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Statistical optimization strategies on waste substrates for solving high-cost challenges in biosurfactants production: a review

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Abstract Biosurfactants are bio-based amphiphilic molecules with extensive applications in various industries. These eco-friendly alternatives possess numerous advantages over chemical surfactants. However, high production costs hinder market competitiveness of biosurfactants. Production costs of synthetic surfactants range between \$1-3/kg, while biosurfactants cost between \$20-25/kg. Principal challenges hindering commercialization of biosurfactants are high costs of media constituents and downstream processing, accounting for 30% and 60-80% of production costs, respectively. Thus, cost-effective biosurfactant production would depend on the utilization of environment-friendly low-cost substrates and efficient product recovery. To this end, statistical tools such as Factorial Designs (FD) and Response Surface Methodology (RSM), are employed to optimize the production processes. FD as effective screening models comprise Plackett-Burman Design (PBD) and Taguchi design; and involves quantification of various significant factor effects including the main effect and level of dependency of one factor on the level of one or more factors. RSM predicts appropriate proportions of media constituents and optimal culture conditions; and is reportedly effective in reducing production cost and consequently, market price. Central Composite Design (CCD) and Box-Behnken Design (BBD) are common RSM for optimizing biosurfactants production. CCD assesses the relationship between one factor or more and a set of experimental variables. BBD is considered more proficient than CCD as it requires fewer experimental runs. Most recently, Artificial Neural Network which uses artificial intelligence-based tools to predict biosurfactant production using dependent variables of the process is gaining attention.

Keywords: Biosurfactants, Statistical Optimization, Waste Substrates.

1. INTRODUCTION

Surfactants are amphipathic molecules that decrease surface and interfacial tension between liquids or biphasic systems [1], hence their diverse applications in various industries [2]. Based on their source, surfactants are broadly classified as chemical surfactants and biosurfactants. Chemical surfactants are mostly derived from petrochemicals and oleochemicals and have been (utilized as emulsifiers or surface energy reducers, generally applied in industrial settings [3]. Conversely, biosurfactants are obtained from natural sources [4], with microorganisms as leading producers of various types [5]. Bacteria are the largest producers of biosurfactants with *Pseudomonas* and *Bacillus* as the highest-producing genera [6]. Next to bacteria, are fungi, producing the most structurally diverse variants of biosurfactants with a representative share of 19% (12% and 7% from Ascomycetes and Basidiomycetes respectively) [6]. Other producing microbial genera include *Acinetobacter, Clostridium, Thiobacillus, Saccharomyces, Candida, Corynebacterium, Penicillium, Rhodococcus, Ustilago, Aspergillus, Enterobacter, Brevibacterium, Leuconostoc, Lactobacillus* [7].

Biosurfactants typically consist of a hydrophobic (long-chain fatty acid, hydroxyl fatty acid) and hydrophilic component (such as glucose, amino acid, cyclic peptide) [8], [9]. Based on origin and chemical nature, these surface-active microbial agents can begrouped intophospholipids, glycolipids, lipopeptides, polysaccharide-protein complexes, neutral lipids and fatty acids [10]. Another categorization is based on molecular weight, where low molecular weight consists of lipopeptides,trehalolipids, rhamnolipids, sophorolipids [11], phospholipids, polyketideglycosides, spiculisporic acid [12] and high molecular weight comprises of polymeric molecules and lipoprotein [11]. Industrially relevant classes of biosurfactants are rhamnolipids, glycolipids sophorolipids, and mannosylerythritol lipids, and lipopeptides – surfactin, given their ease of production [12].

1.1 BIOSURFACTANTS VERSUS CHEMICAL SURFACTANTS

Bio-based surfactants possess variousbenefits over synthetic analogues; these include high selectivity, biocompatibility, and tolerance for extreme environmental conditions such as broad pH range, temperature and high salinity [13]. Also, concerns associated with use of chemical surfactants can be circumvented by using biosurfactants. For instance, chemical surfactants are extensively employed in various processes as emulsifiers, detergents, etc, and this has resulted in the manufacture of more than 15 million tons of chemical surfactants yearly, which contributes adversely to the environment in form of soil and water pollution, and penetration of the trophic chain, thus causing deleterious changes [14]. Biosurfactants on the other hand, can be synthesized from a broad range of renewable feedstock [5], and are recognized as environmentally friendly alternatives with lower toxicity when compared with chemical surfactants [15].

