Some enzymes have the suffix-ase (oxidase, carboxylase), while others have the suffix-in (pepsin, trypsin). Enzyme nomenclature is based on their reaction or substrate specificity.Enzymes can be categorized according to their function, such as ligases, hydrolases, oxidoreductases, transferases, lyases, and isomerases. Based on their production system, they are classified into the plant, animal, or microbial sources. Similarly, depending on extraction site, it may be extracellular or intracellular.The food, pharmaceutical, paper, detergent, and textile sectors are among the industries with a significant amount of enzyme applications.

The selection of various factors and subsequent ANOVA analysis of the obtained results constitute the process of medium optimization in a large number of studies.This model's greater productivity than under non-optimized settings has been investigated by examining the values of R2, nearby R2, anticipated R2, F value, and P-value.This review focuses on 13 different Actinobacteria-produced enzymes, with yield optimization accomplished through the use of RSM with CCD and BB techniques. The optimized model only includes important components that have the potential to increase output. Based on the data analysis, it can be inferred that CCD is a more widely utilized model for enzyme manufacturing than BB.The detailed discussions of the fermentation process for various molecules by different researchers are as follows.

Uricase is a prominent enzyme with widespread applications from therapeutics to industries.The recombinant mammalian uricase is used in the treatment of refractory gout disease. Production of uricase from *Streptomyces rochei* NEAE-25 was reported by (EI-Naggar, 2015). Plackett-Burman design (PBD) and Central Composite Design were employed to optimize the starch nitrate medium for uricase fermentation.Approximately fifteen variables were screened with Plackett-Burman, and the final optimization between them was completed with CCD. The impact on enzyme activity was ascertained by examining the interdependence of variables such as uric acid (6 g/L), medium volume (50 ml/250 ml), and incubation time (5 days). A Pareto chart was employed by Naggar, 2015,to evaluate development variables

according to their favorable and unfavorable repercussions.The significance of the F value in the Fisher's Test was 1.66E.06 for CCD and 0.0015 for PBD. Following medium optimization, uricase output of 47.49 U/ml -1 was observed, which was determined to be significantly greater than the unoptimized medium. The model's substantial significance is indicated by regression values that were reported between 0 and 1.

L-asparginase, which can be produced by *S. parvulus* KUAP106, is frequently employed to treat acute myeloid and acute lymphoblastic leukemia. It also serves as a common food ingredient. The optimum variables for improvised L-asparginase production are aspargine (0.05%), tryptone (0.5%), dextrose (5%), and NaCl (0.05%) in the tryptone glucose yeast extract medium (Usha et al.,2011). The medium ingredients Asparagine, tryptone, dextrose, and NaCl that have a positive effect on output were used in CCD optimization with the influence of the factors discussed in the Pareto chart .Following CCD optimization, the highest output recorded was 135 U/mL, and the significance of the model was confirmed by the Fisher's test.

The enzyme thrombinase enables fibrin to be converted into fibrinogen, thereby facilitating the blood coagulation.A high yield output was achieved by optimizing *Streptomyces venezuelae*, a thrombinase producer isolated from marine materials, using CCD.The optimal factors reported a pH of 7, the temperature at 35°C, and 6 percent (w/v) salt(Naveena et al.,2012)

Pirzadah *et al*. enhanced the synthesis of the cellulase enzyme used in the textile industry from Actinomycetes and Trichoderma (Pirzadah et al., 2014). The Pareto chart can be used to allocate four elements to secondary level screening. The CCD optimization revealed that the cellulase activity was influenced by the ingredient values of KH2PO4 (3 g/l), CMC (12 g/l), peptone (2.5 g/l), and pH of 5.This culminated in a 1.85-fold increase in yield output. Amylases are extensively utilized in the food, pharmaceutical, and textile industries to improve the surface quality of fabric, make syrups, and glucose conversion. Nithya *et al*., (Nithya et al.,2017)examined CCD and its application for enhanced development of amylase from *Streptomyces fragilis*, isolated from the desert, with selected variables of 3.07% dextrose, 1.08 % peptone, pH 6, and temperature 27°C.

