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Fermentation Process Optimization

[1]Bibhu Prasad Panda, [1]Mohd. Ali and [2]Saleem Javed
[1]Pharmaceutical Biotechnology Laboratory, Faculty of Pharmacy,
Jamia Hamdard, Hamdard University, Hamdard Nagar, New Delhi, 110062-India
[2]Molecular Biology and Biotechnology Laboratory, Faculty of Science,
Jamia Hamdard, Hamdard University, Hamdard Nagar, New Delhi, 110062-India

Abstract: Many optimization techniques are available for optimization of fermentation medium and fermentation process conditions such as borrowing, component swapping, biological mimicry, one-factor-at-a-time, factorial design, Plackett and Burman design, central composite design, response surface methodology, evolutionary operation, evolutionary operation factorial design, artificial neural network, fuzzy logic and genetic algorithms. Each optimization technique has its own advantages and disadvantages.

Key words : Fermentation, optimization techniques, evolutionary operation, fuzzy logic, genetic algorithms

INTRODUCTION

For an industrial fermentation process fermentation medium and fermentation process condition plays an critical role because they effect the formation, concentration and yield of a particular fermentation end product thus effecting the overall process economics therefore it is important to consider the optimization of fermentation medium and process conditions in order to maximize the profits from fermentation process (Schmidt, 2005).

There are many challenges associated with optimization of fermentation process, it is laborious, expensive, open ended and time consuming process involving many experiments. In bioprocess industry it is often needs to conduct optimization experiments because new mutants and strains are continuously being introduced. In fermentation process optimization different combinations and sequence of process conditions and medium components are needs to be investigated to determine the growth condition that produces the biomass with the physiological state best constituted for product formation (Stanbury *et al*., 1997).

The present review explores the different well-known and newer optimization method applied in fermentation process.

OPEN AND CLOSE ENDED SYSTEMS FOR PROCESS OPTIMIZATION

In close-ended system, a fixed number and type of component parameters are analyzed for optimization, this is the simplest strategy but many different possible components/parameters which are not considered, could be beneficial in the medium. In open-ended system any number and type of components/parameters are analyzed for optimization of fermentation process. The advantage of this system is that it makes no assumption of which components/parameters are best for fermentation process. The ideal method would be to start with an open-ended system, select the best components/parameters for optimization of fermentation process then move to the close-ended system (Kennedy and Krouse, 1999).

Corresponding Author: Bibhu Prasad Panda, Pharmaceutical Biotechnology Laboratory, Faculty of Pharmacy, Jamia Hamdard, Hamdard University, Hamdard Nagar, New Delhi, 110062 -India
Tel: +91-11-26059688/5627 Fax:+91-11-26059663

DIFFERENT METHODS FOR FERMENTATION PROCESS OPTIMIZATION

Borrowing

This is an open-ended system for process optimization. The medium components and process conditions are obtained from the literatures and what other workers were used to grow the same genus, species or strains are analyzed. The problem with this method is that there are too many options for a given fermentation process. Therefore short listing is necessary and advantage of this method is that it is simple, easy and requires no mathematical skill (Kennedy and Krouse, 1999).

Component Replacing

This is an open-ended system for process optimization and only used to compare the component of one type in a fermentation medium (Nandi and Mukherjee, 1988). In this method, one of component of the medium was replaced by a new one at same incorporation level. However this method does not consider the components interactions. But this method can useful for screening different carbon, nitrogen and other source for improving the medium utilization (Kennedy and Krouse, 1999; Jatinder *et al*., 2006; Tavares *et al*., 2005). Screening of suitable carbon source for mevastatin and citric acid production by solid-state fermentation was carried out by component replacing techniques (Ahamad *et al*., 2006; Kumar *et al*., 2003).

Biological Mimicry

Biological mimicry is a close-ended system for fermentation process optimization. This method is useful for optimization of various components of fermentation media and based on concept that cell grow well in a medium that contains every things it needs in right proportion (mass balance strategy). The medium is optimized based on elemental composition of microorganisms and growth yield. The limitation of this method is measuring elemental composition of microorganisms is expensive, laborious and time consuming moreover it does not consider the component interaction however this method gives an idea about different micro and macro elements level require in the media for optimal growth of microorganisms (Kennedy and Krouse, 1999).