Furthermore, in terms of parameters indicative of surfactant's efficiency such as critical micelle concentration and emulsification, biosurfactants outdo synthetic analogues. Generally, surfactants with lower CMC can self-associate better to form micelles capable of solubilizing hydrophobic compounds, and emulsification is indicative of how well these agents stabilize immiscible mixtures [3]. Biosurfactants possess lower CMC [11] and have been noted to possess better emulsifying abilities than chemical surfactants [3]. Given these qualities, they have extensive application in various industries as summarized in Table 1 below.

TABLE 1: APPLICATIONS OF BIOSURFACTANTS

Industry	Biosurfactant Type	Application	Reference
Medicine	Glycolipids	Antimicrobial, anti-inflammatory, anti-oxidant, anti-coagulant activity, cytotoxic, immunological and neurological property	[1]
	Lipoproteins	Anti-coagulant, anti-mycoplasma, anti-inflammatory, anti-viral, anti-bacterial	
Pharmaceuticals	Glycolipids and Lipopeptides	Microemulsions drug systems, antiviral, antibacterial, antifungal, adhesive and immunomodulatory properties, and utilized in vaccines and gene therapy	[16]
Cosmetics	Glycolipids	Moisturizing and enhanced water retention properties in skin care products, Repair ability for damaged hair and hair strengthener, thus an excellent ingredient in hair products, Anti-oxidative effects	[17]
	Lipopeptides	Emulsification of oils given their heat resistance and capacity	[4]
Textile	Glycolipids	Textile dye degradation	
Bioremediation	Glycolipids and Lipopeptides	Increase bioavailability of substrate for microbial degradation during hydrocarbon bioremediationy.	[18]
Food Industry	Glycolipids	Improvement of food consistency, texture and overall appearance, food preservatives, shelf life extension of bread	[19]
Agriculture	Glycolipids and Lipopeptides	Pest control, plant protection	[20]

1.2 CHALLENGES ASSOCIATED WITH BIOSURFACTANTS

Production costs of synthetic surfactants are reportedly lower than that of biosurfactants, with a kilogram priced in the range of $1-3, whereas biosurfactants are valued between $20-25/kg depending on quantity productivity of the fermentation process [21]. The principal challenges hindering the commercialization of biosurfactants are the expensiveness of fermentation media constituents and downstream purification and recovery processes [22], [23]. Thus, cost-effective biosurfactant production depends on utilization of inexpensive substrates and production methods.

Substrates for biosurfactants production account for up to 30% of production, hence, cheap substrates such as wastes from industrial and agricultural sources can be used, thus, providing a sustainable waste management option [24], [25]. Industrial wastes such as agro, refinery and food waste are the best replacement for costly feedstock as it decreases production costs and enhances economic and environmental sustainability [25]. Agricultural wastes have been noted as the ‘low-cost substrate candidate’ [26]. The use of agricultural waste in production of biosurfactants reportedly decreases production costsby 10% [27].

For fermentation types, solid-state fermentation (SSF) and submerged fermentation (SF) are highlighted as the most effective types for biosurfactant production [28]. However, SSF possesses many advantages over SF, which includes the cultivation of bacteria in their natural habitat, thus facilitating the production of secondary metabolites which are only synthesized in low quantities or not at all produced in SF [29]. Also, it overcomes the challenge of foam production encountered in submerged fermentation [30].

Downstream processing is another critical production point as it plays an important role in market competitiveness of bio-based surface active agents as it accounts for 60-80% of the total production costs[9]. For instance, rhamnolipids with about 90 % purity costs around \$1250 kg^{-1}, while its chemical counterpart with about 99 % purity costs between \$10 - \$20 kg^{-1}[31]. Purification and recovery methods currently in use include acid precipitation, foam fractionation, salts or organic solvents, etc,and chromatographic techniques such as ion exchange and high-pressure liquid. Challenges associated with methods involving the use of acids/salts include toxicity, and relatively low yields [32].

To circumvent challenges explained above, this review highlights statistical models employed to optimize growth media and fermentation conditions, for maximum productivity and yield, stating findings from previously conducted research.