The best thermally stable enzyme, alkaline xylanase, is produced by submerged fermentation of *Streptomyces violaceoruber* and is widely employed in industries.Wheat bran 3.5% (w/v), peptone 0.8% (w/v), and beef extract 0.8% (w/v) have been recommended through CCD-based optimization for 36 hours at 250 rpm in order to enhance productivity (Khurana et

al.,2007). It was discovered that Streptomyces sp. produced inulin by hydrolyzing inulinase. Initially, the fermentation medium contained eighteen nutrient factors; however, PBD and CCD were used to identify the most significant factors. Yeast extract, FeSO4.7H2O, and NH4NO3 were determined to be the most appropriate factors for enhanced development (Dilipkumar et al.,2011). Three of the initial eighteen variables were selected for CCD and maximum output, with an optimum medium of 89 U/gds was obtained.Protease, which is widely used in the textile, detergent, and leather industries, is produced by *Streptomyces* sp.A6 isolated from shellfish waste. PBD and CCD were used to optimize the medium, and the results showed that pH 8, 14g/L of shrimp waste, FeCl3 0.035 g/L, and ZnSO4 0.065 g/L were the ideal conditions (Singh and Chhatpar,2010).There was a 63.4 percent increase in productivity when four production-affecting variables were selected for CCD out of the initial 13 variables used for screening in PBD.

The oxidation and isomerization of the cholesterol group is facilitated through cholesterol oxidase mediated catalysis. The required amounts of yeast extract, dextrose, starch, and ammonium carbonate were optimized by the PBD in conjunction with the Central CCD to be 0.99, 0.8, 0.1, and 0.05 g/100 ml, respectively (Srivastava et al.,2018). ANOVA test used for the confirmation of significance and the following details were observed after the analysis. The screening process was initially conducted using the traditional One Factor at a Time (OFAT) approach. This was followed by PB with seven factors and CCD with three factors, which resulted in a 3.6-fold increase in production compared to the unoptimized medium and was quantified at 5.41 U/Ml. In the food business, xylanase is frequently used for fermentation, bread production, fruit juice clarifying, and feed preparation. Cellulase-free xylanase synthesis using *Streptomyces* sp. Ab106 from cane bagasse was reported. CCD-based optimization verified that temperature and pH interact to determine the optimum conditions for increased production, that are 50 °C and pH-7, respectively (Techapun et al.,2002). Plackett-Burman and Central Composite Design were utilized to optimize production of keratinase, an enzyme that is commonly employed for water treatment. It has been observed that keratinase activity increased by 15 times when chicken feathers were used. For improved output, the optimal combination was found at 28°C and 5 grams per 50 milliliters of chicken feathers for 5.5 days (Demir et al.,2015). The optimal medium for *Streptomyces erumpens* MTCC 7317 was developed using cassava fibrous residue. This was reported in a study utilizing Central Composite Design for amylase production, which verified the maximum hydrolysis of soluble starch (85%) and cassava starch

(70%), resulting in 5 ml of crude enzyme (17185 units), after 5 hours of incubation along with 60 hours of moisture (60%) and 50°C temperature (Shaktimay et al.2010)The xenobiotic degradation, paper, textile, and bioremediation industries are among the various sectors that use laccase enzyme. *Streptomyces psammoticus* produces lacaase in a starch casein media. Temperature, yeast extract, and agitation have been shown to interact favorably to increase laccase synthesis using the Box-Behnken design. Six of the eleven parameters that were found to have a beneficial impact on production were chosen for BB after they had first been screened using PB(Niladevi et al.,2009)

According to the aforementioned studies, several physical and media components can be optimized to improve productivity.The three most often mentioned physical parameters for improved output are agitation, temperature, and pH.Likewise, the enhanced production is largely influenced by the NaCl concentration with varying carbon (yeast extract and dextrose) and nitrogen sources (peptone).

ANTIBIOTICS FROM ACTINOBACTERIA USING RESPONSE SURFACE METHODOLOGY

Following the discovery of penicillin in 1928, the period described as the "golden age" of natural antibiotics emerged. The available antibiotics are classified as follows: those associated with bacteria and fungi, those linked to Actinobacteria, and lastly, synthetic antibiotics. Microbes in their bodies create these antibiotics as a molecule for an offensive or defensive mechanism.Antibiotics act by many mechanisms, which may involve blocking the synthesis of proteins, cell walls, nucleic acids, or ATP.Herein, we have discussed the optimization of the medium for increased synthesis of antibiotics derived from Actinobacteria detail. It was determined that CCD is the optimal model after analyzing nine different antibiotics with RSM optimization.The industrial application of *Nocardiopsislitrovalis* VSM8 has been traced back to its identification in the development of the antimicrobialcompound.CCD was utilized to aid enhance production of antimicrobial compound, and the optimal media was found to be 2% sucrose, 1% soy peptone, pH 8, and 11 days of incubation at 25°C (Managamuri et al.,2016). The bioactive ingredient utilized to treat banana *Fusarium* spp. wilt and antibiotics are made from *Streptomyces* sp. MAPS15, which was isolated from *Spongia officinalis*.The production conditions were optimized using CCD because agro-waste paddy straw, pH, and (NH4)2SO4 (Sathinarayanan et al.,2014) showed good interaction with antibiotic production of 62.41 mg/L