One-factor-at-a-time

One-factor-at-a-time is a close-ended system for fermentation process optimization. This method can be applied for optimization of medium components as well as for process condition and it is based on the classical method of changing one independent variable while fixing all other at a certain level (Ahamad *et al*., 2006; Alexeeva *et al*., 2002; Patidar *et al*., 2005). This strategy has the advantage that it is simple, easy and the individual effects of medium components and process condition can be seen on graphs (Kar *et al*., 1999; Kumar *et al*., 2003) but the limitations of this method are interaction between the components are ignored, extremely time consuming, expensive for large number of variable as it involves a relatively large number of experiments. Because of its easy and convenience one-factor-at-a-time method has been the most popular method for improving the fermentation medium and process condition.

Factorial Design

Factorial design is a close-ended system for process optimization. In this method, level of factors/parameters are independently varied, each factor at two or more levels. This effects that can be attributed to the factors and their interactions are assessed with maximum efficiency in factorial design more over it allow for the estimation of the effects of each factor and interaction.

The optimization procedure is facilitated by construction of an equation that describes the experimental results as a function of the factor level. A polynomial equation can be constructed in the case of a factorial design where the co-efficient in the equation are related to the effects and interactions of the factors. In a full factorial (complete factorial) design every combination of factor level was

tested. Typical factors are microbial strain, medium components, temperature, humidity, initial pH and inoculum volume. The most commonly used full factorials in medium improvement experiments are two factorial designs (denoted by 2^n when there are n factors). These designs are the smallest capable of providing detailed information on factor interaction (i.e., antagonistic or synergistic effects) (Xie *et al*., 2003).

A partial factorial design provides a compromise when the number of runs required in full factorials is impracticable. These are usually two-level factorial design. Two level fractional factorial are denoted by 2^{n-k}, where n is the number of factors and ½ k is the fraction of the complete factorial used. This notation gives an immediate idea of the number of runs required. For example, 2^{6-1} is a half fraction of complete factorial 2^5 and requires 32 (i.e., 2^5) runs per replicate (Rajendhran *et al*., 2002). In most case factorial design were in combined with other different optimization techniques such as central composite design (Park *et al*., 2005) and evolutionary operation (Tunga *et al*., 1999) to optimize fermentation process.

Plackett and Burman's Design

Plackett and Burman' s design may be useful to find out the important variable in a system this design is suitable when more then five independent variables are to be investigated. Plackett and Burman' s design are useful to screen out important factor, which influence the fermentation process (Naveena *et al*., 2005). Which are optimized by response surface methodology in further studies (Sayyad *et al*., 2006; Singh and Satyanarayana, 2006). This technique allows for evaluation of n variables by n+1 experiments. n+1 must be multiple of 4 e.g., 8, 12, 16, 24, etc. therefore the number of independent variables which can be investigated by this method are 7, 11, 15, 19, 23, etc. Any factors not assigned to a variable can be designated as a dummy variable. The incorporation of dummy variable into an experiment makes it possible to estimate the variance of effects (Plackett and Burman, 1946).

Central Composite Design

Central composite design (CCD) was introduced by Box And Wilson; CCDs are formed from two level factorials by addition of just enough points to estimate curvature and interaction effects. The design can be viewed as partial factorials with factors at five levels. The number of runs in CCD increases exponentially with number of factors. Optimization of media components for compaction production in complex and chemically defined production medium using CCD has been reported (Kennedy and Krouse, 1999).

CCD can be combined with response surface methodology, in which experiments were designed by CCD and thereafter optimized by response surface methodology (Chakravarti and Sahai, 2002; Dahiya *et al*., 2005).