2. STATISTICAL OPTIMIZATION STRATEGIES

Statistical design tools such as Factorial designs and Response Surface Methodology improve various factors of the production process [33]. Factorial designs include multifactor linear models, which allow for quantification of various factor effects; the main effect and interaction between the factors [32]. Factorial designs used for optimization include Taguchi design and Plackett-Burman Design (PBD) [30]. Response Surface Methodology (RSM) involves use of models obtained from data in experimental design which describes the connectionbetweenresponse (dependent parameters) and factors (independent parameters) [34]. Box-Behnken Design (BBD) and Central Composite Design (CCD) are the most frequent RSM implemented for optimizing biosurfactant production [32].

2.1 FACTORIAL DESIGNS

2.1.1 PLACKETT-BURMAN DESIGN

PBD is a two-level factorial design used to evaluate ‘n’ factors in n+1 experiments with the assumption that no interaction(s) exist between the factors [33]. Specifically, it is one of the effective screening statistical models utilized to first identify significant factors among many potential factors. [35], [36]. Selected parameters can then be optimized using other statistical designs such as response surface methodology. A study by Ekpenyong et al. [37] demonstrated PBD and Response Surface Methodology which is described below, as reliablemodels for idnetifying and improvingnutrient conditions for biosurfactant production. PBD was used to select trace minerals for biosurfactant production, and using RSM 84.44g/L yield (~3.54fold increase) was obtained. Steps involved in this screening design as noted by [38] include:

i. Selection of factors
ii. Definition of levels for these factors
iii. Identification of responses to be measured
iv. Creation of design matrix
v. Randomization and experimental runs as stated in the set-up
vi. Design replication
vii. Model development
viii. Statistical and graphical analysis of effects
ix. Interpretation of statistical analysis
x. Improvements where necessary based on interpreted findings

2.1.2 TAGUCHI DESIGN

Taguchi Design is utilized in studying the effects of several variables concurrently in achieving optimum productivity and yield [39]. Davis and John, 2018 broadly explain the Taguchi method in four phases: (1) determining parameters important to product/process, (2) design of experimentation sequence and execution, (3) statistical analysis to determine optimum conditions, (4) confirmation test with optimum conditions. [40] elaborates on these steps further:

i. Output variable(s) response(s) to be improved are selected
ii. Factors affecting these variables are identified and levels of these factors are chosen
iii. Using arrays found in literature, the appropriate orthogonal array is selected
iv. Interactions andfactors are assigned to columns of the array
v. Experimentation is carried out in a randomized format to reduce systematic error
vi. Results are analyzed usinganalysis of variance (ANOVA) and signal-to-noise (S/N) ratio analysis
vii. Optimal process parameters are determined
viii. Confirmatory experiments are conducted if required.

A work by Raza et al. [41] evaluated the effect of multi-response optimization of process parameters using Taguchi design. Their experimentation reported yield increase from 1.45 to 1.50g/L with lesser biomass formed and substrate utilized reduced from 26 to 14% (w/v). In another study by Marchut-Mikołajczyk et al. [42], the Taguchi method was employed to identify optimal conditions for biosurfactant production from *Bacillus pumilus* strain 2A. Experimentation was conducted using identified optimal conditions, and their findings were partially consistent with outcomes predicted by the Taguchi design. However, the experimental optimal pH condition was inconsistent with suggestions by the Taguchi design, as highest values for examined parameters occurred at a much lower pH than predicted [14].

2.2 RESPONSE SURFACE METHODOLOGY (RSM)

RSM comprises a set of statistical techniques which can be used to design experiments, build models, and concurrently determine the effect of factors and establish optimum conditions [23]. Correlation (data obtained from experimental data) are used in understanding the effect of factors (which can be dependent or independent), and process optimization [34]. As described by [43], the steps involved in RSM include:

i. Determine variables governing biosurfactant production, that is, factors.
ii. Identifythe significance of the factors on the process
iii. Data is collected following an experimental design – Central Composite Design and Box Behnken Design
iv. Response variables are measured.
v. Evaluate if the experimental region has a curve
vi. A. Curvature present - Add axial points to the experimental design
    B. No curvature – Redefine factor levels in the direction of the stationary point
vii. Carry out experimental runs
viii. Obtain an estimated model for each response variable and analyze the models: Data collected from experimentation are fit into a mathematical model that appropriately represents the process studied in function of its control parameters.