for biomass of 6.13 mg/L of Streptomyces sp. MAPS15.It has been observed that *Rhizoctoniasolani* AG-3 is susceptible to the antifungal compound produced by *Streptomyces diastatochromogenes* KX852460.Utilizing CCD, factors were optimized for enhanced production utilizing peanut meal (4.88%), soluble starch (4.40%), NaCl (0.52%), yeast extracts (0.47%), and ammonia sulphate (0.036%). Each combination's effect on metabolite formation was illustrated, along with the minuscule variations between expected and real values(Ahsan et al., 2017)*Streptomyces avermitilis* 14-12A has been found to be a source of avermectins, which are frequently employed as insecticides in crops.Using CCD, the optimal factor for higher production was identified. It was determined that the variables 149.5 g/L of maize starch and 8.92 g/L of yeast extract had a significant effect on output (Gao et al.,2009).Neomycin is an antibacterial drug, the effect of variables on neomycin production by *Streptomyces marinesis* NUV-5 was investigated using Central Composite Design, and the optimum conditions for solid-state fermentation were dextrin 14.1 g/Kg, raspberry seed powder 64.91g/Kg, and mineral salt solution 172.6 ml/Kg (Adinarayana et al.,2003).Poly-epsilon-lysine is an emulsifier, coating agent for biochips, and detergent ingredient.Using CCD, it was possible to determine the ideal concentrations of yeast extract, glucose, and ammonium sulphate for antibacterial activity against *Streptomyces albus* IFO14147. These values were established to be 2.86, 23.82, and 0.14 g/L, respectively (Shih and Sen,2006). An R2 of 0.816 substantiates that the output rose from 0.75 g/L to 8.13 g/L. Actinorhodin is an antibiotic polyketide that is produced by *Streptoycescoelicolor* A3 (2). It has 33.9 g/L sucrose, 1.95 g/L glucose, 2.72 g/L yeast extract, and 19.5 g/L peptone. It was developed and optimized using CCD. Elibol, 2004 saw a 32% rise in output following optimization when compared to baseline conditions. Using response surface methods and CCD, *Streptomyces aureofaciens* was used to produce tetracycline, a bacteriostatic drug. For increased production, two components were investigated: potassium chloride (0.3, 0.7, and I.1 M) and carrageenan (1.0, 1.5, and 2.0%).peptide antibiotic called enterocin is used to treat infections that are spread through food. Hassan et al., 2017, explored the application of chemical elicitation approach and utilization of heavy metals on *Streptomyces* sp. H-1003 for synthesis of enterocin. The culture conditions for enhanced production, such as starch 20 mg/L, were optimized using Gause's medium and the Box-Behnken design. The enhanced production was supported up to 5.33 mg/L for 10 days at 180 revolutions per minute.The results are significant for the same design, as indicated by the F value of 15.42, p value of 0.0008, and lack of fit of 0.0497. Pikromycin is a polyketide antibiotic with significant clinical utility. Jeong et al. (2015) reported that the response surface methodology was successfully used to design

pikromycin from *Streptomyces venezuelae*. The final variables were fixed at 139 g/L sucrose, 5.29 g/L K2HPO4, and 0.081 g/L CaCO3, with a maximum pikromycin yield of 35.5 mg/L. Positive influence on production was observed for sucrose, K2HPO4, and CaCO3 with the use of a Pareto chart for the same model utilizing an ANOVA p value of 0.000, R2=91.25, and modified R2=79.1.

Thus far, Streptomyces sp. has been the source of the widely reported antibiotics. The medium's constituents, which include ammonium sulfate, peptone, and yeast extract as nitrogen and carbon sources and glucose and sucrose as carbon sources, were mostly tailored to enhance the generation of antibiotics.