Response Surface Methodology

Statistical experiment design is a powerful method for accumulating information about a process rapidly and efficiently from a small number of experiments, thereby minimizing experimental costs. Box and Wilson introduced Response Surface Methodology (RSM). RSM seeks to identify and optimize significant factors with the purpose of determining what levels of factors maximize the response (Sayyad *et al*., 2006; Singh and Satyanarayana, 2006). RSM uses statistical experimental design such as Central Composite Design (Chakravarti and Sahai, 2002; Dahiya *et al*., 2005), Box-Behnken Design (Sayyad *et al*., 2006) etc. in order to develop empirical models that relate a response and mathematically describes the relationships existing between the independent and dependent variables of the process under consideration.

The contours of a response surface optimization plot show lines of identical response. Response means the results of an experiment carried out at particular values of the variables being investigated. The axes are the contour plots are the experimental variable and the area with in the axes is termed the

response surface. To construct a contour plot, the results (response) of a series of experiments employing different combination of variable are inserted on the surface of the plot at the points delineated by the experimental conditions, points giving the same results (equal response) are joined together to make a contour line (Kumar *et al*., 2004).

The purpose of response surface methodology was to obtain a predicted model and this model can be useful for optimizing the fermentation media formulation or for optimization of fermentation process condition, to carry out simulation with model equation and for better understanding the fermentation process.

Evolutionary Operation

Evolutionary operation employs factorial design sequentially to improve yield. The changes made to variable from one cycle to the next are restricted and can only be made when the estimated improvements are greater then the estimated experimental error. Optimization of production of protease by *Rhizopus oryzae* using Evolutionary operation has been reported (Banerjee and Bhattachaaryya, 1993).

Evolutionary Operation Factorial Design

The evolutionary operation (EVOP) factorial design methodology was a hybrid of evolutionary operation and factorial design technique here, experiments are designed based on factorial technique and results are analyzed by EVOP. This methodology is considered to be a multi variable sequential search technique, in which the effects of n variable factors are studied and response analyzed statistically. The decision-making procedure is easy and clear-cut it directs the change of variable to wards the objective maximum or minimum values. Evolutionary operation factorial design technique combines the advantage of factorial technique for designing experiments with n parameters and that of evolutionary operation methodology for systematic analysis of experimental results and facilitate the selection of optimum condition or direct the change desired for individual parameters for design of subsequent experiments. For a study of a five variable system, the total number of new experiments to be conducted is 2^5, apart from two control experiments (search level regions). The parameters for the above experiments are arranged in both higher level (+) and lower level (-) compared to search level regions (0), the parameters and the total number of experiments are represented in a [5 X (2^5+2)] matrix Which has been divided in two blocks i.e., overall negative blocks and overall positive blocks. All experiments were replicated for two cycles. The magnitude of effects, change in mean effects, standard deviation and error limits (of average, of effects and of change in mean effects), analyzed as per the decision making procedure of Evop to arrive at the optimum. When the experimental results of the first set did not satisfy the optimum conditions, a second set of experiments was planed selecting the best condition of the first set as the new search level for second set. This procedure was repeated till the optimum condition was obtained (Tunga *et al*., 1999; Panda, 2001)

Optimization of protease enzyme production under solid-state fermentation by *Rhizopus oryzae* and optimization of gallic acid production under solid-state fermentation using evolutionary operation and factorial design technique has been reported (Tunga *et al*., 1999; Kar *et al*., 2002; Mukherjee and Banerjee, 2004).

Artificial Neural Network

Artificial neural network is the model and trained on a given set of data and then used to predict new data point and provide a mathematical alternative to quadratic polynomial for representing data derived from statistically designed experiments. Artificial neural network's strong points are that they work well with large amount of data and handles them easily with out requiring no mechanistic description of system, this make artificial neural network particularly well suited to medium optimization (Kennedy and Krouse, 1999).

First data generated by conducting a series of experiments and a network is constructed and getting the network to learn on these data set, once trained, the network is given new data points (media composition or fermentation process condition) and the out put (microbial performance or product formation) predicted. Artificial neural networks are well suitable for predicting the outcome from the fermentation process there by saving time and efforts (Patnaik, 2005).