$$y = \beta_0 + \sum_{i=1}^{K} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{\substack{i=1 \\ j=1}}^{k} \beta_{ij} x_i x_j + \varepsilon$$

where,

ε = the residual
β_0 = the constant term of the model
β_{ij} = theinteraction terms coefficient
y = the modeled function
β = the linear terms coefficient
β_{ii} = the quadratic terms coefficient.

ix. Formulate and solve the optimization problem

Assessing determinants that ultimately lead to peak performance, facilitate production at optimum conditions with minimal cost [44]. Computerized software that can be used to apply RSM includes Design expert, Statistica, and Minitab [34].

2.2.1 CENTRAL COMPOSITE DESIGN (CCD)

In CCD which is also recognized as Box-Wilson Central Composite Design, each numeric factor is evaluated over levels: + and – alpha (axial points), + and – 1 (factorial points), and the centre points [32]. A study by Onlamool et al., 2020 combined CCD and RSM to optimize glycolipid biosurfactant production, and although they reported a low yield of 2.31 g/L, it was a 2.1 fold increase achieved under optimal conditions predicted by RSM [45].

2.2.2 BOX BEHNKEN DESIGN (BBD)

BBD are extensively used for second-order models in absolutesplit-plot experiments,randomized experiments and in robustfactor design settings [46]. It requires three levels for each processing factor, and experimental runs are executed depending on the combination of the factors [47]. BBD is considered more powerful and proficient than other designs such as three-level FFD and CCD as it demands fewer experimental runs than both [48]. Another advantage is that it requires a small set of parameters in determining the complex response function and avoids experiments performed under extreme conditions [49].

Table 2 below describes the strengths and deficiencies of each of these strategies.

2.3 Artificial Neural Network (ANN)

ANN has gained increased attention, as a non-linear multivariate modelling tool, which predicts based on experimental data. Data processing in this tool imitatesthe modality of the human brain and this model is noted to be more effective than Response surface methodology (RSM) [54]. In a work by Ekpenyong et al. [37], both methods (RSM and ANN) were used to predict and optimize product yield considering process physicochemical parameters. Performance was based on the nearness of the prediction models to real-life systems usingmean squared error (MSE) and values of coefficient of determination (R2). RSM returned an R2 value of 0.9923 and an MSE of 3.6661, while ANN gave an R2 value of 0.9964 and an MSE of 1.7844, thus outperforming RSM in predictive modelling capability [37].

TABLE 2: STRENGTHS AND DEFICIENCIES OF VARIOUS TYPES OF STATISTICAL DESIGNS

TYPE	NAME	Strengths	Deficiency	References
Response Surface Methodologies (RSM)	Central Composite Design (CCD)	• Better accuracy • Maximum information in a minimum experimental trial.	• Inability to estimate individual interaction terms,	[49]
	Box-Behnken Design (BBD)	• More proficient and powerful than other designs, as it requiresfewer experimental runs than three-level full factorial design and CCD, and is consequently less exclusive.	• poor coverage of the corner of non-linear design space	[48]
Factorial Designs	Plackett-Burman Design (PBD)	• Allows screening of several factors Can be utilized as an initial screening design tool since production involves multifactorial variable	• Two-factor interactions cannot be studied. • Interactions are partially confounded or "aliased" with all main effects.	[51]
	Taguchi Design	• Reduced time and budget.	• For dynamically changing processes this method is not appropriate. • Deals with designing quality rather than correcting for poor quality	[52]

ANN-based software consists of neurons, arranged as input, hidden and output. Neurons in the input layer accept information from the independent (input) variables, the hidden layer is connected fully to all neurons in the input and output layers and estimates the strengths of relationships between variables, calculates weights to be assigned to each to explore their effects and the output layer gives suggested values [54].