OTHER BIOMOLECULES FROM ACTINOBACTERIA USING RESPONSE SURFACE METHODOLOGY

A red pigment produced by *Streptomyces* sp. PM4 has been utilized to treat cancer (Karuppiah et al., 2013). The International Streptomyces Project medium (ISP-2) was used to extract the pigment. The processing medium was effectively standardized using CCD, and it was found that the maximum production required tyrosine (2.8 g/L), peptone (7.34 g/L), yeast extract (4.34 g/L), and maltose (4.06 g/L). The combined parameters listed above produced a maximum output of 1.68 g/L.Glycolipid biosurfactants, which are mostly utilized to lower surface tension, are produced by *Streptomyces* sp. MAB36, which was isolated from a sample of maritime sediment. Starch casein was used as the development medium, and CCD was used to optimize the medium's constituent parts. The study's findings showed that for improved yield, the interplay of fructose, yeast extract, casein, starch, and incubation time was essential (Manivasagan et al.,2014). Floculants are often used to remove flocculation from water channels. A combination of *Streptomyces* and *Celllomonas* sp. was used to create the biofloculant. Both BBD and CCD were employed in the study. CCD indicated enhanced activity, and an adjusted medium comprising sucrose (16 g/L), peptone (1.6 g/L), and magnesium chloride (4.45 g/L) had a good impact on production (Nwodo et al.,2014) and the overall output was 4.45 g/L.The chemicals that facilitate insoluble substances to dissolve in water are called emulsifiers. The need for microbial emulsifiers is emerging. The emulsifier synthesis of *Streptomyces* sp. MC1 was first described. The development of metabolites was found to be improved by up to 65% with an increased emulsification index of up to 3.5 times when using a factorial design study culture medium optimized at pH-8, phosphate 2 g/L, Cal g/L, and 5 g/L of Cr

[11].*Brachybacteriumparaconglomeratum* MSA21 was identified by Kiran et al. 2014 in a marine location and claimed to produce glycolipid-biosurfactant. Using response surface methods, the fermentation medium was designed, and it was found that among the five components, there were significant interactions between glucose, yeast extract, copper sulfate, and inoculum size.The generation of clavulanic acid by *Streptomyces clavuligerus* was enhanced through the use of glycerol and ornithine in conjunction with Response Surface Methodology (Domingues et al.,2010). This mechanism was established upon the suppression of β lactamase.

SIGNIFICANCE OF THIS STUDY

A highly challenging problem in bioprocess technology is the economical optimization of medium. This study is focused on CCD, BB, and RSM in particular while examining the different organic and inorganic substrates utilized by Actinobacteria to synthesize diverse metabolites. Given the importance of parameter selection in medium design, the data produced in the study can serve as a roadmap for future research.Once the medium formulation factors have been assigned, a pareto map can be used to screen the effects of each factor on the final product. Some of the models with ANOVA can be used to undertake additional validation based on the observation. A design's relevance can be ascertained using the contour plot, R2 value, p value, and lack of fit. To validate the confidence of the model, each of these factors should be assessed before moving on to a pilot scale plant.

It is clear from the data that the most widely utilized model for optimization is CCD. R2 values, which indicate the model's accuracy, should range from 0 to 1, and the significance of the p value should be less than 0.05. In a similar vein, the optimized model has fewer inputs than the unoptimized condition, which will increase the yield of biomolecules.

Full factorial or fractional factorial designs, known as central composite designs, are made up of "points" in the experiment's center and "stars" outside of it. The experiment software's nature was predicted using these stars and points.

CONCLUSION

A wide range of investigations can employ experiment design because it is a flexible technique. This approach can be applied to many other tasks, such as variable screening, optimization, and product creation. The platform's ability to produce sustainable goods will speed up as a result. This paper helps researchers conceptualize research goals, data analysis, and

output, and it assesses the traditional components of DOE in addition to contemporary models and implementations. Many biotechnology experiments make use of response surface methodology and its concepts. Response surface approach and related concepts are used in many biotechnology experiments. It has been reported that there are various effective designs for medium optimization in Actinobacteria research. It has also been reported that important factors with enhanced production, like pigment, antibiotic, protein, enzymes, and plant growth agents, can be screened. These points are emphasized and explicitly outlined here. For the purpose of creating more dependable and repeatable procedures, this strategy would be quite helpful.

CONFLICTS OF INTEREST

There is no conflict of interest from authors

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