However artificial neural networks are simply a modeling tool and does not work properly when input data sequence are missing neural network s confused when different data are generated for same set of experiments but averaging the data can solve the problems.

Fuzzy Logic

Fuzzy logic utilizes and executes a series of rules using Fuzzy membership functions. At first the Fuzzy memberships are defined. This defines what should be the level of the components in a fermentation media whether it is in low or high. Then next sets of experiments are defined based on results obtained from the first set of experiment (Ul-haq and Mukhtar, 2006). When a new medium composition is entered in Fuzzy logic programme, it predicts the result or the out come (microbial performance or product formation) (Anderson and Jayaraman, 2005; Kennedy and Krouse, 1999).

Genetic Algorithms

In recent years non-statistical optimization techniques such as genetic algorithms are used in fermentation technology. This is a powerful stochastic search and optimization technique, this technique can be used to optimize fermentation process with out need of statistical designs and empirical models and based on the principle that after a continuous process of mutation only best individual exist. These individuals strive for survivals. After some number of generations only the best individual hopefully represents the optimum solution. In fermentation media or fermentation process optimization rules of genetic algorithms can be applied successfully where the set of one experiment i.e. medium composition are coded in one chromosome and each medium constituent level represents one gene after completing the first generation of experiments chromosome with highest productivity are selected and replicated proportionally to the productivity then cross over of chromosome and mutation of some randomly chosen genes are performed. In such a way, new generations of experiments are obtained. But main disadvantage of genetic algorithms is that it does not store the information generated at each stage of the optimization process (Zuzek *et al*., 1996). A hybrid of genetic algorithms and artificial neural network approach was realized to optimize fermentation process. This technique based on principle that after a satisfactory neural network model and input space which is generated over the range of independent parameters, can be optimized using genetic algorithms the advantage of this technique is that neural network provide better fits to experimental data then quadratic polynomial equation and model optimized by genetic algorithms approach which provide a better alternative to the conventional RSM approach to optimize fermentation process (Nagata and Chu, 2003).

CONCLUSIONS

Designing a fermentation media or optimization of fermentation process can be never ending task and every optimization techniques have their own advantages and disadvantages (Table 1). Historically one-factor-at-a-time used mostly fallowed by full factorial technique and response surface methodology but Plackett and Burman's design and component replacing can be useful for screening medium components. Recently nural networks fuzzy logics, genetic algorithms and different hybrid techniques such as CCD-RSM, Plackett and Burman-RSM, factorial design-RSM, evolutionary operation-factorial design and genetic algorithms-artificial neural network techniques used efficiently to optimize fermentation medium and fermentation process parameters.

Table 1: Type of fermentation optimization techniques and their advantages and disadvantages

Optimization techniques	Advantages	Disadvantages
Borrowing	It is simple, easy and requires no mathematical skill.	There are too many options for a given fermentation process.
Component replacing	Useful for screening	Does not consider the component interaction.
Biological mimicry	Gives an idea about different micro and macro elements require for growth of microorganisms.	Does not consider the component interaction.
One-factor-at-a-time	The individual effects of medium components and process condition can be seen on a graph.	Interactions between the components are ignored.
Factorial design	It allow for the estimation of the effects of each factor and interaction.	Requires mathematical skill.
Plackett and Burman's design	Useful to screen out important factor which influence the fermentation process	Does not consider the components interactions
Central composite design	It allow the estimation of Interaction between the components	Requires mathematical skill
Response surface methodology	Visualization of results by 3D graphs and predicts models.	Requires mathematical skill
Evolutionary operation	Easy decision making procedure.	Requires mathematical skill
Evolutionary operation and factorial design	It analyzes interaction of n variable factors.	Requires mathematical skill
Artificial neural network	Large numbers of data are easily handled.	Requires mathematical skill
Fuzzy logic	It has ability to tolerate highly variable data.	Requires high mathematical skill
Genetic algorithms	Systematic and steady improvements of factors.	Requires high mathematical skill

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