Table 3 below cites examples of these statistical strategies employed for optimized biosurfactant production using agro-industrial wastes. In Table 3, RSM indicates Response Surface Methodology, CCRD indicates Central composite rotational design, PBD indicates Plackett Burman Design, CCD indicates Central Composite Design, TD indicates Taguchi Design

3. FUTURE PERSPECTIVES

Given the advances in renewable solutions and the implementation of regulatory requirements for environmental safety, there is a growing demand for bio-based surfactants [64]. Studies have shown that most biosurfactant industries are based in Europe, Asia and North America, with Europe as the market leader controlling about 52.5% of the global share in 2019 [36]. This was largely attributed to heightened consumer awareness concerning the negative impacts of chemical surfactants in these climes [36]. Hence, as knowledge of environmental sustainability increases, it is expected that this market share would increase.

TABLE 3: Various statistical optimization strategies used for biosurfactants production

S/N	Microorganism	Statistical Design employed	Waste substrate utilized	Results	Biosurfactants Nature	References
1	*Lactobacillus paracasei* subsp. *tolerans* N2	PBD and RSM	Sugar cane molasses	four-fold increase in yield post-optimization	Possible glycolipoprotein	[55]
2	*Candida tropicalis* UCP0996	CCRD and RSM	Sugar cane molasses, Corn steep liquor, Waste frying oil	4.19g/L	Unidentified	[23]
3	*Pseudomonas aeruginosa* strain CGA1	RSM	Molasses	2.31-fold increase	Possible lipopeptide	[56]
4	*Pseudomonas aeruginosa* strain PBS29	RSM	Rice water	0.59 fold increase resulting in improved yield of 9.35 g/l	Possible rhamnolipid	[57]
5	*Bacillus subtilis* SPB1	RSM-CCD	Olive leaf residue flour and olive cake flour	30.67 mg of crude lipopeptide	Lipopeptide	[58]
6	*Pseudomonas aeruginosa* OG1	RSM	Waste frying oil and Chicken feather peptone	2-fold increase yielding approximately 13.31 g/L	Rhamnolipid	[59]
7	*Pseudomonas sp.* F5	CCD	Raw Orange peel	2.4 g/L	Glycolipid	[60]
8	*Pseudomonas putida*	TD	Waste frying oils	Yield increase from 3.4 to 4.1 g/L	Rhamnolipid	[61]
9	*Bacillus subtilis* SPB1	CCD	Orange peels, Soya bean and diluted seawater	2-fold increase yielding approximately 4.45 g/L	Unidentified	[62]
10	*Achromobacter sp*. PS1	CCD	Rice straw hydrosylate	5.46 g/L	Rhamnolipid	[63]

Although the current production costs of these microbial surfactants pose a major challenge, particularly when compared to their chemical counterparts, this is expected to change in the future through the integration of optimization methods [64]. While statistical optimization methods are not entirely expedient for improving biosurfactant production processes, they confer numerous benefits both in experimental efficiency and optimization of process parameters [32]. Also, they are not without their limitations, hence must be utilized based on the desired outcome. With increased knowledge of bioeconomy and circular economy globally, the search for waste as feedstock and renewable bioresources and has intensified greatly across various industries including biosurfactant production [19].

4. CONCLUSION

Biosurfactants have proven to be ideal candidates and promising alternatives to synthetic surfactants for various purposes across different sectors, however, ample research is still required to uncover sustainable production optimization methods, particularly on an industrial scale. This would significantly drive their preference in the global market, and consequently, reduce heavy dependence on their synthetic analogues. Optimization methods ranging from statistical design, strain improvement and process optimization show immense prospects in enhancing the production and applicability of biosurfactants. Although each of these methods hasits limitations, they provide ways to circumvent obstacles related to current production processes. Hence, these optimization techniques should be amply developed and applied to facilitate more efficient and cost-effective production. Waste management constitutes a major problem across the world as man’s activities generate waste in one form or the other. Biosurfactants production shows prospects in translating these discarded materials into valuable products by utilizing them as substrates. This is particularly important in developing countries like Nigeria, striving to attain environmental balance while experiencing exponential population growth. This could open frontiers in waste management methods which hitherto, have not been explored or deployed. Also, this would contribute to economic capacity as it opens up various opportunities for profitability. Finally, the concerted efforts against COVID-19 reinforced the age-long need to breach the gap between academia and industry as this is expedient to effective research and development; hence collaborations are required from the above listed to create a sustainable preference for biosurfactants using these recommendations.

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